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ORIGINAL ARTICLE

Histopathological Effects of Carbaryl on Liver in Albino Rats

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ABSTRACT

Pesticides are one of the most alarming toxic substances that are deliberately added to our environment. Carbaryl, a synthetic 1-naphthyl-N-methylcarbamate insecticide, is being used extensively or its broad-spectrum activity in commercial agriculture, poultry, livestock, home and garden pest control. However, there is paucity of information about the role of carbaryl on the liver. Wistar rats were administered with carbaryl and the effects of this liver histology were analyzed. Profound damage of the liver of rats administered with carbaryl was noted. More studies would be required to assess the real implication of this pesticide on the liver.

Keywords: Albino rats, Liver histology, Liver, Carbaryl, Intoxication.

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INTRODUCTION

Food and agricultural organization (FAO) has defined the term pesticide as substances or mixture of substances intended for preventing, destroying or controlling any pest including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs or substances which may be administered to animals for control of insects, arachnids or other pests in or on their bodies.[1,2] A pesticide may be a chemical, biological agent (such as a virus or bacterium), antimicrobial, disinfectant or device used against any pest. Although, there are benefits to the use of pesticides, some also have drawbacks, such as potential toxicity to humans and other animals. According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are pesticides.[3] Pesticides are categorized into four main substituent chemicals: Herbicides, fungicides, insecticides and bactericides. The commonly used pesticides in India are those belonging to the organophosphorus groups, carbamate groups, organochlorines and pyrethroids. In the recent years, use of carbamate insecticides has gained importance due to ban of the insecticides belonging to organochlorine groups that is dichlorodiphenyltrichloroethane (DDT), aldrin, lindane and endosulfan. These pesticides have a tendency to persist and have potential to bioaccumulate in the body.[4] Carbaryl, a synthetic 1-naphthyl-N-methylcarbamate insecticide is being used extensively for its broad-spectrum activity in commercial agricultural, poultry, livestock, home and garden pest control. It was the most frequently detected carbamate in juice samples studied.[2] Carbaryl is a reversible cholinesterase inhibitor and is toxic to humans. It is classified as a likely human carcinogen by the United States Environmental Protection Agency (EPA).[5] A study conducted on rats, dogs and monkeys to see effect of carbaryl on kidneys showed epithelial changes in proximal convoluted tubule.[6]

Various experimental studies reported congenital malformation in chicken and duck embryos with carbaryl.[7-9] The histopathological changes were seen in various organs of male Wistar rats like heart, liver, kidney, lung and brain on dermal exposure to carbaryl for 4 weeks.[10] An increase in the activities of transaminase and acid phosphatase suggesting hepatocellular damage was also recorded.[11] Inhibition of liver enzymes with carbaryl was also reported.[12] This study was conducted to develop insights about the role of carbaryl on liver histology.

MATERIALS AND METHODS

The study was conducted in Government Medical College, Jammu, India in Anatomy Department. Forty albino rats were procured from the Central Animal House of Government Medical College, Jammu.

The animals were divided into three groups; group 1 (N = 10) receiving intraperitoneal injection of distilled water. Rats of group II received coron oil (200 mg/kg for 5 days a week for 5 weeks). Rats of group III received intraperitoneal injection of carbaryl in corn oil (200 mg/kg for 5 days a week for 4 weeks).

The animals were housed (12 hours light–dark cycle) with ad libitum access to food and water. The body weights were recorded before the onset of experiment and prior to the sacrifice of animals. The animals of all groups were sacrificed within 24 hours of last injection. After deeply anesthetizing the animals, the liver was removed. The liver were cut into smaller pieces (5 mm) and immediately fixed in 10% formalin. The blocks were prepared for section cutting with a microtome by paraffin wax embedding

method. Sections of 5 to 7 µ thickness were cut and stained with hematoxylin and eosin (H&E) stain.[13]

RESULTS

Groups I and II did not show any physical signs while group III showed physical signs in the form of irritability, sneezing, lacrimation, shivering and tremors for about 1 to 1.5 hours for the first 7 to 8 doses of the drug.

