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Research Article

Toxico-Pathological Alterations in the Liver of Cadmium Treated Rats

Tripathi Sarojni, Srivastav Ajai Kumar*

Department of Zoology, DDU Gorakhpur University, Gorakhpur 273 009, India

A B S T R A C T

Male Wistar rats were randomly divided into three groups -- A, B and C. Cadmium chloride was administered orally to two groups at a dose of 5 mg/kg b wt/day to group B and 10 mg/kg b wt/day to group C. Rats from group A served as control. Rats were sacrificed on 1st, 2nd, 4th, 6th, and 8th week after initiation of the experiment. Liver were removed immediately, fixed in Bouin's fixative, routinely processed and stained with hematoxylin and eosin (HE). In rats administered with cadmium, histopathological changes have shown that the hepatic parenchyma is a target to the toxic action of this heavy metal. The most frequent alterations noticed were hepatocytic hypertrophy and vacuolation, degeneration of hepatocytes and their nuclei, hyperchromatic and hypertrophied nuclei, sinusoidal dilation and focal necrosis. However, variable intensities of these changes were noticed depending upon the doses and duration of the treatment.

Key words: Heavy metal; Liver; Cadmium; Toxicity

INTRODUCTION

Heavy metals threaten human health through environmental and occupational exposure[1,2]. Cadmium has been recognized to pose a considerable environmental concern that provoke liver damage[3]. Under normal conditions, the intake of cadmium depends on the cadmium concentration in natural sources such as air, land and water and varies from 50 to 100 µg/day, however, the intake may be higher (150-200 µg) in some environmental conditions[4]. In the organisms which are exposed to cadmium, the highest levels of cadmium have been detected in liver and kidney cortex[5,6].

Histopathological investigations have been considered as an efficient tool for the monitoring of environmental pollution in water resources[7]. There exists many studies in which histopathological analysis has been related with the animals' health more effectively than biochemical analysis and considered to be better in understanding the effects of toxicity at community- and ecosystem-level[8].Liver is a key organ which plays a vital role in physiology and acts as a detoxification organ for the metabolism and excretion of toxic substances. The production of metallothionein (MT) in the liver – a small cystein-rich metal binding protein induced by cadmium, binds most of the cadmium in the form of Cd-MT complex and protects tissues from cadmium toxicity [9]. Cadmium absorption from the gastrointestinal tract is the main route of its entry into human beings[10].

In order to elucidate the importance of food-borne metal contamination, the present study has been carried out to investigate the cadmium-induced toxico-pathology in the liver of rat (orally administered with cadmium chloride at different doses for 8 weeks) with the intention to provide a basis for understanding similar pathology in humans. Oral administration seems to be the most appropriate in long-term experiment of cadmium as it enters the animal/human body through food and water. Also, oral route for cadmium exposure to rats rather than injecting (subcutaneous, intraperitoneal or intravenous) it, would better reflect the dietary exposure that most human population (non-smokers) experiences. Hence, the effects due to repeated dose administration by gavage have been investigated in this study.

MATERIALS AND METHODS

Male Wistar rats (procured from the Indian Institute of Toxicology Research, Lucknow, India) weighing approximately 145-160 g were used in this study. The animals were housed in polypropylene cages under natural photoperiod. Food and tap water were given during the study period. All treatments were started after almost 2 weeks of stabilization from arrival of the rats in the laboratory. The animals were randomly divided into three groups -- A, B and C, each consisting of 25 animals. Cadmium chloride was dissolved in distilled water and administered orally to two groups at a dose of 5 mg/kg b wt/day to group B and 10 mg/kg b wt/day to group C. Rats from group A employed as control. Treatments were given at 08:00 each day throughout the experiment

Rats (five from each group) from all the groups were sacrificed 24 h after last dose on 1st, 2nd, 4th, 6th, and 8th week after initiation of the experiment under light ether anesthesia Animals were fasted overnight before sacrifice. Liver were removed, washed with distilled water and immediately fixed in Bouin's fixative. These fixed tissues were dehydrated in an ethanol gradient, treated with a clearing agent, infiltrated and embedded in paraffin, sectioned at 6 µm, floated on a heated water bath and mounted to glass slides. After drying overnight, paraffin was removed with a clearing agent, tissue was

