

The Protective Role of Vitamin C against Formaldehyde induced-hepatotoxicity and nephrotoxicity in Male Rats

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Abstract: Formaldehyde was administrated by gavage into male albino rats for 7 day as a single dose (1ml), and the histological changes in liver and kidney were investigated using paraffin method. This compound has caused hepatotoxicity which included degeneration of the hepatocytes, and appearing of inflammatory leucocytes. This compound has also caused nephrotoxicity which included high inflammation degree and degenerative effect on kidney tubule cells. When given to the formalin exposed rats, vitamin C caused recovery of the changes in the liver and kidney, i.e. it has been succeeded in playing a protective role against formalin hepatotoxicity and nephrotoxicity.

Keywords: Formaldehyde, Vitamin C, liver, kidney

I. Introduction

Formaldehyde (HCHO) is a colorless, flammable gas with a pungent, suffocating odor. It is soluble in water, acetone, benzene, diethyl ether, chloroform and ethanol. HCHO used to make plastics and resins for the production of intermediates and for other miscellaneous uses. HCHO also used as disinfectant in many human medicines and cosmetics, as an antiseptic in veterinary drugs and biological and in fungicides, textiles and embalming fluids [1]. Exogenous HCHO is taken up into human body by ingestion, inhalation and dermal exposure. Inhaled HCHO appears to be readily absorbed by the upper respiratory tract but is not distributed throughout the body because of its rapid metabolism [2,3].

HCHO may affect the systemic cellular immunity, as well as local immunity in bronchus (BALT): (Bronchus Associated Lymphoid Tissue) [4,5]. However, chronic inhalation of HCHO causes hepatotoxicity [6] and nephrotoxicity [7].

Changes in the liver after absorption of formalin consist of mild or severe grade of cloudy swelling accompanied by vacuolation of the protoplasm, changes in the nuclei and leukocyte infiltration. Focal necrosis may result. Similar changes follow the inhalation of formaldehyde [8]. Furthermore, formalin was found to cause fatty changes through accumulation of lipid droplets in hepatocytes [9]. The toxic effects of HCHO have been reported to cause structural changes in the epithelial biliary cells and damage intrahepatic and extrahepatic biliary ducts [10]. HCHO exposure has led to disorders of oxidant and oxidant-antioxidant systems of the liver tissue and inflicted oxidative damage [11].

The injection of formalin or the inhalation of the vapors of formaldehyde produces cloudy swelling of the parenchyma of the kidney. Focal necrosis may result [8]. Furthermore, formalin was found to reveal the deposition of adipose tissue in kidney [9].

HCHO was found to induce oxidative stress through increasing lipid peroxidation [12], and formation of reactive oxygen species (ROS) [13], that's why it is scientifically accepted to attenuate this action by using a suitable antioxidant. Vit E was already used in previous study to protect liver against the effect of formaldehyde [14]. Vitamin C is considered as a strong antioxidant [15,16] and used against several toxicants such as alcohol [17] and metals [18]. Some vitamins (such as vitamins A, E, and C) are known to play an important role in ameliorating the toxicity effects of reactive species generated by chemical agents in biological systems. Vitamins C and E are known to be potent antioxidants [19,20].

The aim of the present investigation was to determine and investigate the histological effect of formalin on liver and kidney of rat, and the protective role of vitamin c against the previous effect of formalin in male rat.

II. Materials and Methods

Male Albino Rats Were Obtained From Animal House Of Biology Department, Science College, Salahaddin-University –Erbil. Twelve Albino Male Rats (200-230gm) Were Divided Randomly Into Three Groups (4 Animals In Each) And Housed At 22±2c°with 12hr Light/Dark Cycle. They Were Supplied With Standard Laboratory Animal Care ;Fed Rats Diet And Tap Water Ad Libitum. The First Group Was The Control And Given Only Single Gavage Of One Ml Normal Saline, The Second Group Were Given A Daily Gavage Of 1ml Formaldehyde And The Third Group Were Given A Daily Gavage Of 1 Ml Of Vitamin C (1 Mg\ Kg\ B.Wt) And 1 Ml Formaldehyde Intraperitoneal Injection.