A notable clinically significant reduction in the body weight and decrease in appetite of the experimental animals was observed after carbaryl administration.

HISTOLOGICAL CHANGES

Macroscopic Changes

Grossly, the liver in groups I and II was dark, reddish maroon colored large organ suspended under diaphragm by peritoneal ligaments while the liver in experimental group III was reddish brown in color with some pin-point subcapsular hemorrhages over the surface (Figs 1A to F).

The histomorphological study of groups I and II revealed an identical picture of liver. In the liver of experimental rats (group III), the connective tissue capsule was thickened at places, showed fibrotic changes and inflammatory cells. The one cell thick, orderly arranged pattern of the hepatocyte cords was disrupted in many areas. Most of the hepatocytes of group III were enlarged as compared to groups I and II. Many areas showed hepatocytes with dense and pyknotic nuclei. At sites, few of the hepatocytes were binucleated. There were areas of microvesicular and macrovesicular fatty changes. The areas around the central vein showed hepatocytes that had highly eosinophilic cytoplasm with inflammatory infiltration around the portal triads due to hepatocellular degeneration. Many liver cells in areas away from central vein showed increased cytoplasmic basophilia due to higher metabolic activity. There was also proliferation of bile ductules in the portal triads and fibrosis was seen around many portal triads. The sinusoids, the central veins and branches of portal vein appeared dilated and congestion was seen in the central vein and branches of hepatic artery. At places, there were areas of hemorrhage where the normal parenchyma was replaced by large blood-filled spaces.

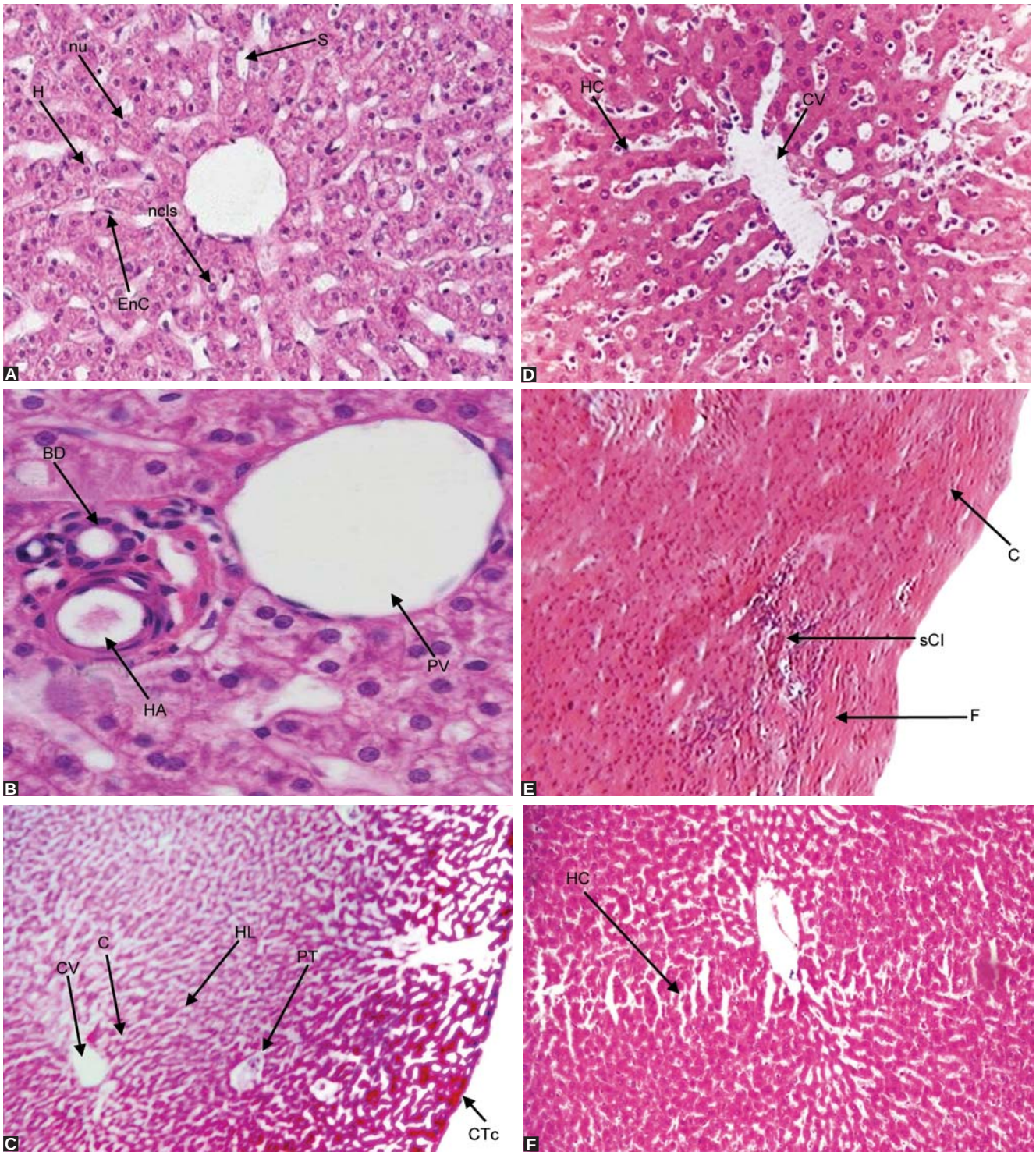
In the present study, the histomorphological changes in the liver of carbaryl-treated rats were significantly different from that of the normal control and the vehicle control rats. The disrupted pattern of hepatocytic cords, capsular fibrosis, subcapsular inflammatory cells, enlarged hepatocytes, evidence of increased cellular metabolism coexistent with ballooning degeneration, microvesicular and macrovesicular fatty change, cytoplasmic basophilia, fibrosis and inflammatory infiltrate around the portal triads along with the dilatation and congestion of the blood vessels and proliferation of bile ductules and areas of hemorrhage are suggestive of toxic hepatitis (Figs 2A to J).

