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Carbaryl, A Pesticide Causes "Toxic Hepatitis" in Albino Rats

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Abstract

Pesticides are one of the most alarming toxic substances that are deliberately added to our environment. Food and agricultural organization (FAO) has defined the term pesticide as: *Any substance 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]. But it is a matter of concern that along with the pests, they prove harmful to many other living beings on this planet as well as human beings including newborns [2]. CARBARYL, a synthetic 1-napthyl-N-methyl carbamate is being used extensively or its broad-spectrum activity in commercial agriculture, poultry, livestock, home and garden pest control. It was the most frequently detected carbamate in juice samples studied [3]. Thorough scanning reveals that there is dearth of literature regarding the histopathological changes in liver associated with administration of carbaryl which has prompted us to initiate this study as liver plays an important role in the first pass metabolism of carbaryl. The present study was conducted on 40 albino rats. The adult albino rats (150-200 gm) were injected with 200 mg/kg body weight of carbaryl intrapeitoneally, five days a week for 30 days. Controls were maintained. There was a significant increase in weight of the experimental rats recorded before the onset of the experiment & prior to their sacrifice ($p<0.001$) as compared to the controls. The rats were sacrificed within 24 hours of the last injection. The blocks of the liver were prepared for section cutting with a microtome by paraffin wax embedding method. Sections of 5-7 µm thickness were cut and stained with Haematoxylin and Eosin stain. In the 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 co-existent with ballooning degeneration, councilman bodies, inflammatory infiltrate around the portal triads along with the dilatation of the blood vessels and the bile canaliculi were seen as signs of degeneration. B enucleated hepatocytes were suggestive of regenerative attempts by the degenerative cells. These findings are highly conclusive of toxic hepatitis induced by an insecticide, Carbaryl.

Keywords: Albino rats, Liver, Carbaryl, Hepatocellular degeneration, Toxic hepatitis

Introduction

Pesticides are substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. 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. Pesticide residues in food, is one of the environmental threats that may alter the delicate organism of a growing child, causing disease, developmental problems or adverse effects later in life [4,5]. According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are pesticides [6,7]. 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, carbamates groups, organochloriness & Pyrethroids. In recent years, use of carbamate has gained importance due to ban of the pesticides belonging to organochlorine groups. That is D.D.T., Aldrin, Lindane & Endosulfan. These Pesticides have a tendency to persist & have potential to bioaccumulate in the body [8].

Carbaryl, a synthetic 1-napthyl-N-methyl carbamate 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 Agency (EPA) [9].

A study conducted on rats, dogs and monkeys to see effect of carbaryl on kidneys showed epithelial changes in proximal convoluted tubule [10].

Various Experimental studies reported congenital malformation in chicken and duck embryos with carbaryl [11-13]. 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 four weeks [14]. An increase in the activities of transaminase and acid phosphatase suggesting hepatocellular damage was also recorded [15]. Inhibition of liver enzymes with carbaryl was also reported [16].

Material and Methodology

The route of administration was selected intraperitoneal to avoid the first-pass metabolism, so that maximum concentration of drug reaches

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to the target. The control group was given distilled water as isotonic saline as distilled water has neutral pH (i.e. 7). The animals were group 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 anaesthetising the animals by keeping them in an inverted glass jar containing large piece of cotton soaked in ether, skin incision extending from xiphoid process to the jugular notch were given and sternum lifted by cutting ribs along the sides of sternum and liver will be removed. The liver were cut into smaller pieces (5 mm) and immediately fixed in 10% formalin [17].

Methodology

The casting and embedding was done with the help of moulds. Two L-shaped blocks were placed on a metallic plate, which acts as a base of the mould and molten wax was poured into it. The tissues were placed in the mould filled with wax and left to solidify. After solidification the blocks of the wax were removed and properly labeled for microtomy.

The slides were subsequently stained by a haematoxylin and eosin. The slides were cleaned beyond the area of tissue implantation, dried and mounted in DPX and examined first under low power and then high power.

Results

Figures 1, 2a and 3b did not show any physical signs while Figures 3a-3h showed physical signs in the form of irritability, sneezing, lacrimation, shivering and tremors for about 1-1.5 hours for the first 7-8 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 figures 1, 2a and 2b was dark, reddish maroon colored large organ suspended under diaphragm by peritoneal ligaments while the liver in experimental figures 3a-3h was reddish brown in colour with some pin-point subcapsular haemorrhages over the surface.

