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HISTOMORPHOLOGICAL AND BIOCHEMICAL STUDIES IN PLASMA AND LIVER OF FIELD RATS INHABITING SOUTH-WEST REGION OF PUNJAB IN NORTH INDIA

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ABSTRACT
The present study was designed to examine the possible effect of environmental contaminants on the biochemical constituents of field rats residing Bathinda district of Punjab. The amount of proteins and enzymes aminotransferases and phosphatases was low in plasma of male and female *R. rattus,* but higher in *T. indica* and *B. bengalensis* residing in Bathinda region as compared to control rats. Protein amount and levels of enzymes ALT, AST and ACP were higher in liver of Bathinda male and female *R. rattus* and *T. indica*. Pesticide residue of malathion was detected in liver sample of these rats. Slight loosening in arrangement of hepatic cords, infiltration of leucocytes, dilation in central vein (CV) and presence of pyknotic cells (PN) was observed in rats collected from Bathinda district. The environmental contaminants/pesticides may be responsible for the biochemical and histological alterations in field rats.of Bathinda.

INTRODUCTION

Globally, use of synthetic pesticides has increased rapidly in the last fifty years due to intensification of farming in order to obtain higher yields. However, over dependence on chemicals not only resulted in high cost of production but also irreparable damage to the environment and long term health problems to human and other forms of life (Xavier *et al.*, 2004). The use of pesticides to manage pests in land and water has posed health hazards to live stock and wildlife (Hashmi and Khan, 2011). Prolonged exposure to insecticides is known to cause chronic neurological syndrome, malignant tumors, immunosuppressive action, teratogenic effect, abortion and decreased fertility in experiments animals (Meeker *et al.,* 2006).

South-Western region of Punjab is known for its high pesticide use and deteriorated ground water quality due to agrochemicals processes and extensive use of phosphate fertilizers (Bhalla *et al.*, 2011). Acute occupational exposure for pesticides among sprayers was also high as they occasionally use protective devices while spraying (Thakur *et al.*, 2008; Singh and Kaur, 2012). Earlier studies give some indication of increased physiological risks of exposure to pesticides/heavy metals, but the epidemiological evidences do not allow any clear inference to be drawn (Thakur *et al.*, 2008, Singh and Kaur 2012, Singh *et al.,* 2012). A number of pathological conditions have been linked to organophosphate exposure in liver of mammals and fish (Indirabai *et al.*, 2010, Sharma and Sangha, 2014). Hence the present study was designed to examine the possible impact of environmental contaminants on the biochemical constituents and histology of liver in male and female *Rattus rattus, Tatera indica* and *Bandicota bengalensis* species of rats inhabiting Bathinda district of South-West of Punjab.

MATERIALS AND METHODS

The present study was conducted on field rats collected from Bathinda district of South-West Punjab and the rats of Punjab Agricultural University (PAU) that served as control rats. Approval of Institutional Animal Ethical Committee, Guru Angad Dev Veterinary and Animal Science University (GADVASU), Ludhiana was obtained for the usage of animals vides letter no. 3901-35 dated 06-08-2012. During the study, rats *i.e., R. rattus, T. indica and B. bengalensis* were trapped from fields of Bathinda district of South West region of Punjab and PAU, Ludhiana Rats were brought to the laboratory and separated according to sex and species.

Biochemical studies

For biochemical studies, blood sample from each rat was collected directly from heart by heparinised syringe in heparinised vials. Blood was centrifuged at 23000 r.p.m. for 15 minutes. Supernatant was obtained as plasma. One gram of liver tissue was also homogenized in 2 mL of phosphate buffer saline (PBS 0.1M, pH 7.4) and homogenate was centrifuged at 3000 r.p.m. for 10 min. The plasma and liver supernatant was used for estimation of total soluble proteins

and various enzymes. Protein content was estimated by the method of Lowry *et al.* (1951). Alanine amino transferase (ALT), aspartate amino transferase (AST) were estimated by the method of Reitman and Frankel as described by Bergmeyer (1974). Acid phosphatase (ACP) and alkaline phosphatase (ALP) activity was measured in citrate buffer (0.05M, pH 10.5) and glycine buffer (0.05M, pH 10.5), respectively using p-nitrophenol phosphate as substrate following the method of Bessay *et al.* (1946).