DISCUSSION

The liver makes up of 4.15% of the total body weight.[14,15] Since liver is the organ where most of the substances undergo first pass metabolism, it becomes an organ of extreme importance to study the effect of various substances. Thomas et al[16] administered a single oral dose of C^{14} carbaryl (24 µCi/kg or 0.9 mg/kg) to normal mice and detected higher amounts of radioactivity in the liver and in the blood as compared to the other organs in the body at various postadministration time intervals. Declume et al[17] also demonstrated the accumulation of C^{14} carbaryl in the liver of rat and mice fetuses. In the present study, the rats became very active and irritable immediately after receiving the first dose of carbaryl. This was accompanied by sneezing, shivering and tremors for half an hour. These findings are in accordance with the reports of Gaines,[18] where carbaryl by a single oral or dermal route produced symptoms typical of cholinergic poisoning such as muscle fasciculations, tremors, excessive salivation and lacrimation, diarrhea and involuntary urination. Similar cholinergic effects were also noted.[19-22]

A statistically significant decrease in the body weight ($p < 0.0001$) was observed in the carbaryl-treated rats as compared to the normal control and the vehicle control rats. It is quite obvious that carbaryl toxicity causes metabolic and structural derangements which in turn lead to wasting of the muscle mass and loss of body weight. Pant et al[23] noted a significant decrease in the absolute weights of testes, epididymis, seminal vesicles, ventral prostate at a dose of 100 mg/kg in young rats as compared to adult rats. The lesser weight gain in the young rats was probably a direct effect on the somatic cells or an indirect influence through central nervous system and appetite. Branch et al[20] observed decrease in the weight in an elderly retired coal miner who was unknowingly exposed to carbaryl dust (10%) for a period of 8 months. The difference in the observations could probably be due to a sustained exposure to higher amounts of carbaryl over a longer duration.

In the present study, many hepatocytes showed an increase in size in response to carbaryl administration. Increase in cell size following administration of carbaryl was also noted by Shtenberg et al (1968),[24] in the hypophysis and in the adrenal glands, which they suggested was due to an increase in the activity of the cells. Thus, in addition to liver, other metabolically active organs also show hyperactivity.

