Histopathological and biochemical changes induced by toluene subcutaneous administration in rabbits

Wasfi Dhahir Abid Ali

Department of basic sciences College of nursing -university of Iraq –Ph.D in physiology

Email- Wasfi.Abid_ali@uobasrah.edu.iq

Abstract

The liver is the main organ responsible for the metabolism of drugs and toxic chemicals, and so is the primary target organ for many organic solvents. The rabbits in group II (treated with 0.3 cm of toluene 97% S/s for 6 weeks, At the end of the experimental period, liver and blood samples were taken from the decapitated animals. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), were determined. Liver tissue sections were stained with routine histological methods and examined under the light microscope. The results revealed significant increase on liver enzymes level as well as liver tissue changes.

Introduction

Although a number of industrial chemicals are known to be hepatotoxins, liver disease from occupational exposure is rarely suspected or diagnosed (Giulia *et al.*,2012). Chemically toluene is a hydrocarbon that is rapidly absorbed through the respiratory and gastrointestinal tracts and, to a lesser extent, through the skin (Campagna et al., 2001). Studies by Franco 1991 have suggested organic solvents may induce liver toxicity] because most chemicals are metabolized in the liver and toxic metabolites generated through the metabolism are the main cause of liver damage. Toxic hepatitis is characterized by different degrees of steatosis and fibrosis, which can lead to cirrhosis(Giulia *et al.*,2012). Sharp(1997) sited that Exposure to inhalants occurs primarily via pulmonary absorption, and significantly less absorption takes place through the

skin or gastrointestinal tract. Most inhaled toluene is metabolized in the liver by conversion to benzoic acid by two enzymes, alcohol dehydrogenase and aldehyde dehydrogenase (Greenberg 1997).

Experimental procedure

Ten adult male rabbits , weighing 950- 1200 g, were allotted in two experimental groups. The animals were purchased from a local market and housed in individual cages (360 · 200 · 190 mm) the animal were available ad libitum Food and tap water for both groups , the first group take normal saline 0.9% Na CL while the second group received 0.3 ml of toluene(97%)/Kg B.W subcutaneously daily for 6 weeks. At the end of the experiment , blood were drown via cardiac puncture after anesthetized by intramuscular injection of mixture xylazine2% (Alfasan-Holland) and ketamine10%(Kepro -Holland), 5 ml of blood samples were collected from each rabbit by heart puncture then they were put in tubes centrifuged (3500 r pm for 15 minute) and the serum that was obtained, transferred to Eppendorf tube kept until analysis for, liver enzymes and biochemical . animals scarified and studied organ were isolated in 10% formalin for path-histological examination .

Results and discussion

The stress of exposure to toluene stimulated liver enzymes . The present study revealed significant elevation in AST, ALT and ALP activation in group of rabbits treated s/c (0.3 /kg B.W toluene) for 6 week compared with control group where rabbits received 1ml normal saline(0.9% NaCl).(0table (1)

Table (1) the effect of s/c administration of toluene on liver enzymes on rabbits after six weeks (Mean ±SE)

Parameter / Group	AST/ UL	ALT/UL	ALP/UL
Control group Normal saline N=5	9.515 ± 0.330	13.785 ± 0.441	24.666 ± 0.774
Treatment group Toluene 97% N=5	38.514± 0.991**	44.770 ± 0.1.105 **	50.758 ± 0.882 **

N=10 , Differences between group p 0.05 vs control

A number of animal studies have reported increased liver sizes or histological changes in mice or rats repeatedly exposed to concentrations , Toluene inhalation significantly increased serum ALT, AST and tissues(Tas *et al.,* 2011).

Increased liver weights and increased serum levels of liver enzymes have been reported in rats repeatedly exposed to toluene at doses greater than 1,125 mg/m3 (300 ppm) for at least 6 hours a day Agency for Toxic Substances and Disease Registry (ATSDR 2000). Toluene and its metabolites have been studied with respect to their reactive oxygen species-enhancing potential (Cara *et al* ., 1983).

Murat *et al.,(*2013) found that the level of plasma transaminase to be increased in toluene administered rats. Additionally, slight degeneration of hepatocyte and mononuclear cell infiltration was observed in the liver tissue sections as well as their study showed that the high dose of toluene triggers apoptosis in the liver of rats via the mitochondrial pathway in acute period.

Toluene alters the lipid structure of the cell wall and interacts with proteins due to its lipophilic nature. In acute doses, it increases membrane fluidity by significantly elevating the Na/K-ATPase activity (Calderón-Guzmán *et al.,* 2005). Some researchers reported that toluene causes tissue damage by increasing the oxygen radicals (Karabulut *et al.,* 2009; Lee *et al.,* 2003).

