Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Kerbala
College of Veterinary
Medicine

Hepatoprodective effect of L-carnitine against cytarabine induced hepatotoxic in male rats

Thesis Submitted to the Council of the Faculty of Veterinary Medicine / University of Kerbala in Partial Fulfillment of the Requirement for the Master Degree of Science in Veterinary Medicine / Physiology

By

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1444 A.H **2022 A.D**

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

﴿ فَتَعَٰلَى ٱللَّهُ ٱلۡمَلِكُ ٱلۡحَقُّۗ وَلَا تَعۡجَلۡ بِٱلۡقُرۡءَانِ مِن قَبۡلِ أَن يُقۡضَىٰٓ إِلَيۡكَ وَحۡيُهُۥۖ وَقُل رَّبِّ زِدۡنِي عِلۡمٗا ﴾

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I hereby declare that this thesis is my origin work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Kerbala or other institutions.

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/ / 2022

Dedication

To the messenger of mercy, the Prophet Muhammad "Allah

blessing and peace be upon him and his pure family" ...

To my homeland, Iraq, which is bleeding with martyrs…

To my loving father and mother, who were of help and support for me, and their blessed supplication had the greatest effect in facilitating the search ship to dock on this image…

To those with whom I have shared all my life, and from whom I derive my pride and determination, my dear sisters and brother ...

To my family, friends and everyone who benefits from this work….

Marwa

Acknowledgments

First, thanks and a special gratitude to **Allah** for His entire blessing during pursuit of our academic career goals, Is the first who deserve all thanks and appreciation for granting me with well, strength and help with which this research has been accomplished.

I would like to thank the Dean of the College of Veterinary Medicine **Prof. Dr. Wefaq Jbori Albazi** and my esteemed professors who preferred of me for their advice

A special thanks to supervisor **Asst.Prof. Dr. Mayada Sahib Hassan** and **Prof. Dr. Rana Fadhil Mousa** for her guidance and advisement through the period of the project countless hours of reflecting and reading. My utmost gratitude to **Asst. Prof. Dr. Haider AL-Karrawy** / College of Veterinary Medicine in Kerbala for his help.

Great thank to Dr.**Sadiq Saffa Mahdi** and Dr. **Mohammed Ali** their help during research . Finally, I am also indebted to all persons who exerted any effort in helping me in this work and I had forgotten their name.

Marwa

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abbreviations	Meaning
ACE	Angiotensin Converting Enzyme
ALP	Alkaline phosphatase
ALT	alanine aminotransferase
AML	Acute myeloid leukemia
Ara-C	Cytarabinee,
AST	aspartate Aminotransferase
CAR-T	chimeric antigen receptor cells
CAT	Catalase
CDA	cytidine deaminase
CYP2E1	cytochrome P4502E1
DCK	deoxycytidine kinase
DCMD	deoxycytidyne monophosphate deaminase
GGT	. Gamma-Glutamyltransferase
GIT	gastrointestinal tract
GOT	glutamate oxaloacetate transaminase
GPT	glutamate pyruvate transaminase
ICI	immune checkpoint inhibitors
LFTs	liver function tests
MDA	Malondialdehyde
ROS	reactive oxygen species
ROS	reactive oxygen species
SOD	Superoxide Dismutase
WBCs	white blood cells
ASP	asparaginase

