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RESEARCH PAPER

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## Histopathological Effects of Endosulfan in Liver of Albino Rats

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### ABSTRACT

*Pesticides are one of the most alarming toxic substances that are deliberately added to our environment. Endosulphan, a organochlorine insecticide, is being used extensively and its broad-spectrum activity in commercial agriculture, poultry, livestock, home and garden pest control is in use. In the present study, the histological changes in the liver of Endosulphan-treated rats were significantly different from that of the normal control rats. 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 over actively involved in the metabolism of endosulphan. 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 changes, cytoplasmic basophilia, fibrosis and inflammatory infiltrate around the portal triads along with the dilatation and congestion of the blood vessels and proliferation of bile ducts and areas of hemorrhage are suggestive of toxic hepatitis.*

**Keywords:** *Cytoplasm, Hepatocytes, Fibrosis, and Hepatitis.*

### INTRODUCTION

Organochlorine pesticides are being extensively used in agriculture and disease control purposes for the last more than fifty years worldwide and their long persistence

in soil have been reported. This pesticide residue is capable to affect the soil fertility, crop productivity, ecological imbalance and caused human health problems (Verma *et al.*, 2011).

In developing countries such as India where the economy depends largely on agricultural products, one cannot afford to lose the harvest to pests. In India, fifteen to twenty percentage of the total harvest is destroyed by pests resulting in uncontrolled use of pesticides by the Indian cultivators. Endosulphan is a widely used insecticide in India to maintain crops and food productions fight against infecting pests safeguard humans from vector borne diseases and related epidemics (Ayub *et al.*, 2003).

However, this pesticide has significant toxicity to non-target organisms including humans. The major routes of exposure to these chemicals are through food chain series, dermal contact, respiratory tract etc. The workers in the field and those involved in the application of these insecticides are at high risk of toxicity manifestations by these chemicals (Babu *et al.*, 1990). Various experimental studies reported congenital malformation in chicken and duck embryos with carbaryl.

### **Chemical nature of endosulphan**

Endosulphan is an organochlorine insecticide belonging to the cyclodiene group that is extensively applied in agriculture to protect crops (Li *et al.*, 2009). It is sold under the trade name of Thiodan® which is a mixture of 70% endosulphan- $\alpha$  (endosulphan I) and 30% endosulphan- $\beta$  (endosulphan II) (Butlar *et al.*, 1993). The technical grade endosulphan (1,2,3,4,7,7-hexachlorobicyclo-2,2,1-heptene-2,3-bis-hydroxy methane-5,6-sulfite) is a mixture of two stereoisomers, a  $\alpha$ - and  $\beta$ -endosulphan, in the ratio of 7:3. It is extensively used throughout the world to control the pests on different crops.

### **Endosulphan and its application**

Endosulphan is a broad-spectrum cyclodiene insecticide that has been used extensively for a longer period of time on a variety of crops. This chemical has been used in many countries. This pesticide has been banned in many countries; however, it is still used in the United States (Parbhu *et al.*, 2009). It has its effect on flora and fauna. Throughout the world in different countries, endosulphan is used as a pesticide and in so many countries it is banned because of its ill effects. It was a cause of panic in British Columbia, where it has been sprayed since years. Some other issues were reported in countries like Cuba, Benin and India where it is still in use and has produced very dangerous effects such as congenital birth defects, cancer, loss of immunity, neurological and mental disorders, reproductive health problems, etc. Along with this, in many countries its toxicity was reported (Balgiar *et al.*, 2005). The histopathological changes were seen in target organ of Albino rats liver on dermal exposure to endosulphan for 15, 30 and 45 days. An increase in the activities of LDH and Acid Phosphatase suggesting hepatocellular damage was also recorded. Inhibition of liver enzymes with Endosulphan was also reported. This study was conducted to develop insights about the role of Endosulphan on liver histopathology of Albino rat.

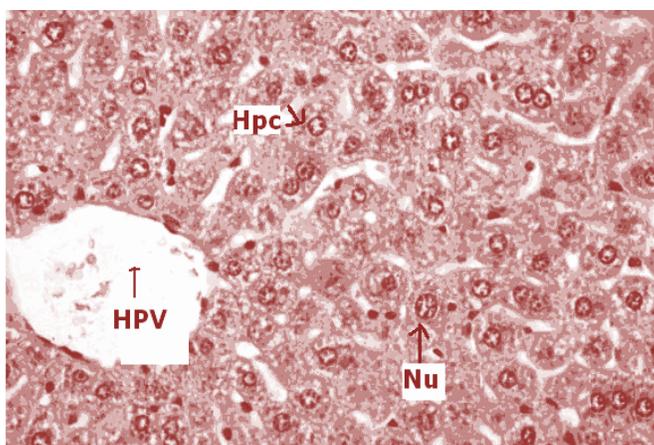
### **MATERIAL AND METHODS**

The toxicity experiments were conducted using the chosen concentration of endosulphan.  $LC_{50}$  was determined using simple graphic (% Mortality Vs. Log Concentration), probit graphic Probit value Vs. Log concentration and regression analysis methods. Sub-lethal concentration of endosulphan was 12.84 ppm. In the present work the histopathological effects

have been under taken. Albino rats were sacrificed after 15, 30 and 45 days of exposure. The liver was dissected out. Tissue must undergo preparatory treatment before microtomy, the slides were stained with eosin for half a minute and then place in 95% alcohol again. The stain sections were place in xylene for 20 minutes, then clean and mounted in D.P.X. The liver of the rat exposed to endosulphan at 30° C temperature showed drastic changes, the

result of present work showed the pathological symptoms.

The body weights were recorded before the onset of experiment and prior to the sacrifice of animals. 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 6 μ thickness were cut and stained with hematoxylin and eosin (H&E) stain.



