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Histological study of L-carnitine effect against acute hepatotoxicity after cytarabine chemotherapy in male rats

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Abstract--Hepatotoxicity is a common clinical manifestation associated with a wide range of anticancer therapies. Because of the inherent toxicity of anticancer therapies, oncologists must maintain a broad understanding of their effects on the body, including the liver. Therefore, the study was conducted to examine the effects of two doses of L-carnitine on damaged liver as a result of giving cytarabine. Twenty-four male rats were used in the experiment. The rats divided to four group each group with six rats, the first group injected normal saline intraperitoneally and set as control group (G1), the second group injected cytarabine 25 mg/kg/bw, intraperitoneally and set as (G2), the third group injected cytarabine 25 mg/kg/bw and 50 mg/kg/bw, L-carnitine intraperitoneally and set as (G3), the fourth group injected cytarabine 25 mg/kg/bw and 300 mg/kg/bw, L-carnitine intraperitoneally and set as (G4), the study showed that use

of L-carnitine reduced the harmful effect of cytarabine in the body when compared to other groups that were injected only with cytarabine at level of (p-value <0.05). The rats treated with L-carnitine have shown significant elevation in CAT and SOD and reduction of the level of MDA. Further, higher significant differences in cytarabine treated group as compared with other groups, after intraperitoneal administration of cytarabine, showed significant increase in serum liver enzymes activities ALT, AST, GGT, and ACE. In conclusion cytarabine reduces liver activity and causes liver injury, and L-carnitine works to reduce cytarabine damage. In addition, L-carnitine 300 mg/kg/bw working better than L-carnitine 50 mg/kg/bw.

Keywords--hepatotoxicity, L-carnitine, cytarabine, histopathological studies in liver.

Introduction

The liver is an important target of the toxicity of drugs, xenobiotics, and oxidative stress. The liver plays a key role in the metabolism of a variety of drugs and toxins and thus is especially susceptible to damage induced by drugs including cytotoxic chemotherapy regimens (1). Hepatotoxicity represents a common clinical manifestation that is associated with a variety of anticancer therapies. The inherent toxicity of anticancer therapies requires oncologists to maintain a broad awareness of their effects on the body, including the liver (2). As a result, proper monitoring and strategies such as discontinuation or dose-modification of pharmacologic agents is commonly required when hepatotoxicity occurs (3). Oncologists and hepatologists must collaborate closely to monitor patients for hepatotoxicity and take appropriate action to prevent long-term liver damage as new, specialized cancer medicines are developed (4).

Treatment with anticancer drugs can have harmful effects, and the toxicity can lead to a worsened condition quality of life and survival time. Cytarabine is one of the most potent cytotoxic drugs used in the treatment of acute leukemia. Cytarabine is an antimetabolite chemotherapy drug that is primarily used to treat cancers of white blood cells (WBCs), such as non-Hodgkin lymphoma, acute myeloid leukemia, acute lymphocytic leukemia, and chronic myelogenous leukemia (5). Its actions are unique to the cell cycle's S-phase. It causes extensive chromosomal destruction causing chromatid aberrations. As a result, quickly dividing cells that require DNA replication, mitosis is the most commonly affected (6). Cytarabine causes the generation of reactive oxygen species (ROS), depletion levels, and inhibition of antioxidant enzyme activity in liver tissues. However, it is known that cytarabine is metabolized in the human liver to a significant degree, and high doses of the drug induce hepatotoxicity.

L-carnitine is synthesized endogenously in the liver, the kidney, and the brain from the essential amino acids lysine and methionine (7,8) or ingested via animal-based food products (9). Its name comes from the Latin *carnus*, meaning meat, because the compound is extracted from meat (10, 11). L-carnitine plays an important role in energy metabolism (12). It transfers long-chain fatty acids to cell

mitochondria for oxidation, which produces energy needed by the body, it also transports harmful substances out of the organelle, preventing them from accumulating in the cell (13). L-carnitine may provide cell membranes protection against oxidative stress, given its pivotal role in fatty acid oxidation and energy metabolism (9). L-carnitine has the potential to protect these cellular events in several manners including decreasing the production of reactive oxygen species at different points and maintaining mitochondrial functions (14). Synthetic drugs used to treat cancer are not only expensive, but also have a complicated mode of administration and a number of side effects. L-carnitine plays important roles in reducing the cytotoxic effects of free fatty acids by forming acyl-carnitine and promoting beta-oxidation (8, 15), leading to alleviation of cell damage.