Abstract

The study was undertaken to examine the effects of two doses of L-carnitine on liver damage induced by cytarabine including cytotoxic chemotherapy regimens. The study conducted at University of Kerbala / College of Veterinary Medicine. Twenty-four(24) adult male rats were used in the experiment. The rats divided randomly in to four groups each group with six rats, the first group were injected normal saline intraperitoneally and set as control group (G1). The second group were injected cytarabine (25 mg/kg/bw) intraperitoneally and set as (G2).The third group were injected cytarabine (25 mg/kg/bw) and (50 mg/kg/bw) L-carnitine intraperitoneally and set as (G3). The fourth group were injected cytarabine (25 mg/kg/bw) and (300 mg/kg/bw) L-carnitine intraperitoneally and set as (G4) ; The study showed that using of L-carnitine reduced the harmful effect of cytarabine in the body when compared to the group that was injected only with cytarabine at level of (p-value <0.05). The rats that were treated with L-carnitine have shown a significant differences in cytarabine treated group if compared with other groups. The cytarabine group (G2) showed significant increase in serum liver enzymes activities alanine aminotransferase (ALT); gama glutamayl transferase (GGT), angiotensin converting enzyme (ACE), aspartate amino transferase (AST),decrease in antioxidant Super oxide dismutase (SOD) and catalase (CAT) also, increase of the level of malondialdehyde (MDA) when compared to other groups .on the other hand, Experience also showed that there is a signefecant (p-value <0.05) decrease in the level of alanine aminotransferase (ALT),gama glutamayl transferase (GGT), angiotensin converting enzyme (ACE), aspartate amino transferase (AST), malondialdehyde (MDA) and increase in serum in antioxidant superoxide dismutase (SOD) and catalase (CAT) in (G4) group as compeers with (G3) group.

In conclusion The concretion of L-Carnitine 300 mg/kg.BW ameliorating the hyoartoxic effect induced by cytarabine better than 50 mg/kg.BW., Hepatic protection effect of L- Carnitine 300 mg/kg.BW better than 50 mg/kg.BW in hypertoxic effect induced by cytarabine by decrease liver enzyme and The hepatoprtective effect of L-carnitine against cytarabine induce hepatotoxicity manifested by increase antioxidant/oxidant balance and decrease liver damage.

Chapter One: Introduction

1. Introduction

The liver is a main organ in the biotransformation of food and drugs, hepatic diseases are a major worldwide issue, the liver serves many metabolic functions. It has enormous regenerative capacity, toxic chemicals are the main cause of hepatic disorders. The liver is an important target of the toxicity of drugs,xenobiotics, and oxidative stress. Furthermore, the active proliferative response of hepatocytes makes the liver an important target of carcinogens,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 (Jaeschke *et al.*, 2002).

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 (Mudd and Guddati, 2021). Anticancer therapies are developed, especially targeted agents, clinicians must remain informed their new side effects, including hepatotoxicity (Choti, 2009). As the hepatotoxicity of cancer related treatments has been further characterized, more focus has been moved to the identification of patient specific risk factors that contribute to increased risk of toxicity especially in the more recently developed targeted agents (Małyszko *et al.*, 2017 and Totzeck *et al.*, 2019). Such unexpected toxicities appear to be the consequence of the unique vascular, secretory, synthetic, and metabolic features of the liver. As a result, proper monitoring and strategies such as discontinuation or dose-modification of pharmacologic agents are commonly required when hepatotoxicity occurs (Fontana, 2014). 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 (Al-Jammas and Al-Saraj, 2020).

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 (Di Francia *et al.*, 2021). Acute leukemia is a type of cancer of the blood and bone marrow characterized by the development of large numbers of immature lymphocytes (Bernard et al.,2017). 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 (Dawood *et al.,* 2020).

Cytarabine commonly known as Ara-C, is a pyrimidine nucleoside analog, antimetabolite, and antineoplastic that inhibits the synthesis of deoxyribonucleic acid (Al-Badr and El-Subbagh, 2009). They are actions unique to the cell cycle's S-shape. It through extensive chromosomal destruction causing chromatid aberrations. As a result, quickly dividing cells that require DNA replication, mitosis are the most commonly affected (Ma *et al.,* 2017). Cyatrabine causes the generation of reactive oxygen species (ROS), depletion levels, and inhibition of antioxidant enzyme activity in liver tissues. However, it is known that cytarabinee 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 (Sabzi *et al.,* 2017) or ingested via animal-based food products (Fielding *et al.,* 2018). Its name comes from the Latin carnus, meaning meat, because the compound is extracted from meat (Sawicka *et al.,* 2020; Dahash and Sankararaman, 2022). L-carnitine plays an important role in energy metabolism (Wang *et al.,* 2021). It is important for heart and brain function, muscle movement, and many other body processes. 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 (Longo *et al.,* 2016; Bota *et al.,* 2021). L-carnitine may provide cell membranes protection against oxidative stress, given its pivotal role in fatty acid oxidation and energy metabolism (Fielding *et al.,* 2018). 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 (Modanloo and Shokrzadeh, 2019; Morimoto *et al.,* 2020).