Corresponding Author: E-mail: ajaiksrivastav@hotmail.com

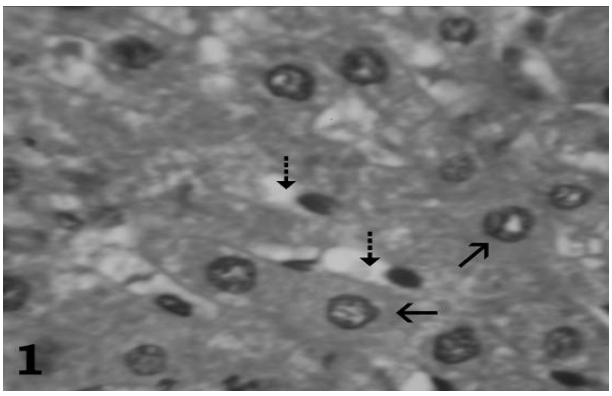

Figure 1: Liver of control rat showing hepatocytes (arrows) and sinusoids (broken arrow). HE x 500.

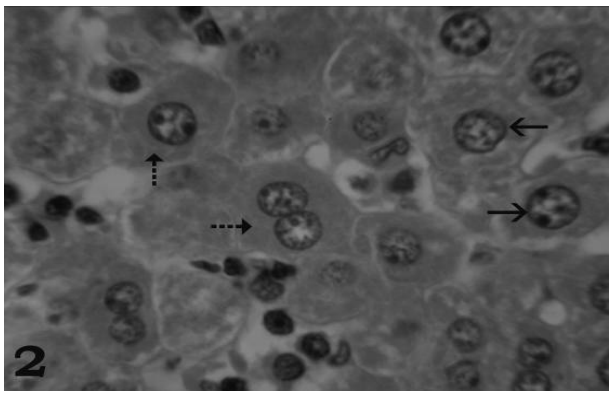

Figure 2: Liver of 2 week cadmium treated (5 mg/kg b wt/day) rat exhibiting hyperchromatic and hypertrophied nuclei (arrows). Note densely stained cytoplasm (broken arrows). HE x 500

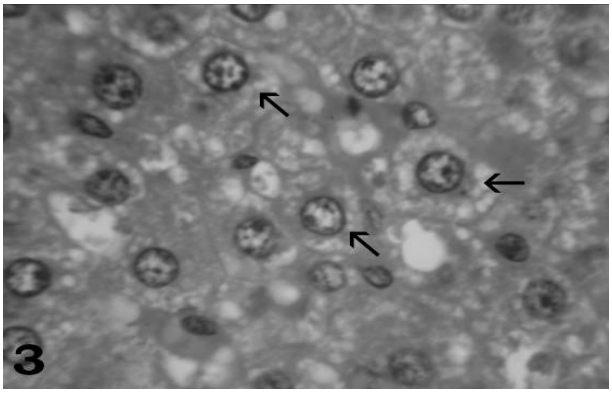

Figure 3: Hypertrophied nuclei and vacuolated cytoplasm (arrows) in the liver of 4 week cadmium (5 mg/kg b wt/day) treated rat. HE x 500

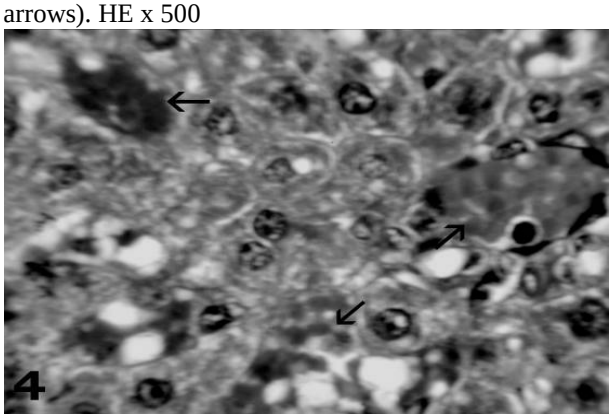

Figure 4: Congestion in the sinusoids and blood vessels (arrows) in the liver of 6 week cadmium (5 mg/kg b wt/day) treated rat. HE x 500.