Materials and Methods

Twenty-four (24) male white albino rat were used in this experiment and their ages between (12-14) weeks and they were (260-300) gm per body weight and the animals were placed in good condition in special plastic cages and provided the animals with the appropriate conditions in terms of temperature around (30 ±5 C°) and ventilation and the light system was 12 hrs. per day. The experiment lasted about during 28 days the rats were administration as the following groups, the first group, which include first group (G1): six male rats were given normal saline (daily, intraperitoneally injection) and served as the control. Second group (G2): six male rats were given single dose of cytarabine (25 mg/kg/bw) intraperitoneally injection. Third group (G3): six male rats were given single dose of cytarabine (25 mg/kg/bw) intraperitoneally and L-Carnitine (50 mg/kg/bw) for intraperitoneally injection for 4 weeks. Fourth group (G4): six male rats were given a dose of cytarabine (25 mg/kg/bw) intraperitoneally and L-Carnitine (300 mg/kg/bw) for intraperitoneally injection for 4 weeks. Blood samples were collected from all rats, and samples were coded to avoid the possibility of bias.

Sample collection and tissue preparation

After the end of the experiment period liver sample has been taken after sacrifice the animal and they were immediately put into a 10% fixative formalin solution and left there for 24 hours before being processed. The samples were first dehydrated in graded alcohol at room temperature for 2 hours each at 70%, 80%, 90%, and 100% concentration before being submerged in xylene for 2 hours and then melted paraffin wax for 3 hours. The samples were then positioned and implanted in brand-new paraffin (paraffin blocks). To examine the sections under a microscope, the blocks were sectioned with a microtome at a thickness of 5 m. The sections were subjected to standard hematoxylin and eosin (H&E) staining procedures. Photomicrographs of each section were taken using a digital camera (canon, japan) using a light microscope to analyze the sections under examination(16).

Statistical Analysis

Statistical analysis of the results was conducted according to SPSS (2016) version 24.00 where one way (ANOVA) was used to assess the significance of changes between the groups' results. The data were expressed as Mean Standard Errors

(SE) and P-value ≤ 0.05 was considered as statistically significant, LSD test was carried out to test the significant levels among means of treatments (17).

Results

Histological Result in Liver

The liver of the control animals group showed normal histology (Figure 1) stained with (H&E), and was revealed normal hepatic architecture and normal hepatocytes cords with slight congestion of the hepatic central vein (40X H and E). The histopathological examination from the second group of the liver tissue treated with cytarabine revealed that there was severe congestion of portal vein with exudation, remarkable heavy infiltration of inflammatory cells in portal area and proliferation of biliary ductule and hyperplasia of portal arteriole endothelia hepatocytes. Along with these outcomes, congestion and the infiltration of a significant number of mononuclear inflammatory cells when compared to the normal histological structure of the liver (Figure 2), as compared to the normal histological structure of the liver (Figure 1).

On the other hand, cytarabine group that treated with L-carnitine 50 mg/kg/bw from third group showed a moderate congestion of central vein, congestion in portal area with moderate inflammatory cells infiltration, remarkable degeneration changes of hepatocytes with keeping the normal arrangements in cords around central vein and some vacillation of hepatocytes when compared to the normal histological structure of the liver (Figure 3), while cytarabine group that treated with L-carnitine 300 mg/kg/bw from G4 group showed mild congestion of central vein with hepatocytes reverse their regular arrangements, remarkable subsides hepatic inflammation with close to normal portal area when compared to the normal histological structure of the liver (Figure 4).