Toluene, after being absorbed, can be found, in a decreasing order, in fat tissue (white and brown adipose tissues), stomach, liver, kidneys, bone marrow, brain, and spleen **Health Protection Agency (HPA),(2007)** . Five hours after toluene is taken, it reaches the maximum level in the adipose tissue. High levels of toluene can be found in the liver and brain of people who have died of glue sniffing **Environmental Protection Agency (EPA)(2005),**

Subcutaneous administration of toluene (0.3cm/Kg B.W) caused histopathological changes in rabbits liver after 6 weeks ,Cross section of liver showed congested central vein , flattening and vacillation of hepatocytes ,enlarged pyknotic nuclei of hepatocytes,with disarrangement of hepatic architecture(Figure 2). compared with control group(Figure 1). Toluene alters the lipid structure of the cell wall and interacts with proteins due to its lipophilic nature. In acute doses, it increases membrane fluidity by significantly elevating the Na/K-ATPase activity (Calderón-Guzmán *et al.*, 2005). Some researchers reported that toluene causes tissue damage by increasing the oxygen radicals (Karabulut *et al.*, 2009; Lee *et al.*, 2003).

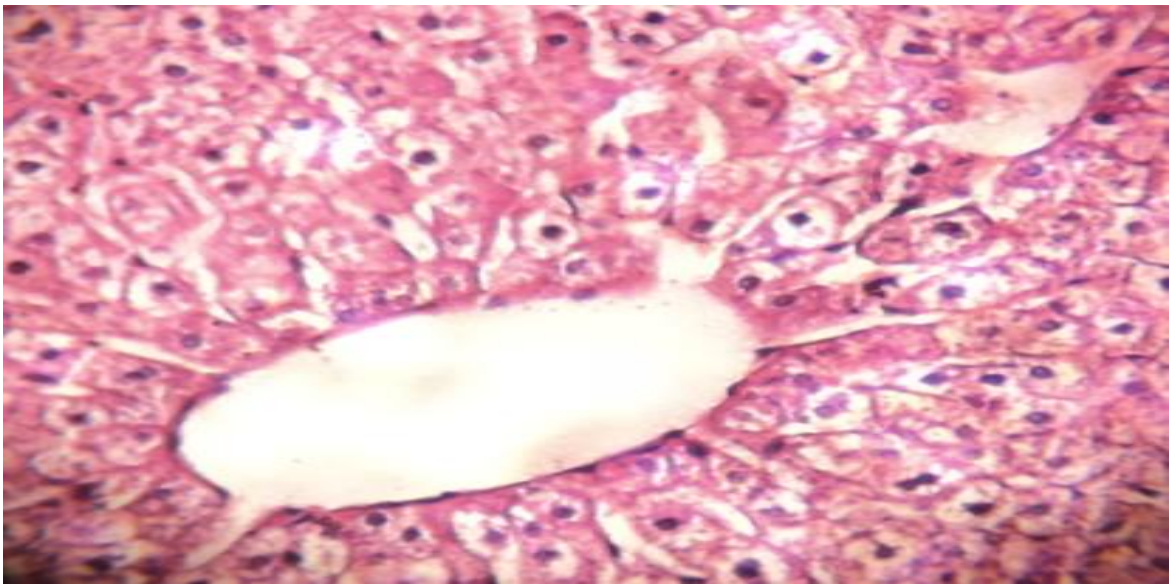

Figure (1) Normal cross section in rabbit liver tissue of control group (H&E) x 400

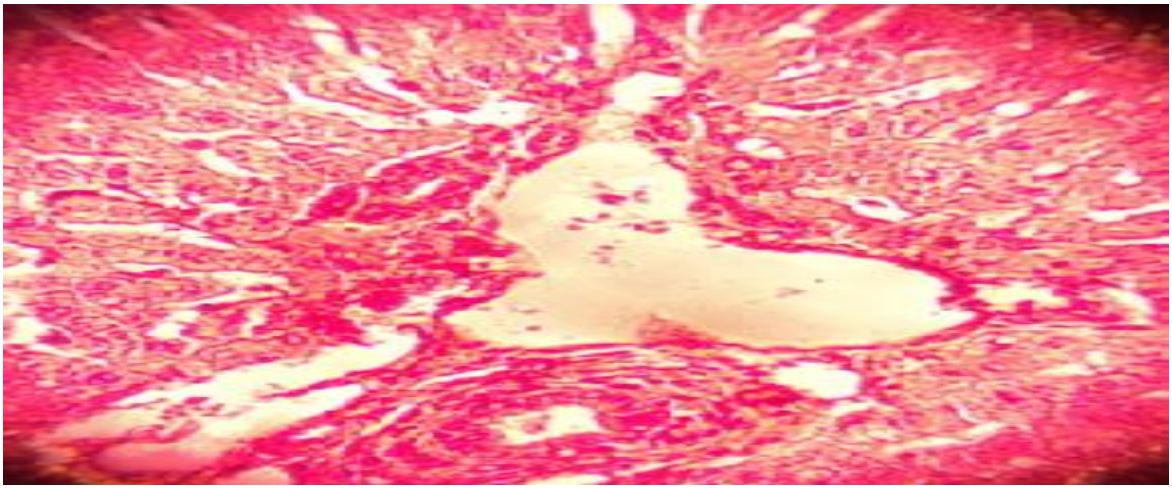

Figure (3) Cross section in rabbit liver treated with toluene administration showing congested central vein and disarrangement of hepatocyte (H&E) x 400

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Acknowledgement

My sincere gratefulness and thanks to Mr Luaa A Shehab and extend my thanks and gratitude to. Prof. of physiology Dr. Ali Abid Allateef

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