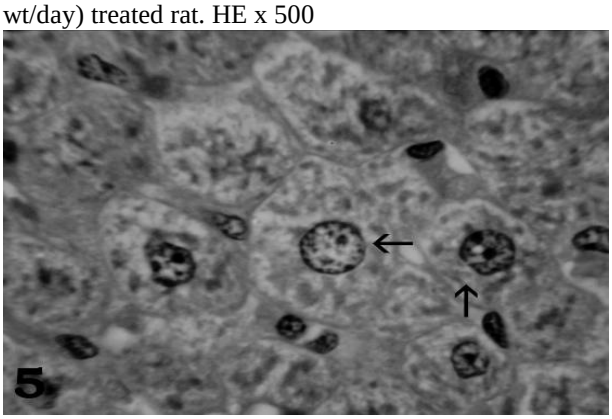

Figure 5: Vacuolated cytoplasm (arrows) in the liver of 6 week cadmium (5 mg/kg b wt/day) treated rat. HE x 500.

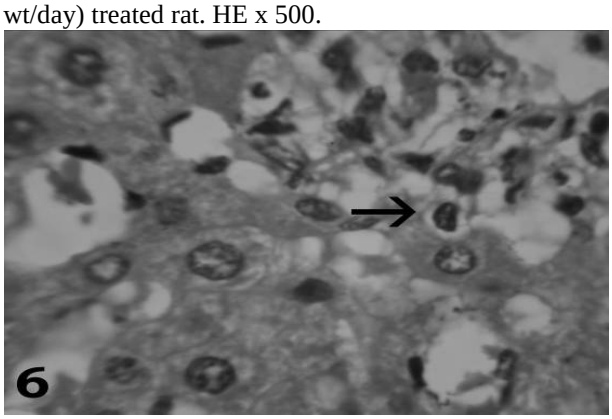

Figure 6: Liver of 6 week cadmium (5 mg/kg b wt/day) exposed rat displaying focal necrosis (arrow). HE x 500

rehydrated in an ethanol gradient and then stained with hematoxylin and eosin (HE) for light microscopic examination (Olympus CH 20i). At least five fields from each liver section (10 sections from each rat) were examined for the appearance of various liver structures. Photomicrographs were taken with the aid of Olympus E 420 camera.

OBSERVATIONS

In control rats liver is composed of cords that extend from the central vein to the portal triads. Hepatocytes are arranged in cords which are separated from each other by sinusoids (Fig. 1). Kupffer cells are also present along the sinusoidal spaces. Each hepatocyte is polygonal cell with a large centrally located spheroid nucleus having chromatin structure and a distinct nucleolus. The cytoplasm of hepatocytes is faintly granular (Fig. 1).

The liver showed no marked changes in its histological structure after 1 week cadmium treatment (5 mg/kg b wt/day). Following 2 week cadmium treatment, hepatocytes exhibited densely stained cytoplasm and

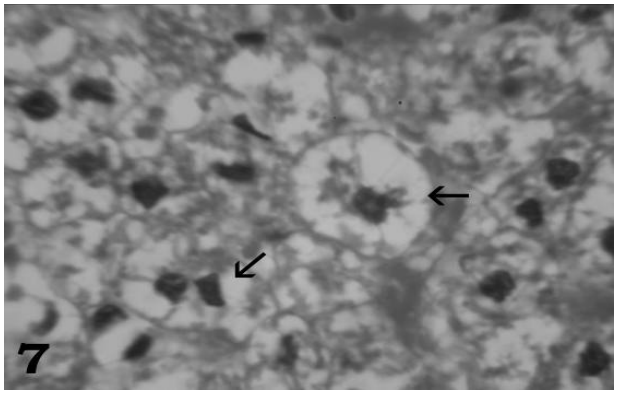

Figure 7: Liver of 8 week cadmium (5 mg/kg b wt/day) exposed rat showing foamy cytoplasm (arrows). HE x 500.

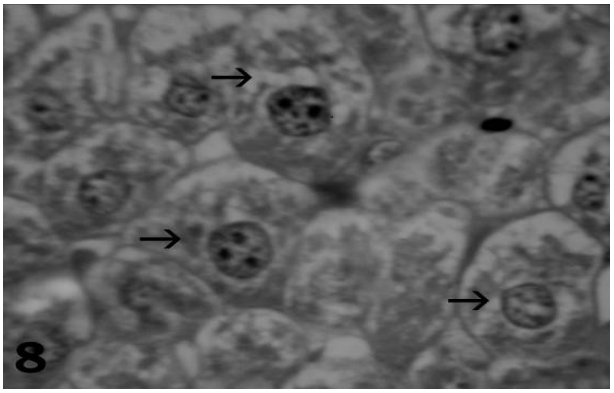

Figure 8: Hypertrophied nuclei and lightly stained cytoplasm (arrows) in the liver of 2 week cadmium (10 mg/kg b wt/day) exposed rat. HE x 500.