Pesticide residue analysis

Liver samples were processed for extraction of pesticide residues following the method of Erney (1974) with some modifications.

Histological studies

For histomorphological studies, liver of rats (*R. rattus, T. indica and B. bengalensis)* collected from Bathinda region and PAU, Ludhiana were fixed in alcoholic bouin's solution for 24 hours. After complete fixation, the tissue was dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax (Melting point between 58-60°C). The 5μm thick sections were cut with the help of microtome and after usual de-waxing and rehydration in descending series of ethanol to water, the sections were stained in haematoxylin, eosin, cleared in xylene and mounted in DPX. The stained sections were observed under Olympus microscope for histopathology and photographed.

Statistical analysis

Results were expressed as Mean $\pm$ standard error of mean (SEM) and subjected to student t-test. Results were considered statistically significant with $p < 0.05$.

RESULTS AND DISCUSSION

The amount of protein was low in plasma of male and female *R. rattus,* but higher in *T. indica* and *B. bengalensis* residing in Bathinda region as compared to Ludhiana district. Levels of enzyme ALT, AST, ACP and ALP was also lower in *R. rattus* but higher in plasma of male and female *T. indica* and *B. bengalensis* inhabiting this region (Table 1, 2). Significant low levels of protein was also observed in liver of male and female *B. bengalensis* rats inhabiting Bathinda district of Punjab. Levels of enzymes ALT and AST were higher in Bathinda male and female *R. rattus* and *T. indica*. Acid phosphatase activity was more in liver of female *R. rattus* and *T. indica* Bathinda rats. Alkaline phosphatase activity was found to be low in male and female *R. rattus* and *B. bengalensis* while in male *T. indica,* it was more as compared to Ludhiana rats (Table 3,4). Biochemical parameters are sensitive index of the changes due to pesticide toxicity and can constitute important diagnostic tool in toxicological studies (Singh and Saxena 2001). Low levels of protein in plasma and liver of Bathinda rats as compared to control rats may indicate the induced degenerative changes in liver of rats or general disturbance of the protein anabolism (Toor *et al.,* 2013, Sharma and Sangha, 2014). Increased transaminase activity is probably the consequence of pesticide induced pathological changes in liver causing hepatic damage. The increased levels of ALT in plasma of rats in present study may indicate their enhanced metabolic activity, perhaps to meet the stress induced by prolonged exposure to the pesticide (Kaur and Dhanju, 2004).

Phosphatases are important and critical enzymes in the biochemicals process and are responsible for detoxificiation processes. A change in enzyme activity is generally related to intensity of cellular damage (Muthuviveganandavel *et al.,* 2008). Raised value of acid phosphatase in liver and blood indicates diseases accompanied by increased osteoblast activity or involvement of the liver. The increase in alkaline phosphatase activity represents the disturbed functional status of vital organs like liver (Murmu and Srivastava, 2011). The rise in alkaline phosphatase and acid phosphatase levels indicate tissue damage and subtle disturbance in hepatic dysfunctions and biliary secretions of animals (Rahman *et al* 2000, Murmu and Srivastava 2011, Toor *et al.,* 2013).

Malathion residues 1.332, 2.253 and 5.523 ppm were detected in the liver samples of *R. rattus, T. indica and B. bengalensis* respectively collected from Bathinda district of Punjab (Table 5 Fig. 2, 3 and 4). Malathion is a widely used pesticide that affects a variety of organs. Epidemiological research into the acute and chronic toxicity of malathion indicates that this chemical is highly toxic to mammals (Abdollahi, 2004). Mammals are observed to be adversely affected by oral, dermal and inhalation exposure to malathion (Edwards *et al.,* 2007, Rezg *et al.,* 2008).

