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Carbaryl induced histological changes in the liver of albino mice

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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 for its broad-spectrum activity in commercial agriculture, poultry, livestock, home and garden pest control. However, there is paucity of the information about the role of Carbaryl on the liver. Albino rats were administered with Carbaryl and the effects on their liver histology were analyzed. Profound damage of the liver of rates 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.

1. Introduction
The spectacular increase in agricultural production in the past 2 decades has been largely accomplished due to the spectacular improvement in the agricultural practices, use of superior and high yield varieties coupled with reduction in losses by the application of herbicides fungicides and pesticides. Most of chemicals used at present are synthetic and alien for the living system. Wide spread and at times excessive use of pesticides has resulted in the occurrence of their metabolites in human and animal tissues including milk, meat poultry, etc which is shearly not acceptable. There could be several pathways by which the pesticides or their residues can travel into the animal and human systems. The main objective of the study is to study the effect of Carbaryl which is an insecticidal carbamate.
The persistence of organo phosphates, carbamates and synthetic pyrethoid pesticides coupled with the tendency to concentrate in non-target organisms as they move up the food chain increases the toxicity in fish's birds, wild life and in turn to men. Thus the overall ecological consequence are very significant and grave [1] (Brown, 1978; Gupta and Gupta, 1976; Muirhead Thomson, 1971).
Substances grouped in the general category of substituted carbanic acid Easters are notable for exerting powerful anti-cholinesterase action and have therefore found extensive application in agriculture as insect oxidants. The inhibitory process is believed to consist of cabamylation of the cholinesterase enzyme by the organic carbamate inhibitor, [2] (sakai *et al*., (1978) contended that N- Methyl carbamates are degraded by human liver.
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 [3]. 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 Agent (EPA) [4]. A study conducted on rats, dogs and monkeys to see the effect of carbaryl on kidneys showed epithelial changes in proximal convoluted tubule [5].
Various experimental studies reported congenital malformation in chicken and duck embryos with –carbaryl [6-8]. The histological changes were seen in various organs of male albino rats like heart, liver, kidney, lung and brain on dermal exposure to carbaryl for 4 weeks [9]. An increase in the activities of Transaminase and acid phosphatase suggesting hepatocellular damage was also recorded [10]. Inhibition of liver enzymes with carbaryl was also reported [11]. This study was conducted to develop insights about the role of carbaryl on liver histology.