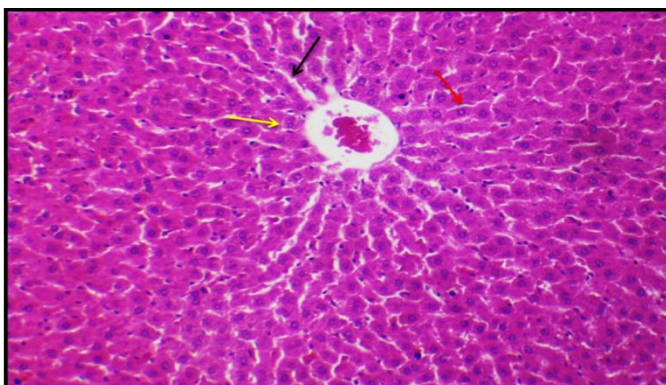


Figure 1. Photomicrograph of liver section: Control group showed the normal histological architecture of hepatic tissue, normal hepatocytes cords (black arrow) radiating around central vein (yellow arrow), significant rounded nuclei with granular cytoplasm (red arrow), (H and E, 10X)

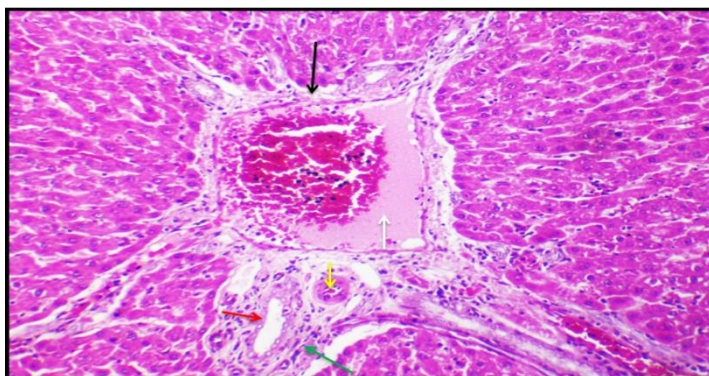


Figure 2. Photomicrograph of liver section: Cytarabine treated group showed severe congestion of portal vein(black arrow) with exudation (white arrow), remarkable heavy infiltration of inflammatory cells in portal area (green arrow) with proliferation of biliary ductule (yellow arrow) and hyperplasia of portal arteriole endothelia (red arrow), (H and E, 10X)

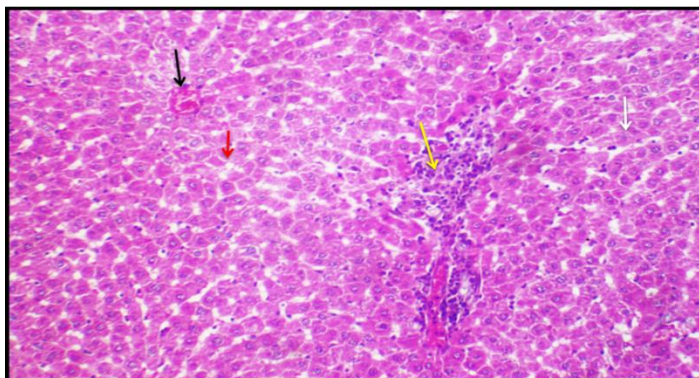


Figure 3. Photomicrograph of liver section: L- Carnitine 50 mg /kg/bw treated group animal showed moderate congestion of central vein(black arrow), congestion in portal area with moderate inflammatory cells infiltration (yellow arrow), remarkable degeneration changes of hepatocytes (white arrow) with keeping the normal arrangements in cords around central vein and some vacillation of hepatocytes (red arrow),(H and E, 10X)

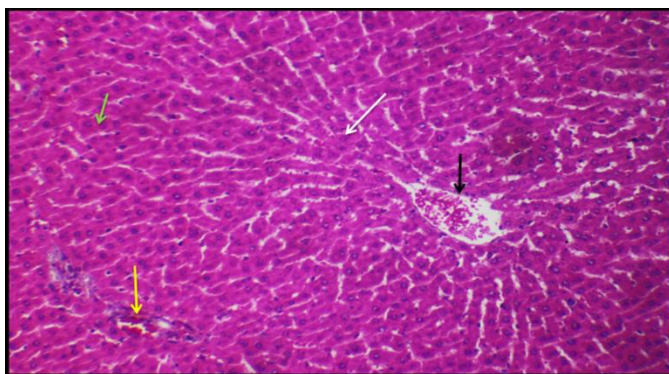


Figure 4. Photomicrograph of liver section: L-Carnitine 300 mg /kg/bw treated group animal revealed mild congestion of central vein(black arrow) with hepatocytes reverse their regular arrangements (white arrow), remarkable subsides hepatic inflammation (green arrow) with improve to normal portal area (yellow arrow), (H and E, 10X)