Liver is the first target organ for toxicological prospects because of its role in detoxification biotransformation and excretion of xenobiotics. Light microscopic observation of the liver sections of the control Ludhiana male and female rats showed a normal histological architecture of liver clearly outlining the sections of anastomosing hepatocytes along with adjacent sinusoids radiating from the central veins towards the periphery of the liver lobules (Plate 1 and 2, Fig a, d and g). Normal outlines of the central vein (CV) can be clearly visualized as shown. Hepatocytes were organized into plates separated by vascular channels (sinusoids). No infiltration of leucocytes was observed in the sinusoids. Each lobule was bounded by scanty

Table 1: Estimation of protein and enzymes from plasma of male *R. rattus, T. indica* and *B. bengalensis*

Biochemical Constituents	*R. rattus* Control	Bathinda	*T. indica* Control	Bathinda	*B. bengalensis* Control	Bathinda
Protein	1.53 $\pm$ 0.11	1.38 $\pm$ 0.09	1.89 $\pm$ 0.08	2.45 $\pm$ 0.08	1.26 $\pm$ 0.09	1.74 $\pm$ 0.22
ALT (IUC)	123.01 $\pm$ 14.04	97.73 $\pm$ 12.04	119.19 $\pm$ 12.14	93.80 $\pm$ 5.69	129.91 $\pm$ 13.71	161.36 $\pm$ 11.53
AST (IUC)	58.47 $\pm$ 5.31	53.30 $\pm$ 7.17	52.67 $\pm$ 3.64	69.53 $\pm$ 10.57	70.42 $\pm$ 7.49	79.54 $\pm$ 8.72
ACP (μmole mg^{-1}protein)	1.43 $\pm$ 0.19	1.01 $\pm$ 0.06	1.67 $\pm$ 0.03	1.98 $\pm$ 0.01	1.17 $\pm$ 0.07	1.27 $\pm$ 0.24
ALP (μmole mg^{-1}protein)	5.05 $\pm$ 0.83	4.62 $\pm$ 0.41	7.18 $\pm$ 0.04	7.76 $\pm$ 0.50	4.34 $\pm$ 0.56	6.34 $\pm$ 1.34

Values are Mean $\pm$ SE; *Significant difference at ($p < 0.05$) as compared to control

Table 2: Estimation of protein and enzymes from plasma of female *R.rattus, T. indica* and *B. bengalensis*

Biochemical Constituents	*R. rattus* Control	Bathinda	*T. indica* Control	Bathinda	*B. bengalensis* Control	Bathinda
Protein	1.64±0.09	1.38±0.09	1.89±0.08	2.46±0.08	1.35±0.12	4.19±0.44
ALT (IUC)	134.27±9.7	99.21±11.07	127.05±10.90	99.43±9.58	129.60±9.17	170.07±12.06
AST (IUC)	64.58±7.31	53.57±4.89	51.94±2.65	72.00±10.14	87.96±6.39	93.073±4.75
ACP (μmole mg^{-1}protein)	1.45±0.14	1.29±0.11	1.79±0.11	2.15±0.19	2.10±0.12	1.18±0.01
ALP (μmole mg^{-1}protein)	4.99±0.21	4.46±0.42	7.39±0.14	9.21±0.29	4.78±0.87	4.89±0.34

Values are Mean ± SE; *Significant difference at ($p < 0.05$) as compared to control

Table 3: Estimation of protein and enzymes from liver of male *R. rattus, T. indica* and *B. bengalensis*

Biochemical Constituents	*R. rattus* Control	Bathinda	*T. indica* Control	Bathinda	*B. bengalensis* Control	Bathinda
Protein	7.48±1.07	7.76±1.21	10.00±0.26	11.58±3.67	10.82±0.56	8.11±0.92
ALT (μmole mg^{-1}wt of sample)	124.39±10.58	170.49±14.04	193.02±4.11	241.99±5.69	162.85±13.71	175.81±13.21
AST(μmole mg^{-1}wt of sample)	22.01±4.70	36.24±3.84	41.40±5.82	54.83±8.82	34.07±2.12	19.12±3.41
ACP (μmole mg^{-1}protein)	6.22±1.31	4.58±0.63	9.66±0.26	7.52±1.00	9.87±0.23	2.61±0.35
ALP (μmole mg^{-1}protein)	2.67±0.01	2.04±0.10	3.03±0.02	3.90±0.36	2.42±0.03	2.39±0.09

Values are Mean ± SE;*Significant difference at ($p < 0.05$) as compared to control

Table 4: Estimation of protein and enzymes from liver of female *R. rattus, T. indica* and *B. bengalensis*