Synthetic drugs used to treat cancer are not only expensive, but also have a complicated mode of administration and a number of side effects. Natural products with antioxidant and anti-inflammatory properties have been shown to protect against the toxicity of chemotherapy (Ringseis *et al.,* 2013; El-Kott *et al.,* 2014). Laevo (l)-carnitine plays an important role in reducing the cytotoxic effects of free fatty acids by forming acyl-carnitine and promoting beta-oxidation leading to alleviation of cell damage. (Bremer, 1983; Yamanaka *et al.,* 2011),

Aims of the Study:

The present study was undertaken to observe protective effects of L-carnitine on a hepatotoxicity due to high dose of cytarabine in liver male rats via performing the following:

1- Evaluating the effect of L-carnitine as antioxidant on liver hepatotoxicity in male rats.

2- Measuring the liver enzyme activities (ALT, GGT, ACE, and AST) in serum .

3- Antioxidant indices assessment of (catalase and superoxide dismutase) and the level of oxidant (Malondialdehyde) in liver rats.

4- Histopathological examination of liver.

Chapter Two: Review of the Related Literature

2. Review of the Related Literature

2.1. Liver

The liver is a major organ only found in vertebrates which performs many essential biological functions such as detoxification of the organism, the synthesis of proteins , biochemical necessary for digestion and growth (Xu *et al*., 2019). The liver is a remarkable organ that usually protects the individual against injury from xenobiotic compounds. However, this organ is often the site of metabolism and it is where some chemicals concentrate and become bio activated, leading to hepatic injury. Although its capacity for repair and regeneration, makes it a quite robust organ, if the ability to regenerate is not adequate, or if injury to the liver is very severe, liver damage can progress to liver failure and death. There is continued interest concerning the higher incidence of liver damage caused by prescription/ injectable drugs, over-the-counter medications, dietary supplements, and special diets, in addition to environmental chemicals/xenobiotic (Bischoff *et al*.,2018).

2.1.1. Diverse Function of the Liver

The liver is a functionally diverse organ. The liver is directly involved in nutrient homeostasis, including glucose regulation, cholesterol synthesis and uptake (Manella *et al*., 2021), storage of glycogen, lipids, minerals and vitamins (Morris & Mohiuddin, 2021), and synthesis of clotting factors, albumin, very low density lipoprotein , other proteins (Huang *et al*., 2020) , metabolism and excretion of such things as hemoglobin breakdown products, and xenobiotic (Oliviero *et al*., 2020). Blood from the gastrointestinal tract (GIT), via the portal vein, is filtered for bacterial products, such as endotoxin and ammonia, and xenobiotic (Bischoff *et al*., 2018).

The liver has a dual blood supply as noted above. The hepatic artery brings blood from the systemic circulation and the portal system brings blood directly from the gastrointestinal system. The portal system is involved in the “first pass effect,” meaning that nutrients and xenobiotics absorbed by the digestive system are filtered through the liver before entering the systemic circulation (Mohammed, 2019). The space of Diseases allows close contact between circulating plasma, plasma proteins, and hepatocytes, allowing rapid diffusion of lipophilic compounds across the hepatocyte membrane (Banerjee, 2020).

2.1.2. Bio activation and Metabolism of the liver

One of the major functions of the liver is the elimination of both endogenous and exogenous compounds (Li *et al*., 2019). Some xenobiotic agents absorbed by the small intestine are highly lipophilic (Banerjee, 2020). Renal excretion is the primary mechanism of removal for many xenobiotic (Chen, 2020), but renal excretion of lipophilic compounds, which are frequently protein bound in the circulation, is poor (Watanabe *et al*., 2019). Therefore, in order for these xenobiotics to be eliminated, biotransformation needs to occur in the liver first to increase their water solubility (Orhan, 2021).