Discussion

Cytarabine is one of the most active cytotoxic agents in the treatment of cancer. Liver toxicity are major complications. Cytarabine caused severe damage in the liver, such as degenerative hepatocytes and moderate enlargement of sinusoids, occlusion of vascular and ductal structures, toxic metabolite formation, and inflammatory cell infiltration into the liver parenchyma can induce damage, which was observed by microscopic examination. The histopathological study of the liver in cytarabine group show that there was a damage in the liver which is represented by congestion, exudation and inflammation of the liver multiple foci of apoptotic cells and minimal inflammatory response were noticed. This current result may be occurred by oxidative damage cytarabine that leads to mitochondrial DNA and those changes related to DNA fragmentation and apoptosis initiation damage and cause a necrotic of the cells. This results agree with the previous study which show that hepatic apoptosis and cell necrosis which can lead to acute or chronic hepatic failure(18, 19). Hepatotoxicity can result from damage to structures such as the liver sinusoids, vasculature, bile ducts, and direct damage to hepatocytes themselves (20).

Drug-induced liver toxicities are characterized by systemic and local inflammation with recruitment of macrophages and neutrophils into the liver vasculature, the main function of these phagocytes is to destroy invading microorganisms and to remove dead cells and cell debris in preparation for tissue regeneration. Because of the nature of the toxic mediators generated by these phagocytes, healthy cells may also be affected, which can aggravate the original liver injury (21, 22, 23). Jaeschke et al. (1) confirmed the suggestion that drug-induced injury to hepatocytes and showed that Kupffer cell activation and neutrophil infiltration extend toxic injury, Kupffer cells release reactive oxygen species (ROS), cytokines, and chemokines, which induce neutrophil extravasation and activation. Kupffer cells and neutrophils are a source of proinflammatory cytokines and chemokines and of reactive oxygen and nitrogen species, which promote oxidative stress in injury induced by toxicants and ischemia/reperfusion.

On the other hand, the study showed that there is a significant improvement in the liver histological section in the 50 and 300 mg/kg/bw L-carnitine. L-carnitine is known to target the antioxidation mechanisms of cell damage in the body by serving as free radical scavengers and therefore promoting hepatoprotection (24). L-carnitine has been proven to have potent antioxidative and cytoprotective properties. It has several pharmacological properties such as anti-inflammatory, antioxidative activities and hepatoprotective effects (25). Hepatoprotection of L-carnitine is the ability to prevent damage to the liver, prevent the liver affections prophylactically and maintains balance in liver enzymes. L-carnitine significant hepatoprotective effect in isolated rat hepatocytes with protective effect against hepatocellular degeneration and necrotic changes (26). This may be because L-carnitine maintains cell integrity by controlling the intramitochondrial proportion of acyl-CoA/CoA, suppressing hazardous substances, maintaining the integrity of the mitochondrial membrane's permeability, and promoting the expulsion of free radicals (27). Additionally, damaging to mitochondria, oxidative stress impairs mitochondrial -oxidation, which causes hepatocytes to accumulate fatty acids and causes hepatic damage such as hepatic steatosis. Due to its role in fatty acid -oxidation and the conversion of lipids into energy in the mitochondria, treating animals with L-carnitine may have a reversible effect on the formation of steatosis, this might be how L-carnitine protects against the potentially harmful effects of cytarabine (28, 29).

Conclusion

We conclude from the previous study that L-carnitine showed positive results in protecting the liver from damage caused by cytarabine, and that there is a dose of 300 mg/kg/bw L-carnitine showed better results than the dose of 50 mg/kg/bw L-carnitine.

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