Paraffin Method: Liver Lobes And Kidney Pieces Were Removed And Fixed In Bouin's Fluid, Dehydrated, Cleared, Embedded In Paraffin And Cut Into 4-5µm Thick Section, Then Stained By Hematoxylin And Eosin [21].

III. Results and Discussion

Exposure to formaldehyde appears to be associated with hepatotoxicity in many species, including humans, following injection, ingestion, or inhalation. The present investigation dealt with the study of the effect of formalin exposure by gavage on the liver of rats and the protective role of vitamin C against this hepatotoxicity.

As shown in Fig 1-4, formalin has caused several histological and cytological alterations such as degeneration of the hepatocytes, and appearing of inflammatory leucocytes. The degeneration included shrunken and swelled appearance. The shrunken cells appeared with hypereosinophilic cytoplasm, condensed nucleus, and blebbed plasma membrane (Fig 4). These changes are similar to the apoptotic cell death [22]. Approximately similar microscopic manifestations in the liver include alterations in centrilobular vacuolization and focal cellular necrosis were noticed previously after exposing to formalin dose [23]. Inflammatory mononuclear cells were also found in the liver of formaldehyde exposed rats [12]. When given to the formalin exposed rats, vitamin C caused recovery of the liver structure and maintaining the normal architecture in which no inflammation, healthy hepatocytes and normal sinusoidal structure were revealed (Fig 5). HCHO disturbs the oxidant-antioxidant balance in various tissues and cause oxidative stress in parallel with tissue damage. In previous studies, increased MDA levels in the lung, liver, and testicular tissues of the rats exposed to

HCHO were reported [24-26]. In accordance with our findings, Strubelt et al. [27] have reported increased MDA levels in the liver tissues of HCHO-exposed animals. Similarly, Teng et al. [28] in their experimental study on isolated rat hepatocytes showed that HCHO at low concentrations leads to oxidative stress. Vitamin C is a potent antioxidant agent and exerts a protective effect against oxidative stress [17]. In our study, vitamin C was found to partially prevent the liver damage against HCHO intoxication. Considering the distinctive properties of vitamin C and the results of the present study, it is plausible that both its radical-scavenging and antioxidant actions are involved in preventing tissue damage.

High degenerative changes were found in the kidney of formalin treated rats in which formalin caused high inflammation degree and degenerative effect on kidney tubule cells in comparison to control group (Fig 1 & 2), while such changes were disappeared when vitamin c was administrated to the formalin exposed rats (Fig 3).

It has been found that the main mechanism in the nephrotoxicity of formalin related to the oxidative stress caused by this compound through production of reactive oxygen species (ROS), and depletion of the antioxidant enzymes such as superoxide dismutase and glutathione peroxidase and also caused destruction of mitochondria [11,12,13]. Since vitamin c is considered as a strong antioxidant compound that can strongly scavenge free radicals especially ROS [29], it has been succeeded in playing a protective role against formalin hepatotoxicity and nephrotoxicity.

IV. Conclusion

Certainly formaldehyde caused histological alterations in liver and kidney and in turn, vitamin C showed a protective role against this effect in rat.

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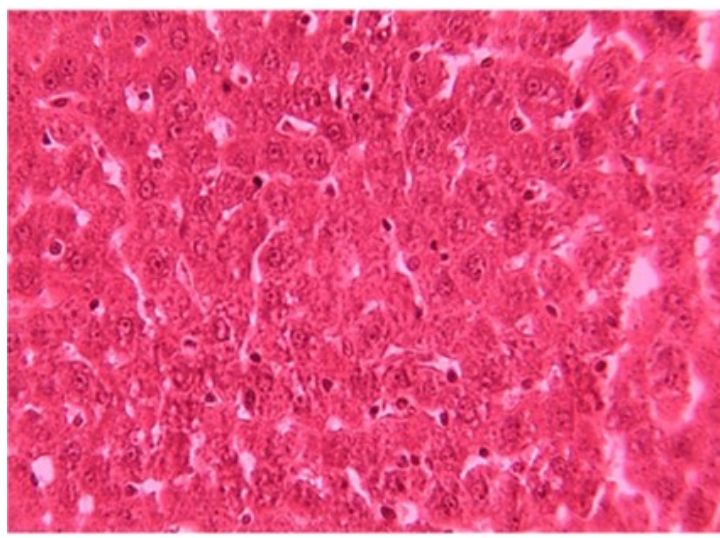


Fig (1): Control liver section showing normal histological structure, 400X.