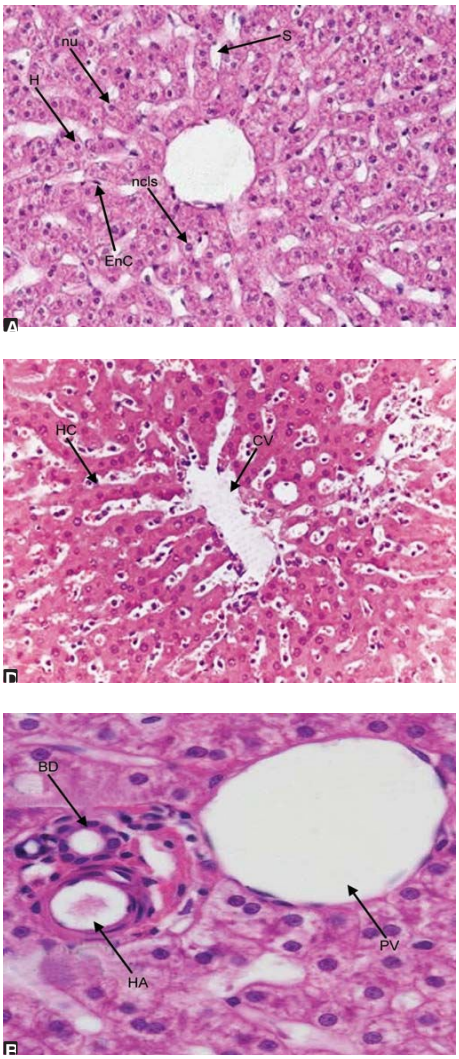
Material and methods

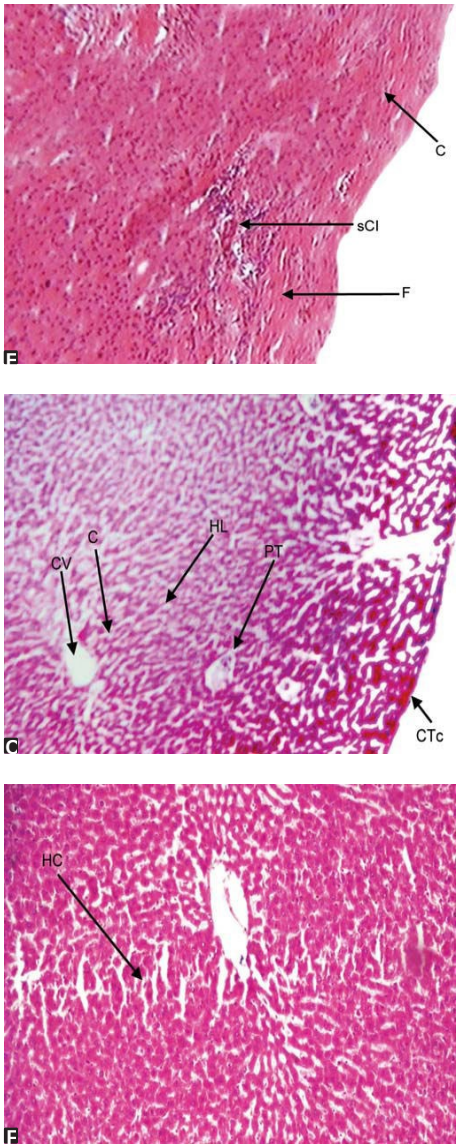
Young mice of body weight 30 Gms were used as a model in the present study. They were dived into three groups; group 1 (N=10) receiving intraperitoneal injection of distilled water. Rats of group II received Coron oil (200 mg/kg for 5 days a week for 5 weeks). Rats of group III received intraperitoneal 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 as libitum access for food and water. The body weights were recorded before the onset of the 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 microtone by paraffin wax embedding method. Sections of 5 to 7 µ thickness were cut and stained with hematoxylin and eosin (H&E) stain [12].

Histological 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 sub capsular hemorrhages over the surface (Figs 1A to F).





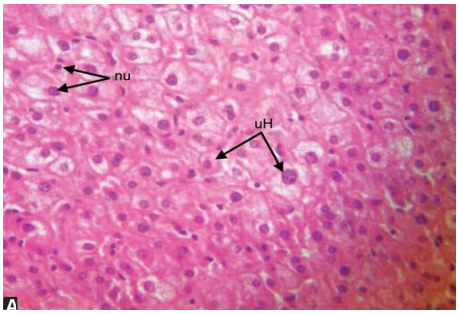
Figs 1A to F: Photomicrograph of transverse section of liver of group I rat showing hepatocytes (H) which are polyhedral in shape placed rounded euchromatic nucleus (nu) and a prominent nucleolus (ncls) with endothelial cells (EnC) lining the sinusoids (S). H&E stain (400×), (B) transverse section of 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×)

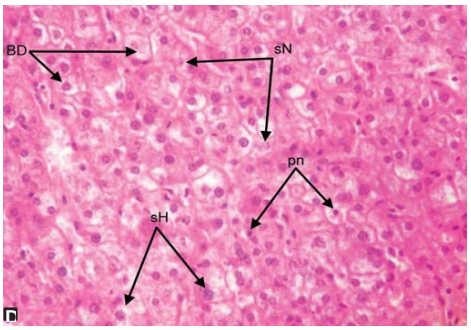
The histomorphological study of groups I and II revealed an identical picture of liver. In the liver of experimental rats (group III), the connective tissue capsule was thickened at places, showed fibrotic changes and inflammatory cells. The one cell thick, orderly arranged pattern of the hepatocyte cords was disrupted in many areas. Most of the hepatocytes of group III were enlarged as compared to groups I and II. Many areas

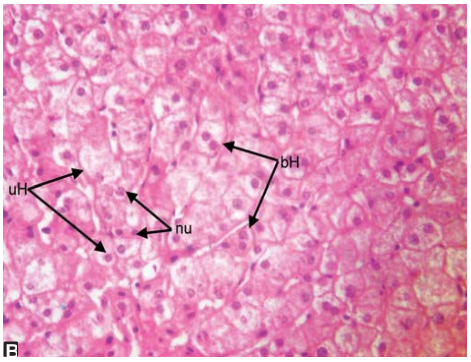
showed hepatocytes with dense and pyknotic nuclei. At sites, few of the hepatocytes were binucleated. There were areas of microvesicular and macrovesicular fatty changes.

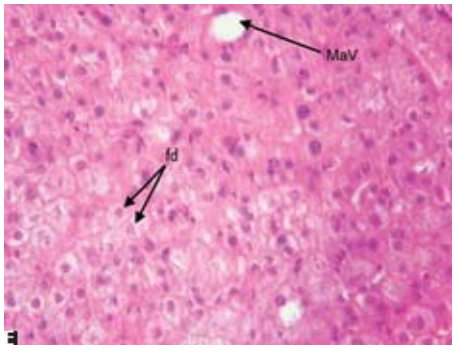
The areas around the central vein showed hepatocytes that had highly eosinophilic cytoplasm with inflammatory infiltration around the portal triads due to hepatocellular degeneration. Many liver cells in areas away from central vein showed increased cytoplasmic basophilia due to higher metabolic activity. 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.

