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Histopathological Studies On The Liver Of Rats Exposed With Toluene And Trichloroethylene Pre-Treated With Phenobarbital

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Abstract: In rats, the effect of phenobarbital Pre-treatment on the toxicity of a variety of halogenated organic solvent has been investigated. The current findings show that Phenobarbital pre- treatment significantly reduces the toxicity of toluene and trichloroethylene. Our observations Show that toluene is more hepatotoxic than trichloroethylene. It causes peculiar ultra-structural changes i.e. involution of nuclear membrane and irregular arrangement of mitochondrial cristae. However, using single high- dose levels of these solvents, a potentiating impact of Phenobarbital on the toxicity of other organic solvents such as methanol, xylene, and methylene chloridecould not be demonstrated. Because barbiturates are commonly used by industrial workers, it's crucial to be aware of the possibility of synergistic effect from inhaling of organic solvents, as well as of potentiation associated with others.

Keywords: Toluene, Trichloroethylene, Phenobarbital, Hepatotoxic, Organic solvents

Introduction

Organic solvents, often known as carbon-based solvents, are distinguished by their colour, volatility, molecular weight and boiling point. These solvents are frequently used in various industries and day to day products, thus making them a potential toxic threat. Knowing about their hazardous effects on human health and to the environment is a must to take appropriate corrective measures. The lipophilic nature of organic solvents promotes their absorption immediately after inhalation or oral exposure and dermal contact (Firestone, et al 2009). Once the solvent is absorbed, exposure route, solvents physical and chemical nature affects the metabolism shorter and longer deposition. This metabolism can occur immediately in the liver, without even entering the systematic circulation. Some of these solvents are metabolized to less toxic components while others yield severely toxic metabolites. The un-metabolized solvents are dispersed largely in adipose tissue resulting in long term effects on human body.

Hepatotoxicity or liver damage is defined as the irregular functioning of liver due to chemicals. Drugs induced hepatotoxicity adversely affects structure of Mitochondria and its function. These drugs can cause severe damage to the Mitochondria and can induce hepatic necrosis. This may cause cytolytic hepatitis and can even progresses into liver failure. (Jain, etal 2010)

Quite a few important metabolic functions are regulated by the liver. It is the key organ of metabolism and excretion. Any Hepatic injury is associated with distortion of these metabolic functions (Wolf 1999). Liver strategic placement in the body subjects it to continuous exposure to varied xenobiotic substances. The toxins absorbed from the intestinal tract have an easy access to the liver resulting in its various ailments. Thus in spite of medical advancements,

liver problem always remains one of the serious health issues. Toluene is a gravely toxic and fatal compound to mammals (Pelletti etal 2018) with the liver and kidney being extremely sensitive to the toxic effects of xenobiotics viz toluene and drugs due to the presence of high detoxifying, degrading and bio activation enzymes and ability of the organ to metabolize the compounds (Nigam, etal 2021) mostly due to the presence of cytochrome P450 enzymes (CYP 450) in liver (Wu, et al 2021) which is responsible for the metabolism of the xenobiotics in the organ.

Trichloroethylene (TCE) is a halogenated hydrocarbon that has been used as a degreasing agent, due to its widespread use; it remains one of the most significant environmental contaminants. It is a causative factor in no. of diseases.

The oxidative stress induced by xenobiotics such as toluene and trichloroethylene maybe one of the mechanisms responsible for numerous liver diseases. (Genchi, et al 2020).

Some of common solvents are toluene; trichloroethylene, carbon tetra chloride, acetone and dimethyl formaldehyde etc are used as industrial solvents.

Phenobarbital is used clinically as a sedative hypnotic and anti-convulsant agent; it accelerates the hepatic metabolism of a variety of xenobiotics by microsomal induction. (Sato and Nakajima, 1985). It is potent inducer of CYP450 and 2B1. (Waxman and walsh, 1983) and does not stimulate CYP450 2E1 (Korcorck et.al, 2010) although effects of toluene and trichloroethylene on liver and other system have been thoroughly studied by (Rana and Kumar 1994) very lesser reports on their effect after microsomal induction are available.

Material and methods

Animals: inbred, adult (150 +- 20 gram), male 4 months old Wistar rats were selected for this study. They were maintained individually in polypropylene cages on a saw dust bed under standard laboratory condition (room temperature 25 + - 5^0C, RH50 $\pm$ 10%) and fed commercial food pellets (Lipton India) and tap water ad libitum following the guidelines of NIH (USA).