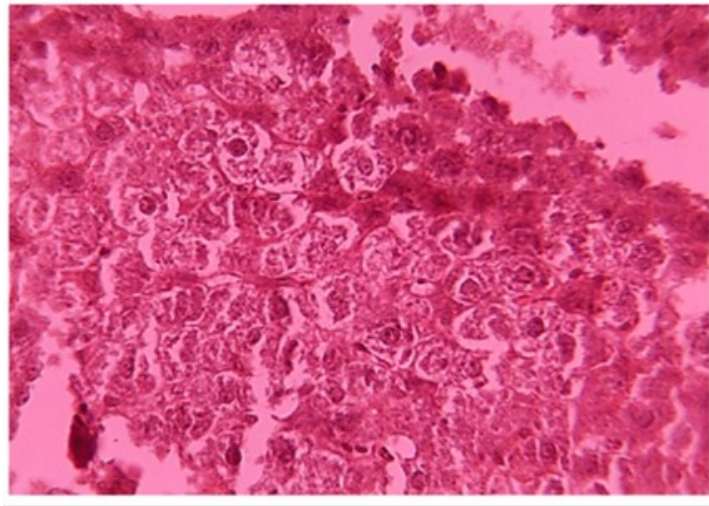


Fig (2): formalin treated rat liver showing degeneration of the liver cells, some **shrunken** and others are seen swelled, 400X

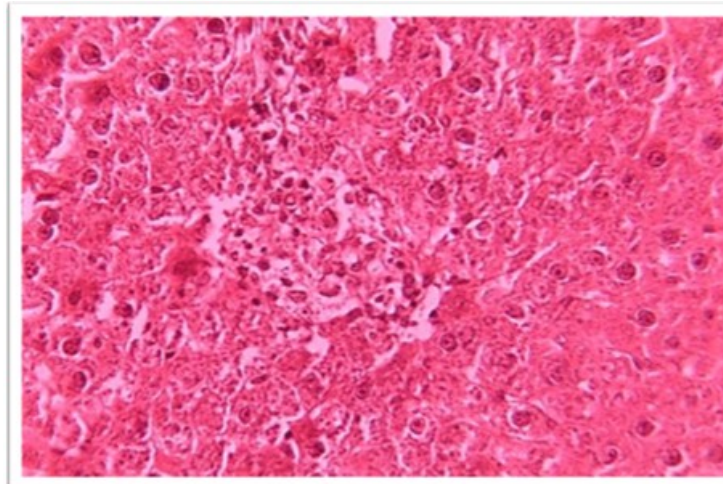


Fig (3): Formalin treated rat liver section shown accumulation of leukocytes **inflammatory cells**, 100x.