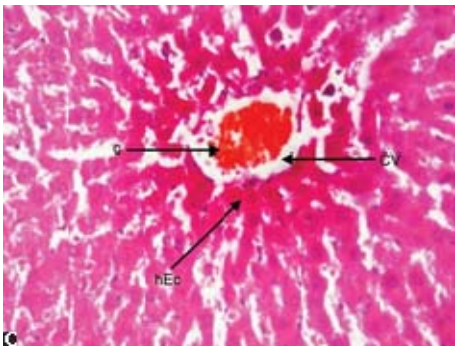
In the present study, the histomorphological changes in the liver of Carbaryl-treated rats were significantly different from that of the normal control and the vehicle control rats. The disrupted pattern of hepatocytic cords, capsular fibrosis, subcapsular inflammatory cells, enlarged hepatocytes, evidence 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).

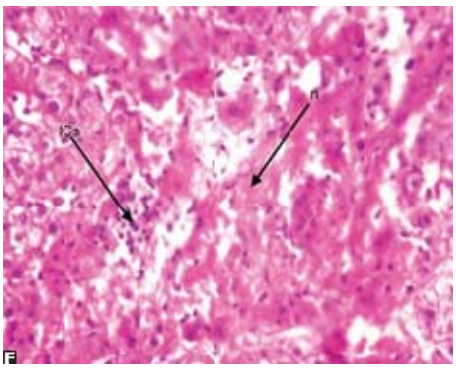


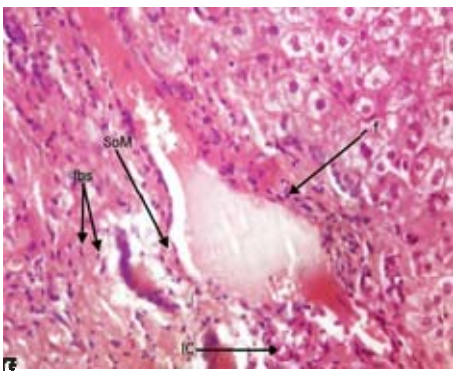


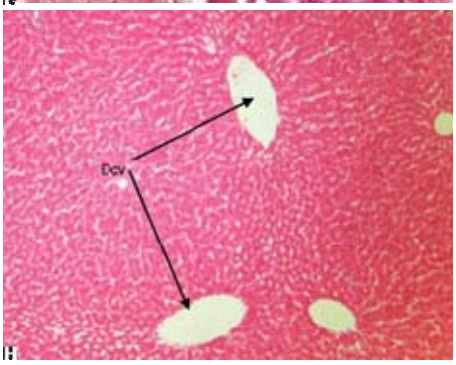


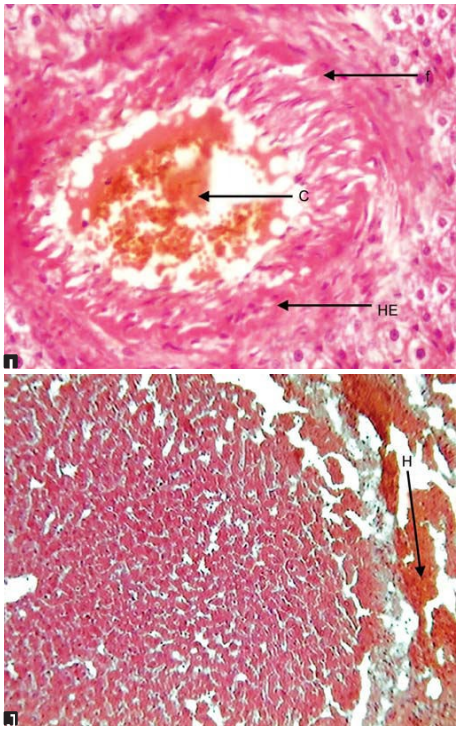












Figs 2A to J: (A) Transverse section of liver of group III rat showing unequal size of hepatocytes and their nuclei (pleomorphism). H&E stain (400×), (B) transverse section of liver of group III rat showing unequal size of hepatocytes (uH) and their nuclei (nu) (pleomorphism) with number of binucleate 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×)

Results and discussion

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 [13] where carbaryl by a single oral or dermal route produced symptoms typical of cholinergic poisoning such as muscle fasciculation's, tremors, excessive salivation and lacrimation, diarrhea and involuntary urination. Similar cholinergic effects were also noted [14-17]. Many hepatocytes showed an increase in size in response to carbaryl administration. In addition to liver, other metabolically active organs also show hyperactivity.

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 overactive involved in the metabolism of Carbaryl. These findings are suggestive of an ongoing ballooning degeneration of the hepatocytes.

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.

Also, 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. Khera [18] noted hepatic degenerative changes when carbaryl was injected in the duck and chick embryos

However, Wills *et al*. [19] 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

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