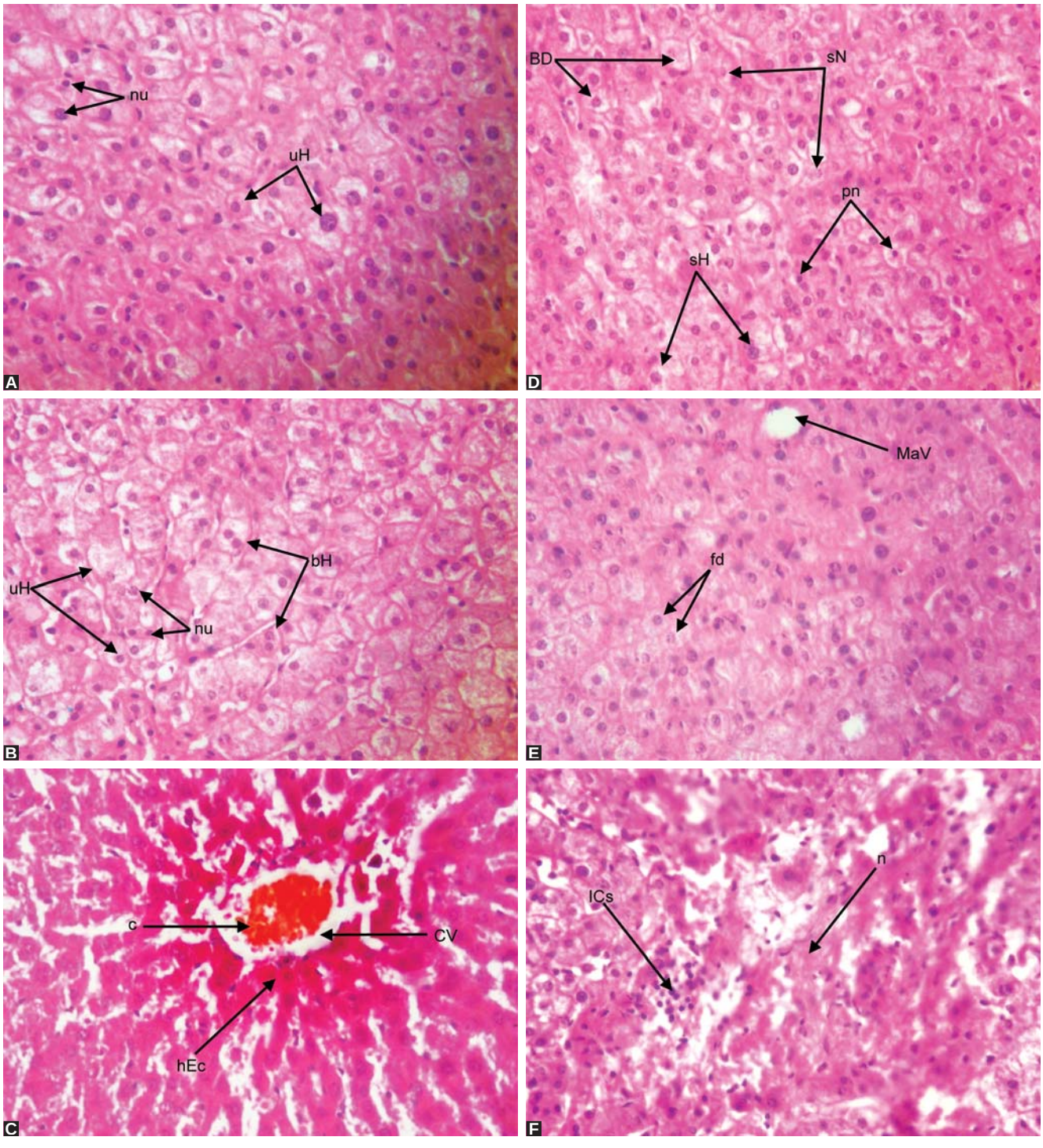


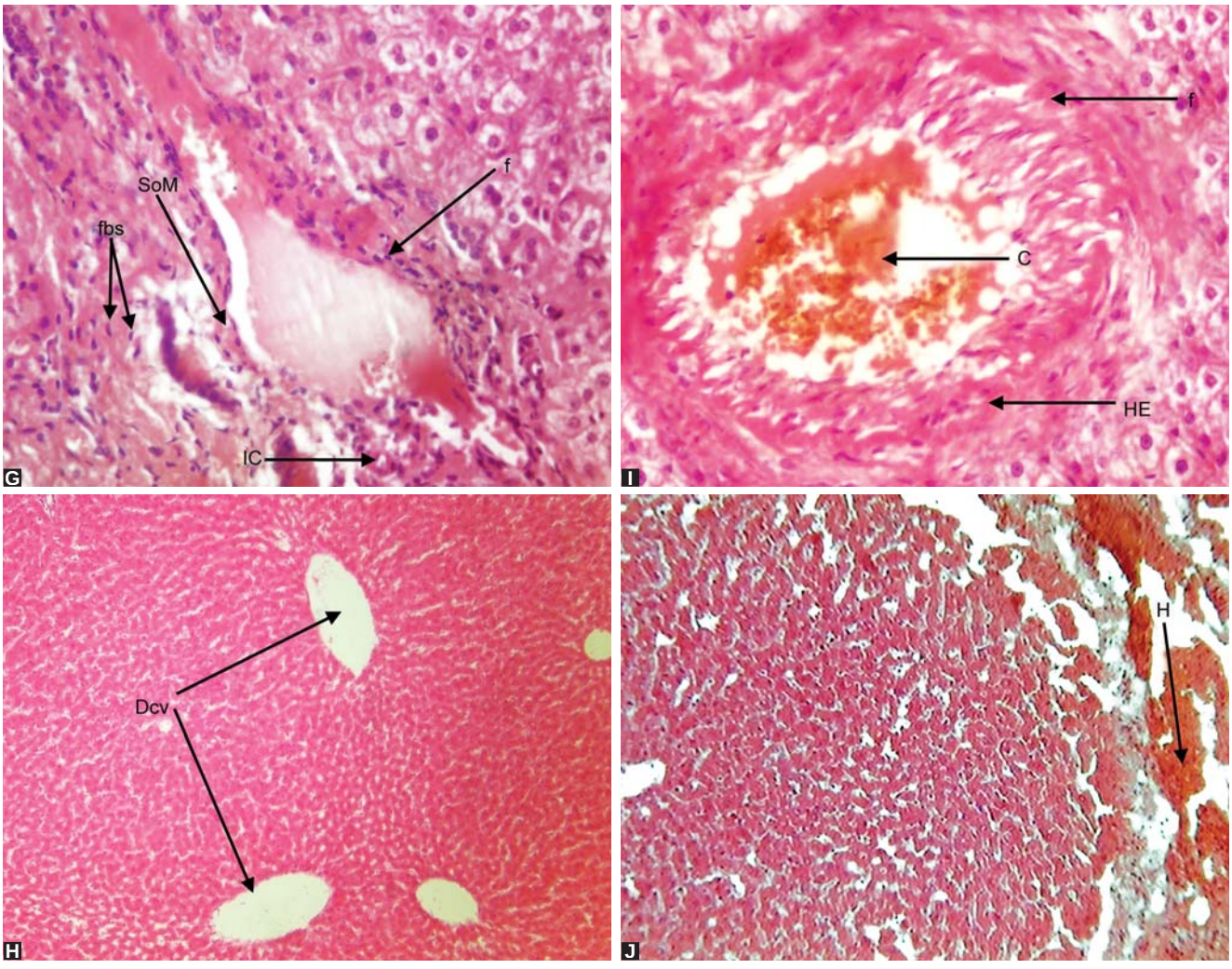
Figs 1A to F: Photomicrograph of transverse section of liver of group I rat showing hepatocytes (H) which are polyhedral in shape placed rounded euchromatic nucleus (nu) and a prominent nucleolus (ncls) with endothelial cells (EnC) lining the sinusoids (S). H&E stain (400×), (B) transverse section of liver of group I rat showing liver parenchyma with branches of hepatic with eccentrically artery (HA), portal vein (PV) and bile duct (BD) forming the portal triad. H&E stain (800×), (C) Photomicrograph of transverse section of liver of group II rat showing connective tissue capsule (CTc) and radial arrangement of cords (C) around central vein (CV) with portal triad (PT) at the periphery of hepatic lobule (HL). H&E stain. (100×), (D) transverse section of liver of group II rat demonstrating the rat demonstrating the radial arrangement of hepatocyte cods around central vein. H&E stain (800×), (E) transverse section of liver of group III rat showing thickened capsule (C) with fibrosis (F) and subcapsular inflammatory cells (sCI). H&E stain (100×), (F) transverse section of liver of group III showing disrupted hepatocytic cords. H&E stain (100×)