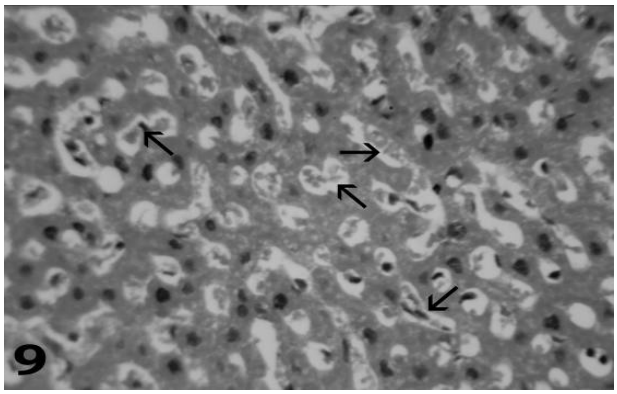

Figure 9: Deposition of eosin-positive material in the sinusoids and blood vessels (arrows) of liver of 2 week cadmium (10 mg/kg b wt/day) exposed rat. HE x 200

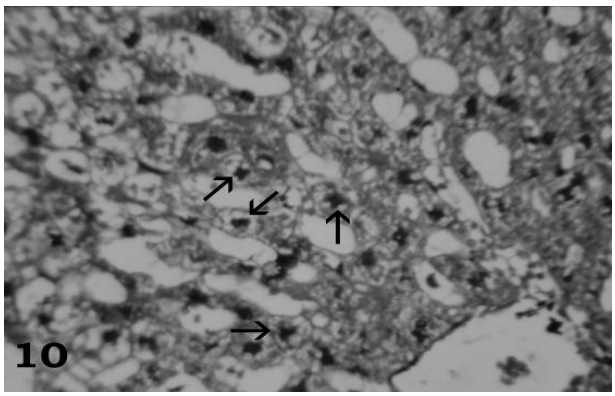

Figure 10: Cytoplasmic vacuolation and nuclear degeneration (arrows) in liver of cadmium (10 mg/kg b wt/day) treated rat for 4 week. HE x 200.

hypertrophy. Nuclei became hyperchromatic and hypertrophied (Fig. 2). The sinusoids were dilated. Rats exposed for 4 weeks to cadmium displayed much enlarged nuclei and light stained cytoplasm with vacuoles (Fig. 3). The sinusoids were more dilated. Nuclear degeneration was noticed at few places. After 6 week cadmium administration, congestion in the blood vessels and sinusoids was noticed due to deposition of eosin-positive materials (Fig. 4). Several crescentric nuclei have been noticed. The cytoplasm of hepatocytes became more vacuolar (Fig. 5). At places many degenerated cells (Fig. 6) were noticed aggregated (focal necrosis). Following 8 week cadmium treatment, the hepatocytes exhibited clumped chromatin, irregular nuclear boundaries and highly increased cellular boundaries having much vacuolated and foamy cytoplasm (Fig. 7). Focal necrosis was more frequent. In the liver of 1 week cadmium treated (10 mg/kg b wt/day) rat, the hepatocytes exhibited densely stained cytoplasm with hypertrophied nuclei. Sinusoids were noticed dilated. After 2 week cadmium treatment, the nuclear hypertrophy was more pronounced. In few cells the cytoplasm became lightly stained and vacuolated (Fig. 8). Sinusoids were more dilated. At places sinusoids were noticed filled with eosin-positive substances (Fig. 9). After 4 week cadmium treatment, clumped chromatin material was observed in few nuclei. The cytoplasm became more vacuolated and at places cellular and nuclear degeneration was encountered (Fig. 10). 6 week cadmium treatment caused complete loss of cord arrangement. Few nuclei became crescentric. Cellular and nuclear degenerations were more pronounced thus exhibiting focal necrosis. Cytoplasm became more foamy and vacuolated. The sinusoids were extremely dilated and contain eosin-positive materials. After 8 week cadmium administration focal necrosis was seen at many places. The changes noticed at earlier duration were exaggerated. At certain places the cytoplasm appeared cloudy thus resulting into hydropic degeneration (Fig. 11).