Hepatic fibrosis is a nonspecific lesion that usually results from chronic inflammation. Chronic inflammation can be the result of continuous exposure to a variety of hepatotoxic chemicals such as organic arsenicals (Bischoff *et al*., 2018), chronic ethanol ingestion and nonalcoholic fatty liver disease. Fibrosis usually occurs around the portal area, in the space of diseases, and around the central vein (Brunt *et al*., 2021). Hepatocytes are lost and replaced with fibrous connective tissue. Per portal fibrosis can lead to portal hypertension (Miller *et al*., 2021).

Toxic liver injury can reproduce virtually any known pattern of injury, including necrosis, steatosis, fibrosis, cholestasis, and vascular injury (Inci and Karatas, 2019). Liver injury during cancer chemotherapy may not always reflect hepatotoxic anticancer drugs; the clinician must also consider reactions to antibiotics, analgesics, antiemetic, or other medications. Preexisting medical problems, tumor, immune- suppression, hepatitis viruses and other infections, and nutritional deficiencies or total parenteral nutrition all may affect a host's susceptibility to liver injury, attributing liver damage to a toxic reaction is difficult. (Floyd et al., 2016). The liver serves many metabolic functions, yet quantitative markers for liver function are not available in everyday practice. Estimation of liver injury is therefore indirect, and recognizing the severity of hepatic damage can also be problem (Grigorian and O'Brien, 2014).

2.2. Hepatotoxicity

Hepatotoxicity refers to liver damage caused by chemicals. Liver is a primary organ involved in biotransformation of food and drugs. Hepatic diseases are a major worldwide problem. Hepatic disorders are mainly caused by toxic chemicals, xenobiotic (carbon

tetrachloride, chlorinated hydrocarbons and gases CO2 and O2), anticancer (azathioprine, doxorubicin, cisplatin), immunosuppressant (cyclosporine), analgesic anti-inflammatory (paracetamol, thioacetamide), anti-tubercular (isoniazid, rifampicin) drugs, biologicals (Bacillus-Calmette–Guerin vaccine), radiations (gamma radiations), heavy metals (cadmium, arsenic), mycotoxin (aflatoxin), galactosamine, lipopolysaccharides, etc. Various risk factors for hepatic injury include concomitant hepatic diseases, age, gender, alcoholism, nutrition and genetic polymorphisms of cytochrome P450 enzymes have also been emphasized (Ingawale *et al*., 2014). Acute and chronic liver disease caused specifically by drugs is known as drug-induced liver injury (Hassan, 2022). The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents (Shakir and Saliem, 2021). The signs and symptoms of liver injury are jaundice, abdominal pain, nausea, vomiting and asthenia. They are not specific enough to ascertain a liver disorder. Therefore, confirmation by laboratory liver testing is required (Zhang *et al*., 2020). The liver's role in detoxification, drug metabolism, and excretion of waste products partially explains why many cancer therapies are harmful to the liver (Grigorian and O'Brien, 2014). However, the specific mechanisms underlying hepatotoxicity of many agents remain to be elucidated. Various therapies are associated with liver toxicity, including irinotecan, cisplatin, oxaliplatin, and irradiation, which can manifest in several ways (Torrisi *et al*., 2011). Hepatitis, cholestasis, and steatosis are some of the most frequent toxic side-effects of chemotherapy (Grigorian *et al*., 2014).

2.2.1. Mechanism of Hepatotoxicity

Drugs continue to be taken off the market due to late discovery of hepatotoxicity (Williams, 2018). Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver is susceptible to injury from drugs and other substances. (Garcia-Cortes *et al*., 2020). Fifty-five percentage of blood coming to the liver arrives directly from gastrointestinal organs and the spleen via portal veins that bring drugs and xenobiotics in near-undiluted form. (Abd El-Ghffar *et al*., 2020). Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process.

Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases excessive amount of oxidants that, in turn, injure hepatic cells. Activation of some enzymes in the cytochrome P-450 system such as

CYP2E1 also lead to oxidative stress (Mansouri *et al*., 2018) Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside the liver. This promotes further liver damage (Cai *et al*., 2020). Non-parenchymal cells such as Kupffer cells, collagen-producing stellate cells, and leukocytes (i.e. neutrophil and monocyte) also have a role in the mechanism (Krenkel *et al*., 2019).

Hepatotoxicity is manifested in a variety of patterns, including hepatocellular necrosis, cholestasis, steatosis, ductal injury fibrosis, peliosis hepatitis, and veno-occlusion (Kleiner, 2017). The most common pattern of hepatotoxicity is hepatocellular injury (Schmeltzer *et al*., 2016). Hepatocellular injury results directly from damage to intracellular functions or membrane integrity or indirectly as a byproduct of immune-mediated collateral damage. These insults present as a clinical spectrum including asymptomatic mild transient transaminitis, veno-occlusion, fulminant hepatic failure, and neoplastic transformation. Chemotherapeutic hepatic injury occurs principally in an idiosyncratic manner Fortunately, hepatocellular injury is generally reversible and non fata (Andrade *et al*., 2019).

2.3. Liver enzyme

The accompanying biochemical picture in a patient with symptoms or signs suggestive of liver disease, toxicity, or hepatic failer, or an isolated, unexpected finding in a patient who has had a wide range of laboratory tests for a non-hepatic disease or for minor, vague complaints, could be liver enzyme alteration (Giannini *et al*., 2005).

2.3.1. Aspartate Aminotransferase:

Aminotransferases, also known as transaminases, are enzymes that catalyze the transfer of amino groups between amino acids and -keto acids. The two aminotransferases of greatest clinical significance are aspartate aminotransferase (AST), formerly glutamate oxaloacetate transaminase (GOT, GOT activity of serum is called sGOT), Transaminase enzymes transition the -amino group of an amino acid to a -keto acid (Tabrizi *et al*., 2012). Aminotransferase is mostly present in the liver, although it is also found in modest levels in the heart, muscle, and kidney. Many physiological tissues, AST is released into the circulation when body tissues or organs such as the liver or heart are damaged. (Huang *et al*., 2006).

Although the epidemiology of ALT and AST are both reported because they are commonly measured together, all subsequent descriptive and prognostic analyses were limited to ALT because this enzyme is known to be a more sensitive and specific marker of liver disease than AST, which is also found in skeletal and cardiac muscle (Ambrosy *et al*., 2013). ALT is only found in the cytoplasm and cytosol, on the other hand both mitochondria and cytoplasm contain AST. The two isoforms of AST, one found in mitochondria and the other in the cytosol, are immunologically separate and unique isoforms. Aminotransferase has a half-life of around 17 hours, whereas ALT has a half-life of about 47 hours. Aminotransferase and ALT play a role in gluconeogenesis by accelerating the transfer of amino groups from aspartate and alanine to -keto glutaric acid to create oxaloacetate and pyruvate, respectively, as well as glutamate (Nsiah *et al*., 2011).

2.3.2. Alkaline Phosphatase:

Alkaline phosphatase (ALP) is a group of closely related enzymes,It's a homodimeric enzyme with a molecular weight of approximately 160 kDa and each active site region contains three metal ions, two zinc and one magnesium ion, all necessary for enzymatic activity (Sharma et al.,2014).

In nature alkaline phosphatases are found in many organisms, both prokaryotes and eukaryotes. The enzymes are present in bacteria and fungi, are relatively abundant in fish and mammals although they are absent from higher plants, This enzyme is present in many tissues, with highest concentrations in liver and biliary tract epithelium, bone, intestinal mucosa, and placenta,Alkaline phosphatases are a family of isoenzymes,They hydrolyze a variety of organic phosphate esters transferring phosphate groups from a donor substrate to an acceptor containing a hydroxyl group. The active center of the enzyme contains a serine residue; the catalytic mechanism involves the formation of a serine phosphate at the active site which reacts with water at alkaline pH to release inorganic phosphate (Pi) from the enzyme (Golub and Boesze-Battaglia, 2007).