In the present study, at some sites, few hepatocytes appeared swollen and empty with indistinct cell membranes. Their, nuclei were also enlarged. The nuclear membrane of a few of these cells was lost. The size of the nucleus is an indicator of functional activity of the cell. Therefore, the observed increase in the size of nucleus suggests that these

cells are overactively involved in the metabolism of carbaryl. These findings are suggestive of an ongoing ballooning degeneration of the hepatocytes. According to observations made by Tośk-Luty et al,[10] after dermal application of carbaryl in rats, there was edema and an increase in vacuolation in the cells of the epidermis and swelling of the mitochondria. Similar changes were seen in the endothelium of the capillary vessels in the lungs and the heart. The cytoplasm of many hepatocytes appeared to contain several tiny vacuoles, giving it a foamy appearance. This is indicative of a fatty change in the liver cells. These ultrastructural changes seen suggest that the cytoplasm might be participating in the metabolism of carbaryl and the overactivity progressively exhausted the cell leading to degeneration. It also suggests that carbaryl affects many organs of the body by transdermal route in addition to the intraperitoneal route used in the present study.

In the present study, areas around the portal triads and central vein showed hepatocytes that become shrunken and had a highly eosinophilic cytoplasm. Their nucleus was dense and pyknotic. These findings are suggestive of hepatocellular degeneration and are in accordance with the





Figs 2A to J: (A) Transverse section of liver of group III rat showing unequal size of hepatocytes and their nuclei (pleomorphism). H&E stain (400×), (B) transverse section of liver of group III rat showing unequal size of hepatocytes (uH) and their nuclei (nu) (pleomorphism) with number of binucleate hepatocytes (bH). H&E stain (400×), (C) transverse section of liver of group III rat showing degenerating swollen and empty hepatocytes (sH) with central vein (CV). H&E stain (400×), (D) transverse section of liver of group III rat showing few hypereosinophilic cells (hEc) and congestion (C) in indistinct cell membrane, few hepatocytes with swollen and partially lysed nuclei (sN), i.e. ballooning degeneration (BD) of hepatocytes and some with dense and pyknotic nuclei (pn). H&E stain (400×), (E) TS of liver of group III rat showing areas of microvesicular (foamy degeneration) (fd) and macrovesicular (MaV) fatty change H&E stain (400×), (F) transverse section of liver of group III rat showing necrosis (n) and inflammatory cells (ICs), predominantly lymphocytes in the liver parenchyma. H&E stain (400×), (G) transverse section of liver of group III rat showing of fibroblasts (fbs) in the space of Mall (SoM) along with inflammatory cells (IC). H&E stain (400×), (H) TS of liver of group III rat showing fibrosis (f) in the number central veins (Dcv). H&E stain (100×), (I) transverse section of liver of group III rat showing Congestion© and fibrosis (f) around the hepatic artery (HE). H&E stain (400×), (J) transverse section of liver of group III rat showing hemorrhage, (H) in the liver parenchyma. H&E stain (100×)

findings of Tość-Luty et al,[10] who also noted degenerative changes in the hepatocytes, pyknotic cells in the stratum spinosum of the epidermis and in the Purkinje cells of the cerebellum following dermal application of carbaryl. Also Khera[9] noted hepatic degenerative changes when carbaryl was injected in the duck and chick embryos, while Smalley et al[25] demonstrated three distinctive morphological patterns of myodegeneration following oral doses of carbaryl. However, Wills et al[26] found no significant histological and biochemical changes of normal bodily functions in men with a dose of 0, 0.06 and 0.12 mg/kg daily of carbaryl when administered orally over a period of 6 weeks. The contradiction in observations can be due to the different (lesser) dose and different route of drug administration, in a larger animal.