Biochemical Constituents	*R. rattus* Control	Bathinda	*T. indica* Control	Bathinda	*B. bengalensis* Control	Bathinda
Protein	7.42±0.19	5.21±0.24	6.99±0.56	7.46±0.36	8.01±0.05	7.00±0.16
ALT (μmole mg^{-1}wt of sample)	130.98±13.30	176.23±10.44	202.26±12.67	236.25±8.92	185.58±9.40	187.81±13.69
AST(μmole mg^{-1}wt of sample)	28.12±3.74	54.14±8.58	35.08±4.57	52.83±1.89	39.34±4.97	19.12±3.41
ACP (μmole mg^{-1}protein)	9.73±0.56	10.70±1.15	6.65±0.32	11.00±0.76	8.83±0.39	7.23±0.86
ALP (μmole mg^{-1}protein)	2.24±0.21	2.23±0.30	3.04±1.10	3.39±0.55	1.90±0.13	1.72±0.04

Values are Mean ± SE;*Significant difference at ($p < 0.05$) as compared to control

Table 5: Pesticide residue analysis (ppm) in liver samples of *R. rattus, T. indica* and *B. bengalensis*

Sr. No.	Species of Rats	Name of the Pesticide detected	Residue (ppm)
1	*R. rattus*	Malathion	1.3327
2.	*T. indica*	Malathion	2.2531
3.	*B. bengalensis*	Malathion	5.5228

connective tissue. Liver of Bathinda field rats showed loosening in arrangement of hepatic cords around central vein which was more prominent in male *B. bengalensis* rats inhabiting Bathinda district (Plate 1 and 2, Fig b,c,e,f,h and i). The loss of radial arrangements of hepatocytes was also seen in all species male and female Bathinda rats. The dilation of CV and sinusoids between hepatocytes were also increased and showed infiltration of large mass of leucocytes inflammatory cells in