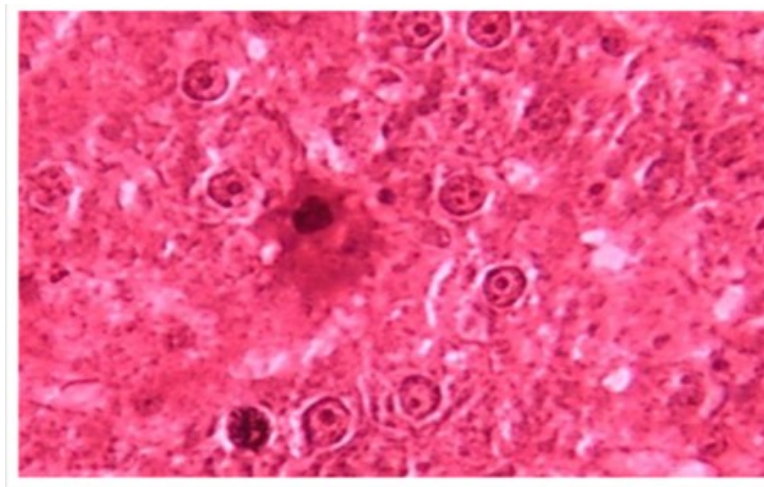
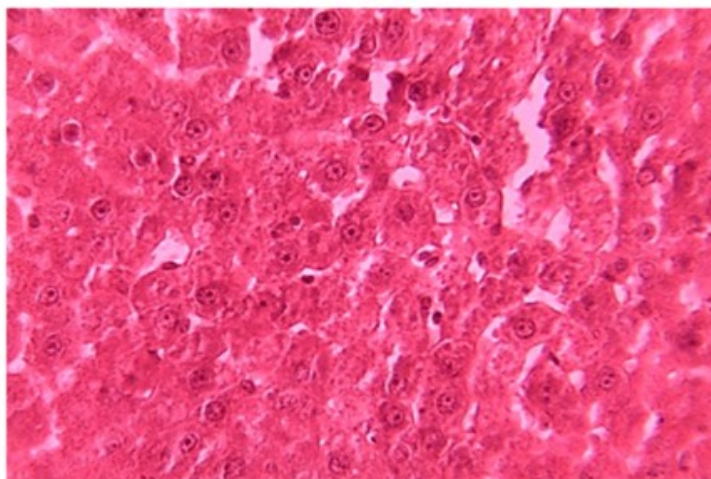
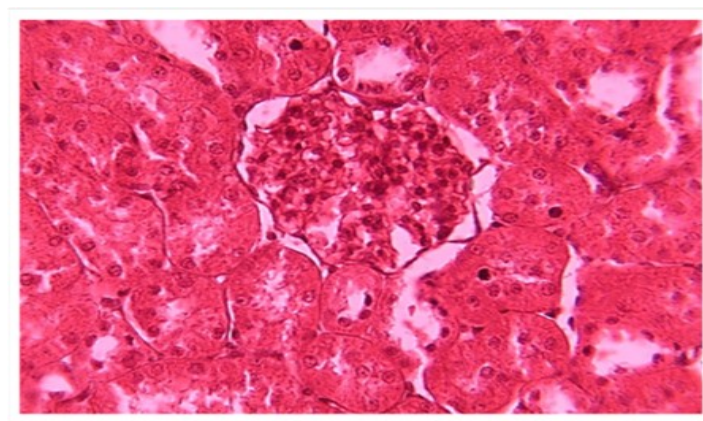


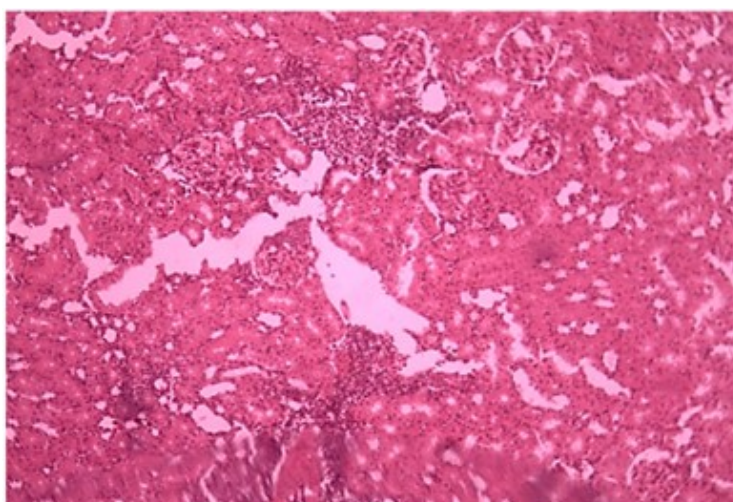
Fig (4): A typical apoptotic hepatocyte with **blebbed plasma membrane**, **condensed nucleus** and **hyper eosinophilic cytoplasm**, 400X.



Fig(5): Section in the liver of formalin plus vitamin C treated rat showing recovered structure, 400X.



Fig(6): Sections in the kidney of control group showing healthy glomerulus and kidney tubule structure, 400X.