Smalley et al[25] demonstrated nuclear regenerative attempts and regeneration of discontinuous type in the skeletal musculature along with myodegeneration on administration of carbaryl in the dose of 150 mg/kg daily for 4 weeks in pigs. Similar findings were also observed in the present study, as, at few sites some of the hepatocytes were seen to be binucleated. This was suggestive of regenerative attempts.

Since, effects of carbaryl on the morphology of liver has not been studied in details, results cannot be compared *per se*. The biochemical studies done by Sharma[11] and Singh

et al[12] revealed that sublethal exposure to carbaryl caused a decrease in the total protein, glucose, glycogen and pyruvate contents in serum of fresh water fish. They suggested that this was due to utilization of these compounds for energy generation, a demand possibly caused by cellular hypoxia, cellular destruction and necrosis and a consequent impairment in the protein synthesis machinery. Sharma[11] also observed an increase in the levels of transaminases and acid phosphatases in fresh water fish serum following sublethal exposure to carbaryl this was due to enhanced protein catabolism and a probable result of hepatocellular damage. Delescluse et al[27] also suggested that carbaryl may generate oxidative signals and cause cellular damage. These findings are in accordance with my observations which reveal areas of necrosis and inflammatory cells due to cellular damage.

The present study also revealed areas of inflammatory infiltration. The portal triad was predominantly infiltrated with lymphocytes macrophages, eosinophils and neutrophils. Few of the inflammatory cells were also seen in between the degenerating hepatocytes. The connective tissue capsule was thickened at places and was infiltrated with inflammatory cells. These findings are in accordance with the findings of Tośk-Luty et al[10] in which carbaryl applied dermally in rats for 4 weeks, produced inflammatory infiltrations in many organs including the liver heart, lungs, kidneys and the skin.

At places, there were areas of hemorrhage where the normal parenchyma was replaced by large blood-filled space and the neighboring sinusoids were hugely distended with hemorrhagic fluid. The available literature did not reveal any evidence of hemorrhage in the liver but similar findings were noted by Smalley et al,[25] where vascular degenerative changes and hemorrhage was observed in the gray matter of central nervous system following administration 150 mg/kg and 300 mg/kg per day of carbaryl for 4 weeks in pigs.

In the present study, the histomorphological changes, in the liver of carbaryl-treated rats was significantly different from that of the normal and the control rats. The disheveled pattern of the one cell thick orderly arrangement of hepatocytic cords, evidence of increased cellular metabolism coexistent with ballooning degeneration, inflammatory infiltrate around the portal triads along with the dilatation of the blood vessels and the bile canaliculi, are suggestive of toxic hepatitis induced by the insecticide, carbaryl.