DISCUSSION

In rats administered with cadmium, histopathological changes have shown that the hepatic parenchyma is a target to the toxic action of this heavy metal. The most frequent alterations noticed were hepatocytic hypertrophy and vacuolation, degeneration of hepatocytes and their

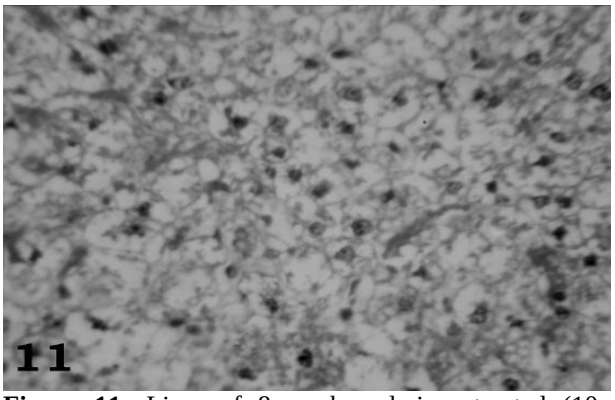

Figure 11: Liver of 8 week cadmium treated (10 mg/kg b wt/day) rat exhibiting cloudy appearance and hydropic degeneration. HE x 200.

nuclei, hyperchromatic and hypertrophied nuclei, sinusoidal dilation and focal necrosis. However, variable intensities of these changes were noticed depending upon the doses and duration of the treatment.

In the present study, cadmium-treated rats exhibited hypertrophy of hepatocytes. This is in accordance with the findings reported in rat[11] and fish[12] after cadmium treatment. The hyperchromatic nuclei in the hepatocytes of cadmium-treated rats derives support from the similar observations reported from rats treated with methamidophos[13] and chloroform[14].

The hepatocytes undergo cytoplasmic vacuolation and degeneration after cadmium treatment. Similar findings have been recorded earlier after cadmium treatment to mammals[6,11,15,16,17], bird[18] and fish[12]. Treatment with various other toxicants also produced vacuolization and degeneration of hepatocytes in rat (malathion[19], dimethoate[20], methamidophos[13], aluminium[21], fenitrothion[22], cypermethrin[23], mixture of metals[24], lamda-cyhalothrin[25]), mice (dimethoate[26,27]), chick (cypermethrin [28]) and fish (nickel[29], chlorpyrifos[30,31]). No changes were noticed in liver of rats treated with chlorpyrifos[32,33] and FYROL[34]. Luty et al.[35] and Gokcimen et al.[36] have noticed mild changes in the liver of rats treated with alpha-cypermethrin and diazinon, respectively.

In the present study foamy cytoplasm was noticed in the rats after cadmium treatment. The porous /foamy cytoplasm of liver cells noticed after treatment with dichlorvos in rats[35] was confirmed by ultrastructural studies of these investigators. Empty spaces were filled with vacuoles of different sizes and lipid-like bodies[35]. Similar findings were also reported by IARC Working Group[37] who noted lipid infiltration of liver cells after dichlorvos treatment. Focal necrosis of liver as observed in this study following cadmium treatment has been reported earlier by Jee et al.[38] and Ayoola and Ajani[39] in fish exposed to cypermethrin. It has been stated that tissue toxicity usually manifests itself in the form of cell degeneration accompanied by formation of large vacuoles, accumulation of fat and tissue necrosis[40]. Mollendroff[41] opined that vascular formation is a cellular defense mechanism against injurious substances to cells, these substances were segregated in vacuoles and thus were prevented from interfering with cellular metabolism. It has also been suggested that cytoplasmic vacuolation is mainly a consequence of disturbances in lipid inclusions and fat metabolism occurring during pathological disturbances[42]. Durham et al.[43] considered vacuolar degeneration as an alternation for the collection of injurious substances in the cells.