Microscopic changes: The histomorphological study of figures 1, 2a and 2b revealed an identical picture of liver. In the liver of experimental rats (Figures 3a-3h) 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 appeared to be enlarged as compared to figures 1, 2a and 2b. 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. 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 haemorrhage 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 co-existent 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 haemorrhage are suggestive of toxic hepatitis induced by the pesticide, Carbaryl.

Discussion

The liver makes up of 4.15 percent of the total body weight [18,19]. 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. [20] 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 post-administration time intervals. Declume et al. [21] 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 [22], where Carbaryl by a single oral or dermal route produced symptoms typical of cholinergic poisoning such as muscle fasciculations, tremors, excessive salivation and lacrimation, diarrhoea and involuntary urination. Similar cholinergic effects were also noted by Robens [23], Branch et al. [24], Wesseling et al. [25] and Punzo [26].

A statistically significant decrease in the body weight ($p<0.0001$) was observed in the Carbaryl-treated rats as compared to the normal

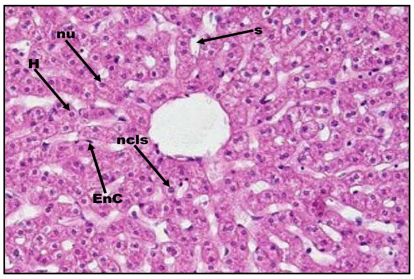

Figure 1: Hepatocytes (H) polyhedral in shape with eccentrically placed rounded euchromatic nucleus (nu) with prominent nucleoli (ncls) & endothelial cells (EnC) lining the sinusoids (s) Stain H & E (400X).

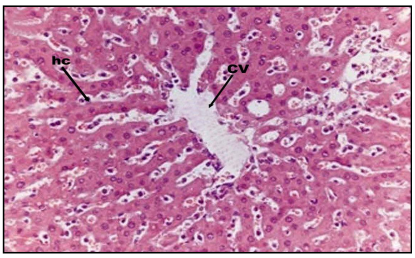

Figure 2a: Radial arrangement of hepatocytic cords (hc) around central veins (cv) H & E (400X).

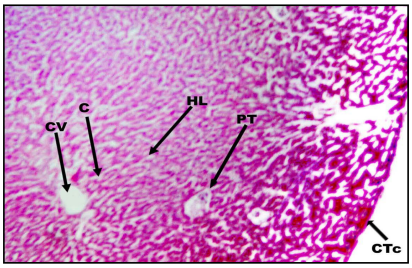

Figure 2b: Connective tissue capsule (CTc) with radial arrangement of hepatic cords (C) around Central vein (CV) & Portal triad (PT) at the periphery of hepatic lobule (HL) Stain H & E (100X).

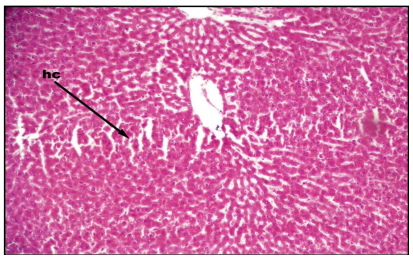

Figure 3a: Disrupted hepatocytic cords. Stain H & E (100X).

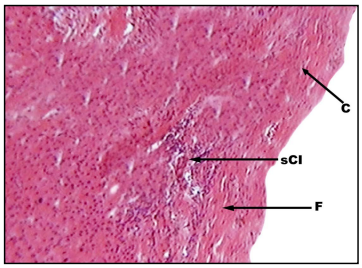

Figure 3b: Thickened capsule (C) with fibrosis (F) & Subcapsular inflammatory cells. Stain H & E (100X).

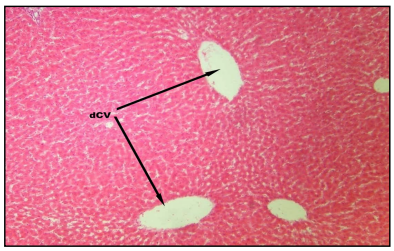

Figure 3c: Dilated Central veins (Dcv) Stain H&E (100X).