Experimental procedure: Rats were divided into 6 groups A-F, each containing eight rats. After two weeks of acclimatization, rats of group B,D and E were administered phenobarbital (90 mg/kg/ day) intraperitoneally in distilled water (4 ml/kg) for three consecutive days (Rana and Gupta1999). Thereafter rats of group A and B were injected intraperitoneally predetermined sub lethal dose of toluene (0. 5 ml/100gm body weight) on each alternate for 30 days as described by (Rana and Kumar 1994). Similarly rats of group C and D injected with the Trichloroethylene (0.5ml/ 100 gm body weight) on each alternate day for 30 days as reported (Kumar and Rana 1998). Rats of group E were treated with phenobarbital and group F were injected olive oil (0.5 ml/100gm body weight) to serve as controls.

Analysis: On the 31st day all the rates were starved overnight and sacrifice the next morning by light ether anaesthesia. For microscopical examination small pieces of liver were fixed in 10% formalin and embedded in wax. 5-6mu thick sections were prepared and double strain with haematoxylin/eosin and following observation was recorded.

Result and discussion

The characteristic feature of a chronic active hepatic lesion is the inflammation in and particularly around the portal tracts designated as piecemeal necrosis. In acute hepatisis of almost any etiology, the structural and functional injury of the hepatocytes has estabished in the first step in liver damage and it mainly depends upon their haemolytic cleavage. (Ugazio. et.al.1973).

Sections of liver from healthy control rates showed hepatic lobules as typical hexagons with a terminal branch, a central vein at the centre.

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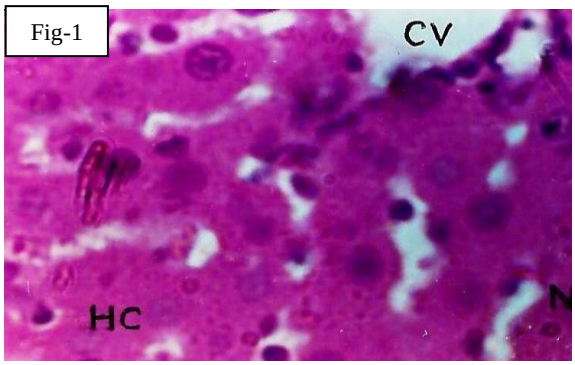
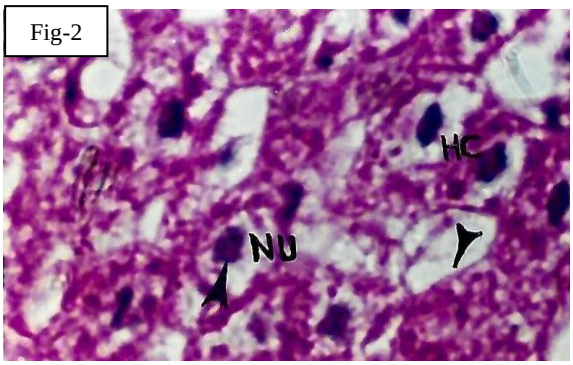
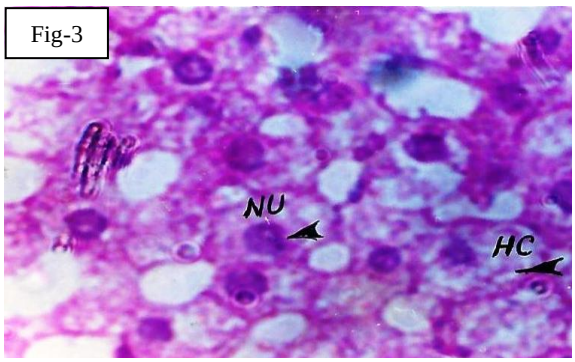
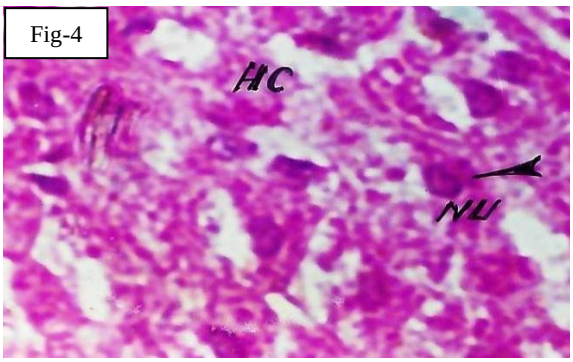
Uniform hepatic parenchyma continued from one lobule to another. Hepatic parenchymal cells possessed round nuclei with centrally placed nucleolus. The blood vessels and bile canaliculi run in between the parenchymal cell (Fig 1).