We conclude that ingestion of cadmium may lead to liver function impairment as assessed by toxico-pathological changes altering the structural integrity of liver in a dose related manner in cadmium-treated rats. Moreover, even low concentrations of cadmium caused histological alterations in the liver of rats and therefore allows the liver of the rats to be used as a biomarker of prior exposure to cadmium.

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REFERENCES

1. WHO. (2000) Cadmium. Air Quality Guidelines, World Health Organization, Regional Office for Europe, Copenhagen, Denmark.
2. Thijssen S, Maringwa J, Faes C, Lambricht I, and Van Kerkhove E (2007) Chronic exposure of mice
3. to environmentally relevant, low doses of cadmium leads to early renal damage, not protected by blood or urine cadmium levels. Toxicology 229: 145-156.
4. Gebhardt R (2009) Prevention of cadmium-induced toxicity in liver-derived cells by the combination preparation Hepeel. Environ Toxicol Pharmacol 27: 402-409.
5. Environmental Health Criteria 134: (1992) Cadmium. Friberg L, Elinder CG, Kjellstrom T, eds.; World Health Organization, Geneva.
6. Kocak M and Akcil E (2006) The effects of chronic cadmium toxicity on the hemostatic system. Pathophysiol Haemostat Thromb 35: 411-416.
7. Borges LP, Brandao R, Godoi B, Nogueira CW and Zeni G (2008) Oral administration of diphenyl diselenide protects against cadmium-induced liver damage in rats. Chemico-Biological Interactions 171: 15-25.
8. Stentiford GD, Longshaw M, Lyons BP, Jones G, Green M and Feist SW (2003) Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Marine Environmental Research 55: 137-59.
9. Au DWT (2004) The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Marine Pollution Bulletin 48: 817-834.