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. [27] noted a significant decrease in the absolute weights of testes, epididymis, seminal vesicles, and 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. [24] observed decrease in the weight in an elderly retired coal miner who was unknowingly exposed to Carbaryl dust (10 percent) for a period of eight 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. [28], 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.

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. These findings are suggestive of an ongoing ballooning degeneration of the hepatocytes. According to observations made by Tośl-Luty et al. [14], 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 over-activity progressively exhausted the cell leading to degeneration. It also suggests that Carbaryl affects many organs of the body by trans- dermal route in addition to the intra-peritoneal 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 findings of Tośl-Luty et al. [14], 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 [13] noted hepatic degenerative changes when Carbaryl was injected in the duck and chick embryos, while Smalley et al. [29] demonstrated three distinctive morphological patterns of myodegeneration following oral doses of

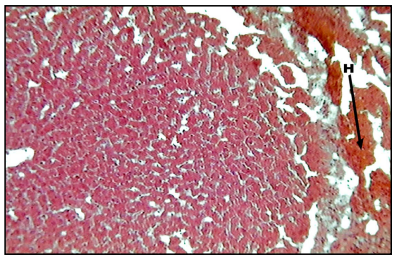

Figure 3d: Hemorrhage (H) in liver parenchyma Stain H & E (100X).

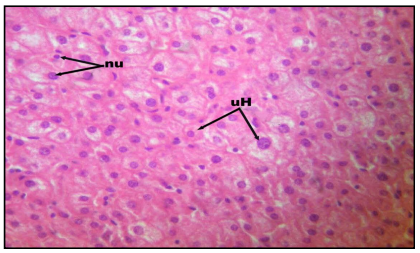

Figure 3e: Unequal size of hepatocytes (uH) & their nuclei (nu) {Pleomorphism}. Stain H & E (400X).

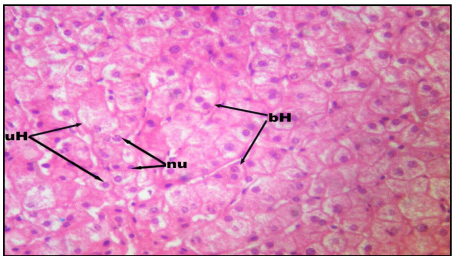

Figure 3f: Unequal size of hepatocytes (uH) & their nuclei (nu){Pleomorphism} with number of binucleate hepatocytes (bH). Stain H & E (400X).

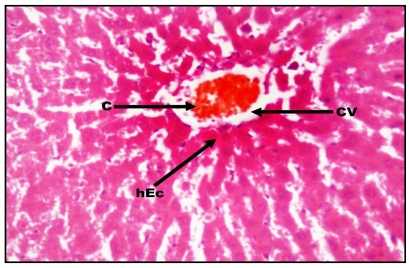

Figure 3g: Degenerating hypereosinophilic cells (hEc) with congestion (c) in central vein (cv). Stain H & E (400X).

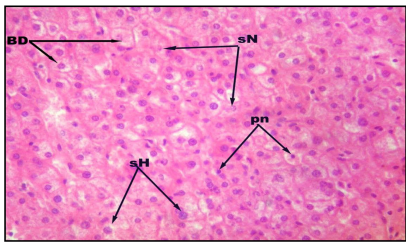

Figure 3h: Few swollen & empty hepatocytes (sH) with indistinct cell membrane, few hepatocytes with swollen & partially lysed nuclei (sN),that is, ballooning degeneration of hepatocytes (BD) & some with dense & pyknotic nuclei (pn) Stain H&E (400X).

Carbaryl. However, Wills et al. [30] 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 six 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. [29] 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 four 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 [15] and Singh et al. [16] 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 [15] also observed an increase in the levels of transminases 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. [31] 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 Toś-Luty et al. [14] 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. Such histological changes & other morphometric measurements were also seen by Munglung M et al. [32].

At places, there were areas of haemorrhage where the normal parenchyma was replaced by large blood-filled space and the neighbouring sinusoids were hugely distended with hemorrhagic fluid. The available literature did not reveal any evidence of haemorrhage in the liver but similar findings were noted by Smalley et al. [28], where vascular degenerative changes and hemorrhage was observed in the grey matter of central nervous system following administration 150 mg/kg and 300 mg/kg per day of Carbaryl for four 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 co-existent 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 Pesticide, Carbaryl.

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