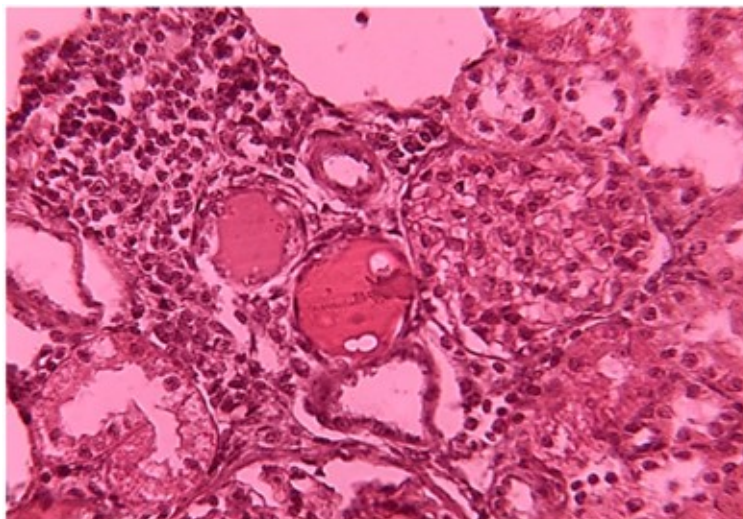


Fig (7): Sections through the kidney of formalin treated rats showing:
A) inflammation (arrows) and dilation of kidney tubules (KT),100X. (above)
B) Higher magnification showing the inflammatory cells and degeneration of the kidney tubule cells (arrow), 400x. (below)

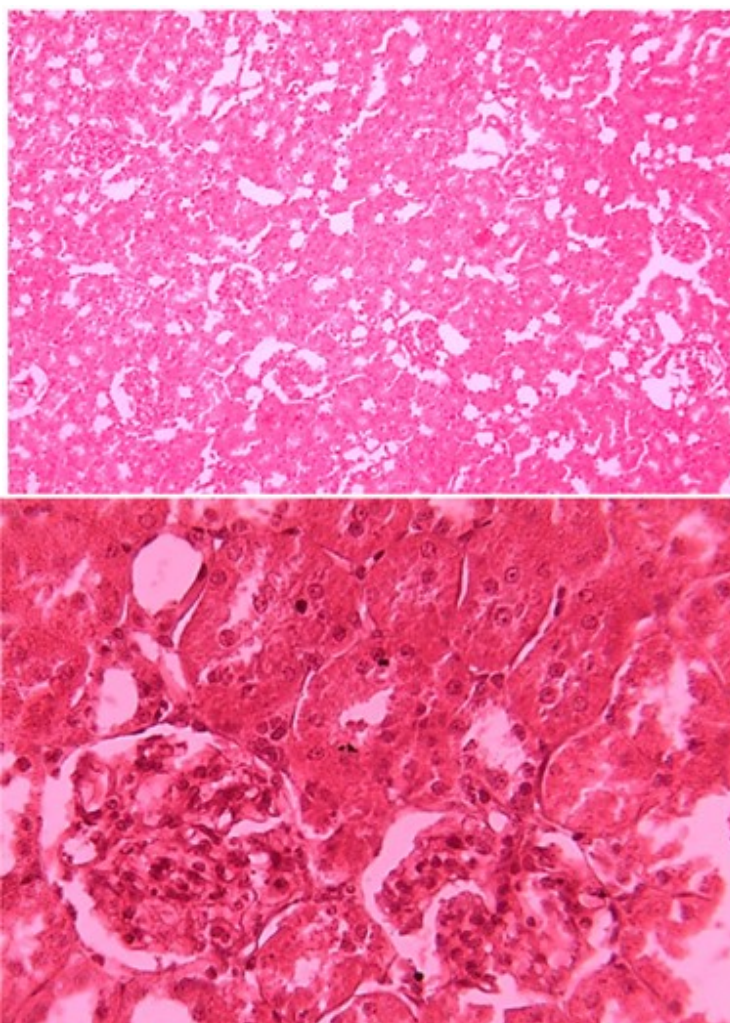


Fig (8): Sections through the kidney of formalin plus vitamin c treated rats showing approximately healthy histological structure, 100X, 400x respectively