10. Nordberg GF (2009) Historical perspectives on cadmium toxicology. Toxico Appl Pharmacol 238: 192-200.
11. Klassen CD, Liu J and Diwan BA (2009) Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 238: 215-220.
12. Jihen H, Imed M, Fatima H and Abdelhamid K (2008) Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver and kidney of the rat: histology and Cd accumulation. Food Chem Toxicol 46: 3522-3527.
13. Dyk JCV, Pieterse GM and Vuren JHJV (2007) Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and zinc. Ecotoxicol Environ Saf 66: 432-440.
14. Satar S, Satar D, Tap O, Koseoglu Z and Kaya M (2004) Ultrastructural changes in rat liver treated with pralidoxime following acute organophosphate poisoning. Mt Sinai J Med 71**:** 405-410.
15. Mostafa AZM, Zehran FM, Guirgis A and Zein N (2009) Hypomethylation and histological changes induced by chloroform administered by oral gavages in corn oil to mice. Aust J Basic Applied Sci 3:1569-1576.
16. Brzoska MM, Moniuszko-Jakoniuk J, Pilat-Marcinkiewicz B and Sawicki B (2003) Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol Alkoholism 38: 2-10.
17. Damek-Poprawa M and Sawicka-Kapusta K (2004) Histopathological changes in the liver, kidneys, and testes of bank voles environmentally exposed to heavy metals emissions from the steelworks and zinc smelter in Poland. Environ Res 96: 72-78.
18. Renugadevi J and Prabu M (2009) cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp Toxicol Pathol doi: 10.1016/j.etp.2009.03.010
19. Chishti MA and Rotkiewicz T (1993) Hepatic and renal ultrastructural changes in cockrels exposed to cadmium chloride and subsequent interaction with organophosphate insecticide. J. Environ. Pathol Toxicol Oncol 12: 35-45.
20. Luty S, Obuchowska-Przebirowska J, Latuszynska J, Tokarska-Rodak M and Haratym-Maj A (2003) Dermal and oral toxicity of malathion in rat. Ann Agric Environ Med 10: 101-106.
21. Sharma Y, Bashir S, Irshad M, Nag TC and Dogra TD (2005) Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. Toxicology 15: 173-181.
22. Bogdanovic M, Begic Janeva A and Bulat P (2008) Histopathological changes in rat liver after a single high dose of aluminium. Arh Hig Rada Toksikol 59: 97-101.
23. Afshar S, Farshid AA, Heidari R and Llkhanipour M (2008) Histopathological changes in the liver and kidney tissues of Wistar albino rat exposed to fenitrothion. Toxicol Indus Hlth 24: 581-586.
24. Muthuviveganandavel V, Muthuraman P, Muthu S and Srikumar K (2008) A study on low dose cypermethrin induced histopathology, lipid peroxidation and marker enzyme changes in male rat. Pesticide Biochem Physiol 91: 12-16.
25. Jadhav SH, Sarkar SN, Patil RD and Tripathi HC (2007) Effects of subchronic exposure via drinking water to a mixture of eight water-contaminating metals: a biochemical and histopathological study in male rats. Arch Environ Contam Toxicol 53: 667-677.
26. Fetoui H, Garoui EM and Zeghal N (2009) Lambda-cyhalothrin-induced biochemical and histopathological changes in the liver of rats: Ameliorative effect of ascorbic acid. Exp Toxicol Pathol 61: 189-196.
27. Khogali FA, Sheikh JB, Rahman SA, Rahim AA and Dagestani MH (2005) Histopathological and hematological effects of dimethoate 4EC on some organs of Albino mice. J King Saud Univ 18: 73-87.
28. Domitrovic R, Jakovac H, Milin C and Radosevic-Stasic B (2009) Dose- and time-dependent effects of luteolin on carbon tetrachloride-induced hepatotoxicity in mice. Exp Toxicol Pathol 61: 581-589.
29. Aslam F, Khan A, Khan MZ, Sharaf S, Gul ST and Saleemi MK (2009) Toxico-pathological changes induced by cypermethrin in broiler chicks: Their attenuation with vitamin E and selenium. Exp Toxicol Pathol (doi: 10.1016/j.ctp.2009.06.004)
30. Patshynski M, Pedlar R, Evan R, Baron C and Klaver Kamp J (2002) Toxicology of dietary nickel in lake white fish (*Coregonus clupeaformis*). Aquat Toxicol 58: 229-247.
31. Kunjamma KPA, Philip B, Bhanu SV and Jose J (2008) Histopathological effects on *Oreochromis mossambicus* (tilapia) exposed to chlorpyrifos. J Env Res Develop 2: 553-559.
32. Velisek J, Svobodova Z and Piackova V (2009) Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*). Veterinarni Medicina 54:131-137.
33. Vodela JK and Dalvi RR (1995) Comparative toxicological studies of chlorpyrifos in rats and chickens. Vet Hum Toxicol 37: 1-3.
34. Wang HP, Liang YJ, Long DX, Chen JX, Hou WY and Wu YJ (2009) Metabolic profiles of serum from rats after subchronic exposure to chlorpyrifos and carbaryl. Chem Res Toxicol 22: 1026-1033.
35. Katz AC, Turnier JC, Zwicker GM and Sprague GL (1988) Hepatic changes in rats following subchronic administration of FYROL 6, an organophosphorus ester flame retardant. J Toxicol Environ Hlth 23: 295-301.

36. Luty S, Latuszynska J, Halliop J, Tochman A, Obuchowska D, Przylepa E and Korczak E (1998) Toxicity of dermally applied alpha-cypermethrin in rats. Ann Agric Environ Med 5: 109-116.
37. Gokcimen A, Gulle K, Demirin H, Bayram D, Kocak A and Altuntas I (2007) Effects of diazinon at different doses on rat liver and pancreas tissues. Pesticide Biochem Physiol 87:103-108.
38. IARC Working Group (1991) Dichlorvos: *IARC* Monographs on the evaluation of the carcinogenic risk of chemicals to humans. 53: 267-307.
39. Jee LH, Masroor F and Kang JC (2005) Responses of cypermethrin-induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Agri Res 36: 898-905.
40. Ayoola SO and Ajani EK (2008) Histopathological effects of cypermethrin on juvenile African Catfish (*Clarias gariepinusi*). World J Biol Res 1: 1-14.
41. Ariens EJ, Simonis AM and Offermeier J (1976) Introduction to general toxicology, Academic Press, Inc. New York.
41. Mollendorf A (1973) Cytology Cell Physiology, 3rd Edition, Academic Press, New York.
42. Zhang LY and Wang CX (1984) Histopathological and histochemical studies on toxic effect of brodifacoum in mouse liver. Acta Acad Med Sci 6: 386-388.
43. Durham SK, Brouwer A, Barelds RJ. Comparative endotoxin-induced hepatic injury in young and aged rats. J Pathol 1990;162: 341-349.