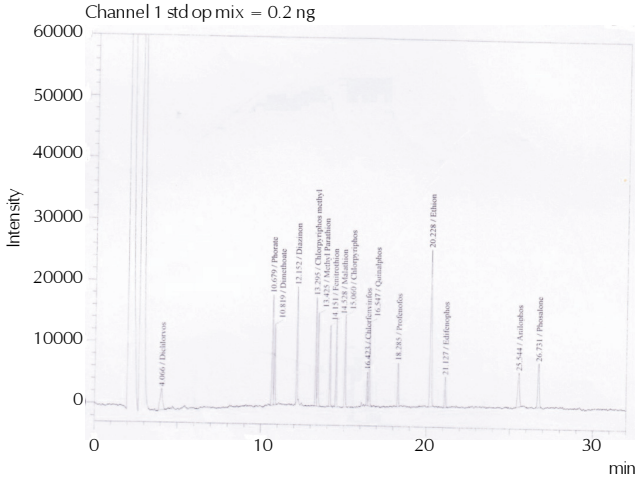

Figure 1: Standard chromatogram of pesticide residue

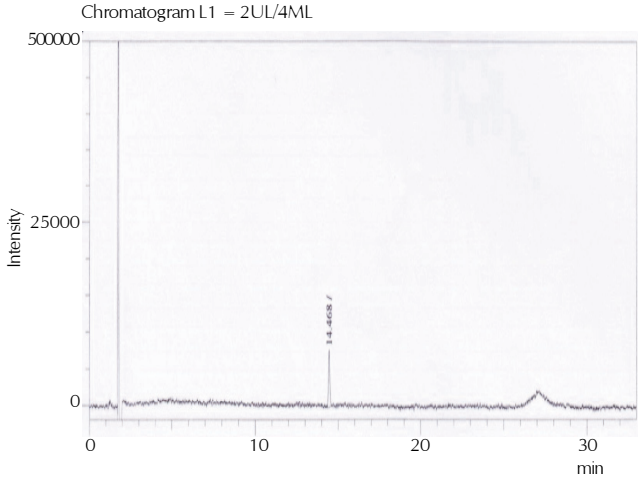

Figure 2: Pesticide residue chromatogram of Bathinda rats *(R. rattus)*

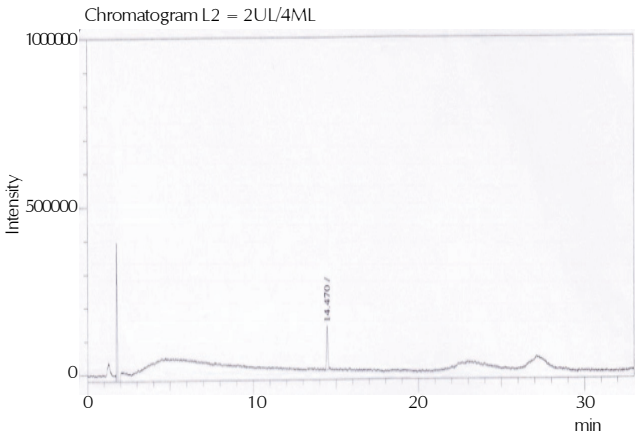

Figure 3: Pesticide residue chromatogram of Bathinda rats *(T. indica)*

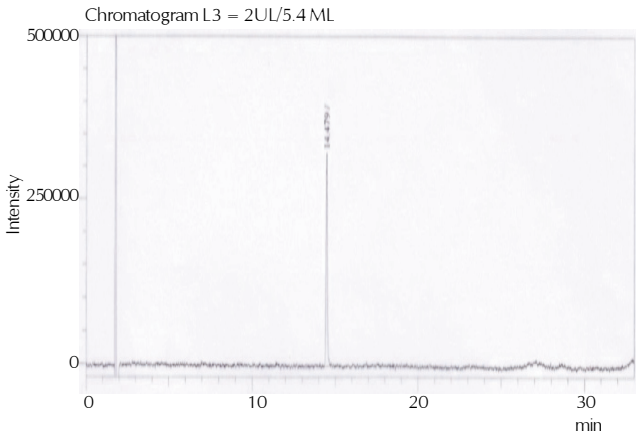

Figure 4: Pesticide residue chromatogram of Bathinda rats (*B. bengalensis*)