REFERENCES

1. Food and Agricultural Organization of United Nations. International code of conduct on distribution and use of pesticides 2002. Retrieved 2007;10-25.
2. Rawn DFK, Roscoe V, Krakalvich T, Hanson C. N-methyl carbamate concentrations and dietary intake estimates for apple and grape juices available on the retail market in Canada. Food Additives Contam 2004;21:555-63.
3. Stockholm Convention on Persistent Organic Pollutants (POPs). 17th May, 2004.
4. Kamrin MA. Pesticide profiles: Toxicity; environmental impact and fate. CRC Press 1997;136-37.
5. US Environmental Protection Agency. Interim Reregistration Eligibility Decision for Carbaryl. Case 0080. Available from: www.epa.gov/oppsrrd1/REDs/Carbaryl_ired.pdf. Accessed: Oct 2003.
6. Carpenter CP, Weil CS, Palm PE, Woodside MH, Nair JH, Smyth HF. Mammalian toxicity of 1-naphthyl-N-methylcarbamate (Sevin insecticide). J Agr Food Chem 1961;9:30-39.
7. Marliac JP, Verrett MJ, Laughlin J Jr, Fitzhugh OG. A comparison of toxicity data obtained for twenty-one pesticide by chick embryos treated with acute, oral LD-50's in rats. Toxicol Appl Pharmacol 1965;7:490-96.
8. Ghadiri M, Greenwood DA. Toxicity and biological effects of malathion, phosdrin and sevin in the chick embryo. Toxicol Appl Pharmacol 1966;8:342-48.
9. Khera KS. Toxic and teratogenic effects of insecticides in duck and chick embryos. Toxicol Appl Pharmacol 1966;8:345-50.
10. Tośk-Luty S, Prezbirowska D, Latuszynska J, Tokarska-Rodak M. Histological and ultrastructural studies of rats exposed to carbaryl. Ann Agric Environ Med 2001;8:137-44.
11. Sharma B. Effect of carbaryl on some biochemical constituents of blood and liver of Clarias Batrachus, a freshwater teleost. J Toxicol Sci 1999;3:157-64.
12. Tripathi PK, Singh A. Toxic effects of dimethoate and carbaryl pesticides on reproduction and related enzymes of the fresh water snail Lymnaea acuminata. Bull Environ Contam Toxicol 2003; 71:535-42.
13. Drury RAB, Wallington EA. General staining procedures. Carleton's histological techniques (4th ed). Oxford University Press 1967;114-37.
14. Caster WO, Poncelet J, Simon AB, Armstrong WD. Tissue weights of the rat. Normal values determined by dissection and chemical methods. Proc Soc Exp Biol Med 1956;91:122-26.
15. Webster SH, Lilijegren EJ, Zimmer DJ. Organ; body weight ratios for liver, kidneys and spleen of the laboratory animals; albino rat. Am J Anat 1947;81:477-513.
16. Thomas JA, Dieringer CS, Schein L. Effects of carbaryl on mouse organ and reproduction. Toxicol Appl Pharmacol 1973; 28:142-45.
17. Declume C, Benard P. Foetal accumulation of (^{14}C) carbaryl in rats and mice. Autoradiographic study. J Applied Toxicol 1977;8(1):95-105.
18. Gaines TB. The acute toxicity of pesticides to rats. Toxicol Appl Pharmacol 1959;2:88-90.
19. Robens JF. Teratologic studies of carbaryl, diazinon, norea, disulfiram and thiram in small laboratory animals. Toxicol Appl Pharmacol 1969;15:152-63.
20. Branch RA, Jacoz E. Subacute neurotoxicity following long-term exposure to carbaryl. Am J Med 1986;80:741-45.
21. Wesseling C, Keifer M, Ahlbom A, McConnell R, Moon JD, Rosenstock L. Long-term neurobehavioural effects of mild poisonings with organophosphate and n-methyl carbamate pesticides among banana workers. Int J Occup Environ Health 2002;8:27-34.

22. Punzo F. Effects of carbaryl-treated bait on maternal behaviour and sprint performance in the meadow jumping mouse, Zapus hudsonius. Bull Environ Contam Toxicol 2003;71:37-41.
23. Pant N, Shankar R, Srivastava SP. Spermatotoxic effects of carbaryl in rats. Hum Exp Toxicol 1996;15:736-38.
24. Shtenberg AI, Rybakova MN. Effect of carbaryl on the neuro-endocrine system of rats. Fd Cosmet Toxicol 1968;6:461-67.
25. Smalley HE, Curtis JM, Earl FL. Teratogenic action of carbaryl in beagle dogs. Toxicol Appl Pharmacol 1968;13:392-403.
26. Wills JH, Jameson E, Stein A, Serrous D, Coulston F. Effects of oral doses of carbaryl on man. Toxicol Appl Pharmacol 1967; 10:390-97.
27. Delescluse C, Ledirac N, Li R, Piechocki MP, Hines RN, Gidrol X, et al. Induction of cytochrome P450 1A1 gene expression, oxidative stress and genotoxicity by carbaryl and thiabendazole in transfected human HepG2 and lymphoblastoid cells. Biochem Pharmacol 2001;61:399-407.

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