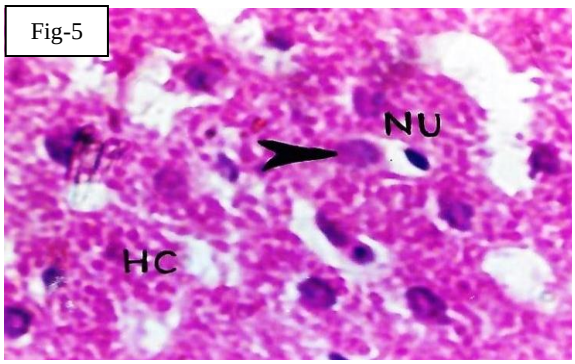
Mild cytoplasmic degeneration was recorded in perilobular region after toluene treatment (Fig 2).











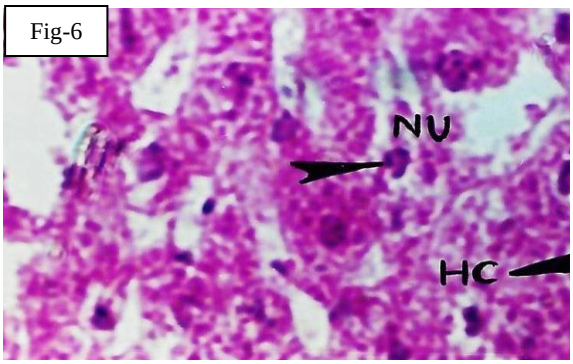


Fig 1: Control rat liver shows uniform hepatic parenchyma cells (HC) with round nuclei (NU) and centrally placed nucleolus.
Fig 2: Liver of toluene treated rat shows cytoplasmic degenerations, nuclei of different shape, sizes and several binucleated cells.
Fig 3: Liver of phenobarbital and toluene treated rat shows inflammation of hepatic parenchyma, binucleated cells and few megalocytes.
Fig 4: Liver of trichloroethylene treated rat shows hyperplasia and hypertrophy and nuclei of different shapes and sizes.
Fig 5: Liver of phenobarbital and trichloroethylene treated rat shows mild cytoplasmic degenerations. Hepatic cells (HC) through possessed irregular shaped nuclei but were better organised.
Fig 6: Liver phenobarbital treated rat shows only hydrophic degeneration and necrosis.

However, well define necrosis was wanting. In the centrilobular region fatty changes were frequently observed. Increased mitotic activity was also recorded. Sclerosis and hepatitis were also witnessed. Nuclei of different shapes and size and several binucleated cells for observed.

Study of liver of phenobarbital and toluene treated showed inflammation of hepatic parenchyma, hyperplasia, hypertrophy, fatty changes and massive necrosis. Binucleated cells and megalocytes were observed and also degeneration of Kupffer cells was frequently observed (Fig 3,4)

Trichloroethylene administration of rats induced inflammation of hepatic parenchyma, hyperplasia and hypertrophy.

Necrosis was wanting in the liver of phenobarbital and trichloroethylene treated rats. However, cytoplasmic degeneration at the perilobular zone was observed. Eosinophilia was also wanting. Canalicular spaces were enlarged. Hepatic cells possessed irregular shaped nuclei (Fig 5,6).

Hydropic degeneration, portal inflammation, hyperplasia and necrosis did occur after phenobarbital treatment.

All these treatments do not promote any tumour formation. Morphological changes observed in liver after these treatments have been summarised in Table 1

Table-1: Summary of Histopathological Observation in Liver

Lesions associated with hepatocytes	**Toluene**	**Phenobarbital +toluene**	**Trichloroethylene**	**Phenobarbital+ Trichloroethylene**	**Phenobarbital**	**Control**
Hydrophic degeneration	+	+	-	-	+	-
Steatosis/Fatty change	+	+	-	-	-	-
Inflammation	+	+	+	+	+	-
Necrosis	+	+	-	-	+	-
Apoptosis	-	-	-	-	-	-
Hypertrophy	+	+	+	-	-	-
Hyperplasia	+	+	+	+	+	-
Nuclear Changes						
Increased mitosis	+	-	-	-	+	-
Karyomegaly/hyperchromatosis	-	-	-	-	-	
Megalocytosis	+	+	-	+	-	-
Multinucleated cells/Binuleated Cells	+	+	-	-	-	-
Intranuclear inclusions	-	-	-	-	-	-

Conclusion

Use of phenobarbital as a sedative hypnotic agent by workers exposed to toluene and trichloro ethylene in their work environment should be carefully monitored.

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