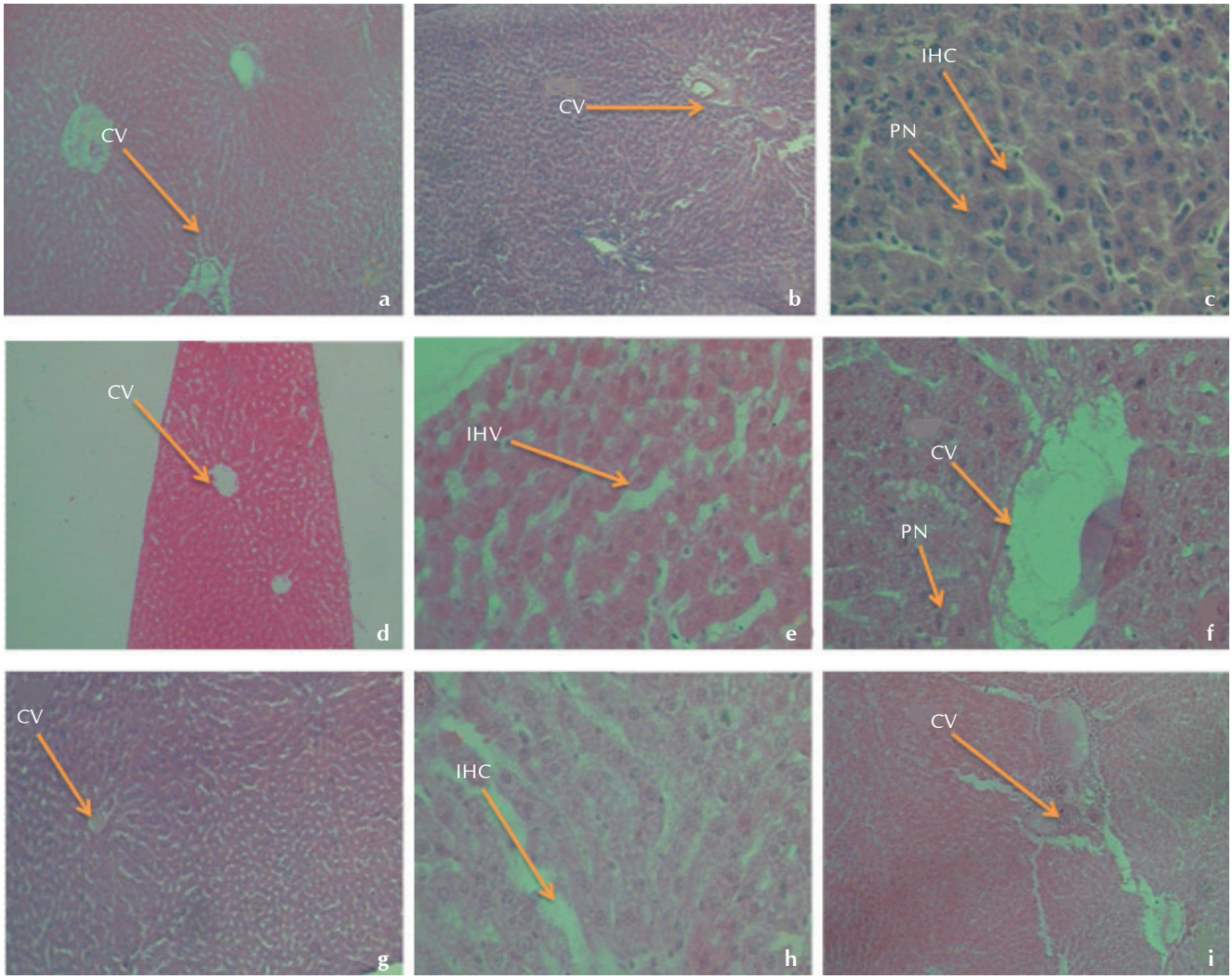

Plate 1: Fig. a, d &g: T.S. of liver of a Ludhiana male *R. rattus, T. indica and B. bengalensis* showing normal outlines of central vein (CV). Hepatocytes nuclei are round with dispersed chromatin and prominent nucleoli (X100); Fig b & c T.S. of liver of a male *R. rattus* collected from Bathinda district showing slight loosening in arrangement of hepatic cords (arrow), irregularly arranged hepatic chords (IHC), infiltration of leucocytes and dilation in central vein (CV) and presence of pyknotic cells (PN) (X100) & (X400); Fig e & f T.S. of liver of a male *T. indica* collected from Bathinda district showing slight loosening in arrangement of hepatic cords (arrow), irregularly arranged hepatic chords (IHC), infiltration of leucocytes and dilation in central vein (CV)) and presence of pyknotic cells (PN). (X100) & (X400)