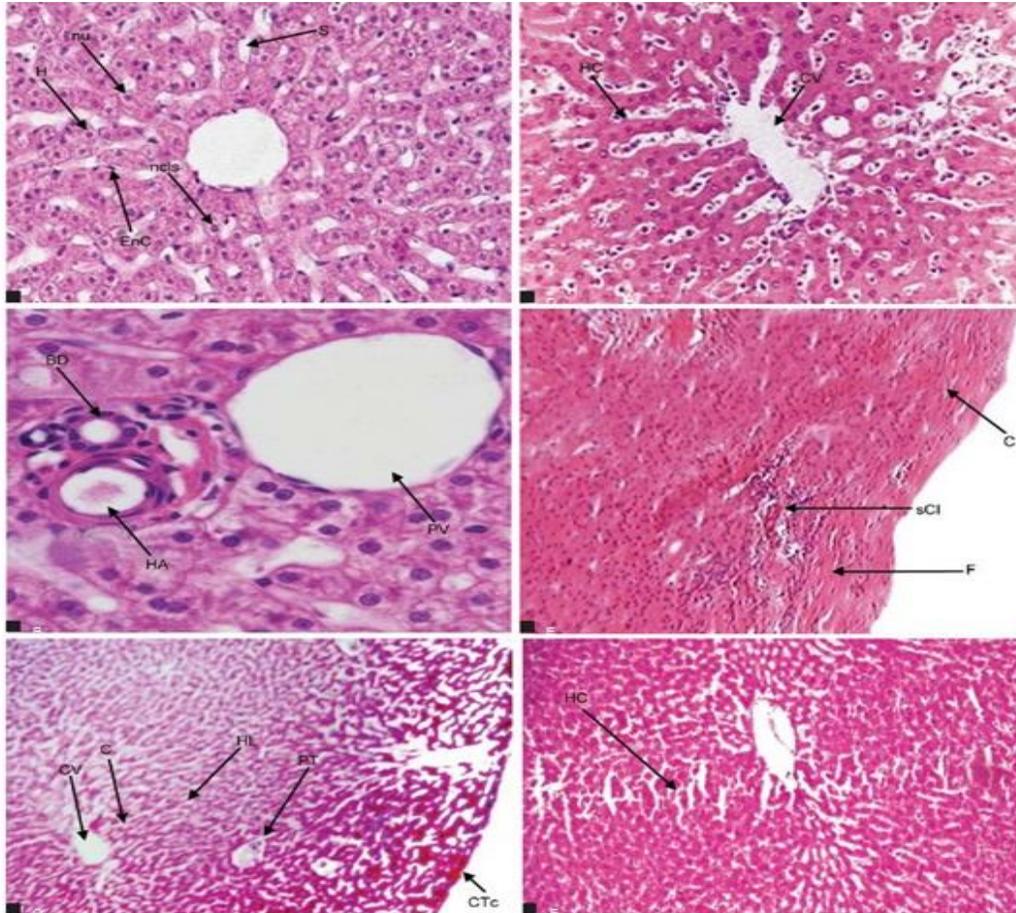
**Fig A. Liver of control rat composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroid nucleus. HPV=Hepatic portal vein, Nu=Nucleus**

## RESULT AND DISSUCION

In the present study, the histological changes in the liver of Endosulphan-treated rats were significantly different from that of the normal control rats.

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 after administration of dose. The liver in control groups was dark, reddish maroon colored large organ suspended under diaphragm by perito-neal ligaments while the liver in experimental

group was reddish brown in color with some pin-point subcapsular hemorrhages over the surface. In the liver of experimental rats group, 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.



**FIG A to F Showing Changes in Histology of Liver of Experimental Albino rat 1**

**Figs 1A** 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).

**(B)** Transverse section of liver of group I rat showing liver parenchyma with branches of hepatic eccentrically artery(HA), portal vein (PV) and bile duct (BD) forming the portal triad. and unequal size of disrupted hepatocytes (uH)

**(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). and their nuclei (nu) showing(pleomorphism)with number of binucleate hepatocytes (bH).

**(D)** Transverse section of liver of group II rat demonstrating the radial arrangement of hepatocyte cods around central vein.

**(E)** Transverse section of liver of group III rat Showing thickened capsule (C) with fibrosis .and pyknotic nuclei.

**(F)** Sub capsular inflammatory cells (sCI)., transverse section of liver of group III showing disrupted hepatocytic cords. Necrosis (n) and inflammatory cells (ICs).

There were areas of micro vesicular and macro vesicular 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. Carrol et al (1956) 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. Khan, et al (2010) observed Effect of organophosphate Insecticide on protein and glycogen content in liver and kidney of *Gambusia affinis* and found decrease in protein content.

The liver makes up 4.15% of the total body weight. Since liver is the organ where most of the substances undergo metabolism, Ton et al (2012) administered a single oral dose of C14 carbaryl (24  $\mu$ Ci/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.

Pant, et al (2011) 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 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 over actively involved in the metabolism of endosulfan. 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. Chaudhary, et al (2003) Khan, et al, (2013) observed histopathological alterations in the kidney of *Gambusia affinis* after exposure to chlorpyrifos showed vacuolated epithelial cells.

## CONCLUSION

Endosulfan, though used for betterment of mankind, has ultimately resulted in being dangerous to environment. The toxic residues of it impairs the development and normal functioning of the hormone-dependent processes in flora, fauna and has lead to severe and chronic human poisoning. It has been banned in many developed countries. Bane of endosulfan in India is in consideration, as it leads to many drastic effects, resulting in mortality. It is the requirement of the present time to reduce the ill effect of this pesticide. Government must come forward with an alternative to reduce the hazardous effect of this pesticide strictly or stop its future use completely.

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