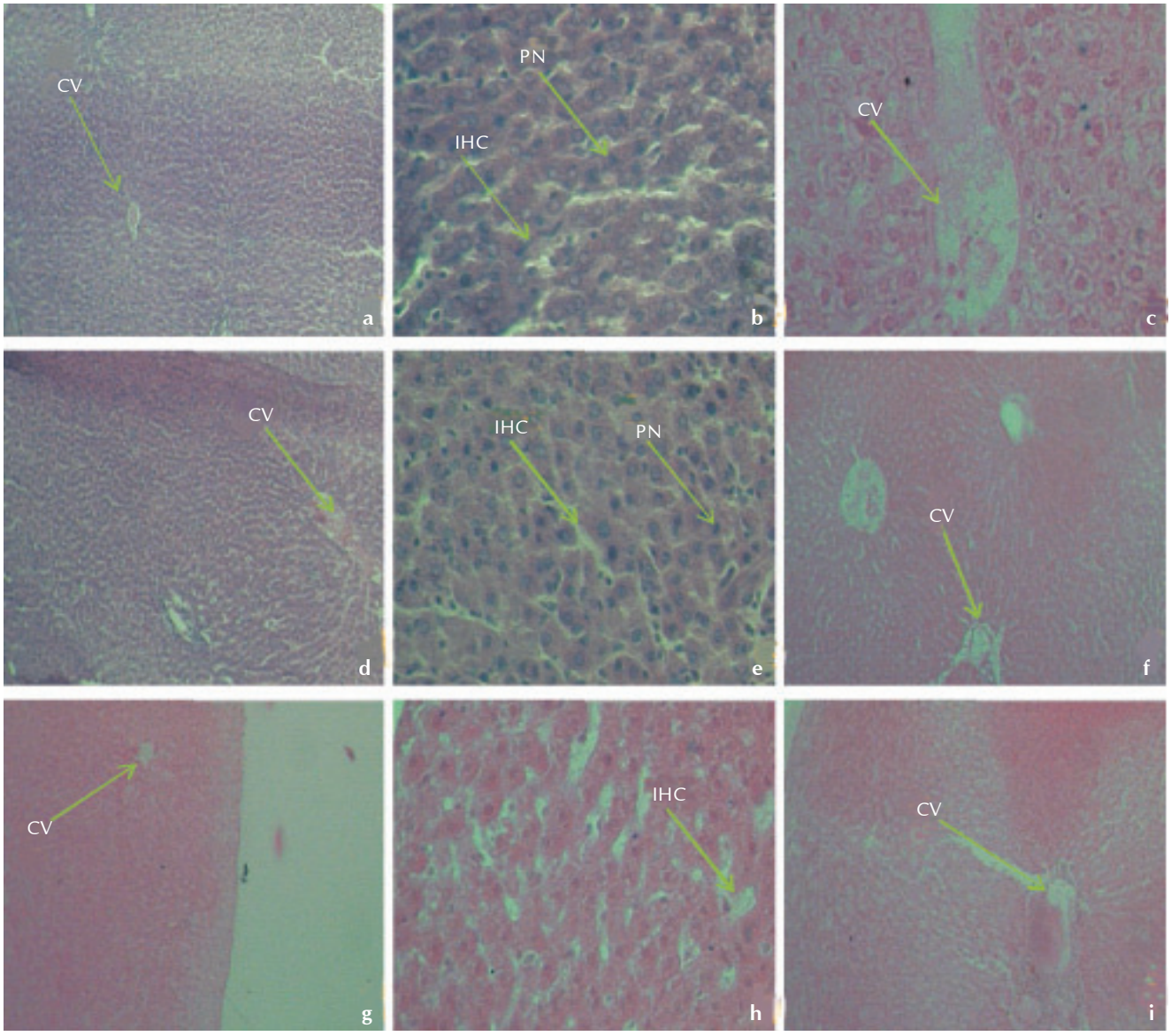

Plate 2: Fig. a,d & g: T.S. of liver of a Ludhiana female *R. rattus*, *T. indica and B. bengalensis* showing normal outlines of central vein (CV). Hepatocytes nuclei are round with dispersed chromatin and prominent nucleoli (X100); Fig b & c T.S. of liver of a female *R. rattus* collected from Bathinda district showing slight loosening in arrangement of hepatic cords (arrow), irregularly arranged hepatic chords (IHC), infiltration of leucocytes and dilation in central vein (CV) and presence of pyknotic cells (PN) (X100) & (X400); Fig e & f T.S. of liver of a female *T. indica* collected from Bathinda district showing slight loosening in arrangement of hepatic cords (arrow), irregularly arranged hepatic chords (IHC), infiltration of leucocytes and dilation in central vein (CV)) and presence of pyknotic cells (PN). (X100) & (X400; Fig h & i T.S. of liver of a female *B. bengalensis* collected from Bathinda district showing slight loosening in arrangement of hepatic cords (arrow), irregularly arranged hepatic chords (IHC), infiltration of leucocytes and dilation in central vein (CV) (X100) & (X400)

CV and sinusoids.

Earlier studies have also reported that the liver of control rats showed a normal structure with hexagonal lobules, central veins and peripheral triads embedded in connective tissue (Owoeye *et al.,* 2012)

Histopathological examination of the liver from animals treated with cypermethrin and cyfluthrin, imidacloprid also revealed various cellular and lobular abnormalities, including intralobular vein (ILV) membrane dilation, presence of hepatocytes in ILV, cytoplasmic vacuolisation, hepatocyte membrane damage, nuclear division, nucleocentricity, pyknosis and necrosis (Bhushan *et al.,* 2013, Toor *et al.,* 2013). Heikal *et al.* (2013) reported histopathological alterations in liver of cyromazine and chlorpyrifos treated rats which include degeneration and coagulative necrosis in the hepatocytes, inflammatory cells infiltration, and kupffer cells proliferation.

Environmental contaminants and pesticides might have altered the biochemical status and distorted the histology of the liver of rats inhabiting Bathinda district of South-West Punjab.

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