Alkaline phosphatase act on large variety of physiologic and non-physiologic substances, Though the precise natural substrate of alkaline phosphatase in the body is not known, it is associated with calcification and mineralization process in bone and probably in lipid transport in the intestine (Srivastava and Chosdol, 2007). In

mammals several functions can be recognized according to its distribution throughout the various tissues. Bone alkaline phosphatase forms an important part in ossification with two possible roles being proposed:

- The precipitation of calcium phosphate is induced by the localized production of high concentrations of Pi due to phosphatase activity.
- The enzyme allows crystal growth at nucleation sites in the matrix by ensuring the continuous removal of PPi which is considered a crystal poison.

In tissues with high concentrations of alkaline phosphatase, like intestine, kidney and placenta, the enzyme location at the absorptive surface suggest a direct role in the transport of nutrients across the epithelial membrane (Badgu and Merugu, 2013).

2.3.3. Gamma-Glutamyltransferase:

Gamma-glutamyltranspeptidase is located mainly in liver cells, to a lesser extent in kidney, and in much smaller quantities in biliary tract epithelium, intestine, heart, brain, pancreas, and spleen. GGT activity is usually higher in males than in females (Crook, 2012).

Gamma-glutamyltransferase (GGT) is a cell-surface protein contributing to the extracellular catabolism of glutathione (GSH). The enzyme is produced in many tissues, but most GGT in serum is derived from the liver. In the serum, GGT is carried primarily with lipoproteins and albumin, Serum levels of GGT are determined by several factors: alcohol intake, body fat content, plasma lipid/lipoproteins and glucose levels, and various medications, The serum determination of gamma-glutamyltransferase (GGT) activity is a low-cost, highly sensitive and accurate, and frequently used laboratory test, Gamma-glutamyl transferase (GGT) is an important predictor of all-cause mortality, development of cancer, diabetes, and cardiovascular disease (Emiroglu *et al*., 2010).

GGT has been regarded as a clinical marker for free-radical formation and proinflammation,GGT-related pathomechanism is that GGT enhances the availability of cysteine to promote intracellular glutathione, the principle thiol antioxidant in humans, and resynthesis, thereby counteracting oxidant stress. Certainly elevations of serum GGT belong on the list of biomarkers linked to the metabolic syndrome (Lee *et al*., 2013).

2.4. Chemotherapy Drug

Chemotherapeutic agents, alone or in combination, may cause hypersensitivity reactions or direct hepatic toxicity, and altered liver function may alter drug metabolism and cause an increased risk of non-hepatic toxicity. Guidelines on dose modification in hepatic disease are largely empiric (Mudd and Guddati, 2021). Other categories of chemotherapy drugs, include Cytarabine, 5-flurouracil, methotrexate, vincristine, vinblastine, bleomycin, doxorubicin, and daunorubicin (Weber *et al*, 2015).

2.5. Hepatotoxicity with Chemotherapy

The liver plays a key role in the metabolism of a variety of drugs and toxins and thus is especially susceptible to damage inducing by drugs including cytotoxic chemotherapy regimens.drug induced liver injury can exhibit a multiplicative effect in which previous damage can feed-forward resulting in impaired drug metabolism and further toxicity. Hepatotoxicity can result from damage to structures such as the liver sinusoids, vasculature, bile ducts, and direct damage to hepatocytes themselves. Additionally, occlusion of vascular and ductal structures, toxic metabolite formation, and inflammatory cell infiltration into the liver parenchyma can induce damage. As a result, proper monitoring and strategies such as discontinuation or dose-modification of pharmacologic agents is commonly required when hepatotoxicity occurs (Hoofnagle and Björnsson, 2019). General mechanisms of anticancer therapy hepatotoxicity as in figure (2-1).

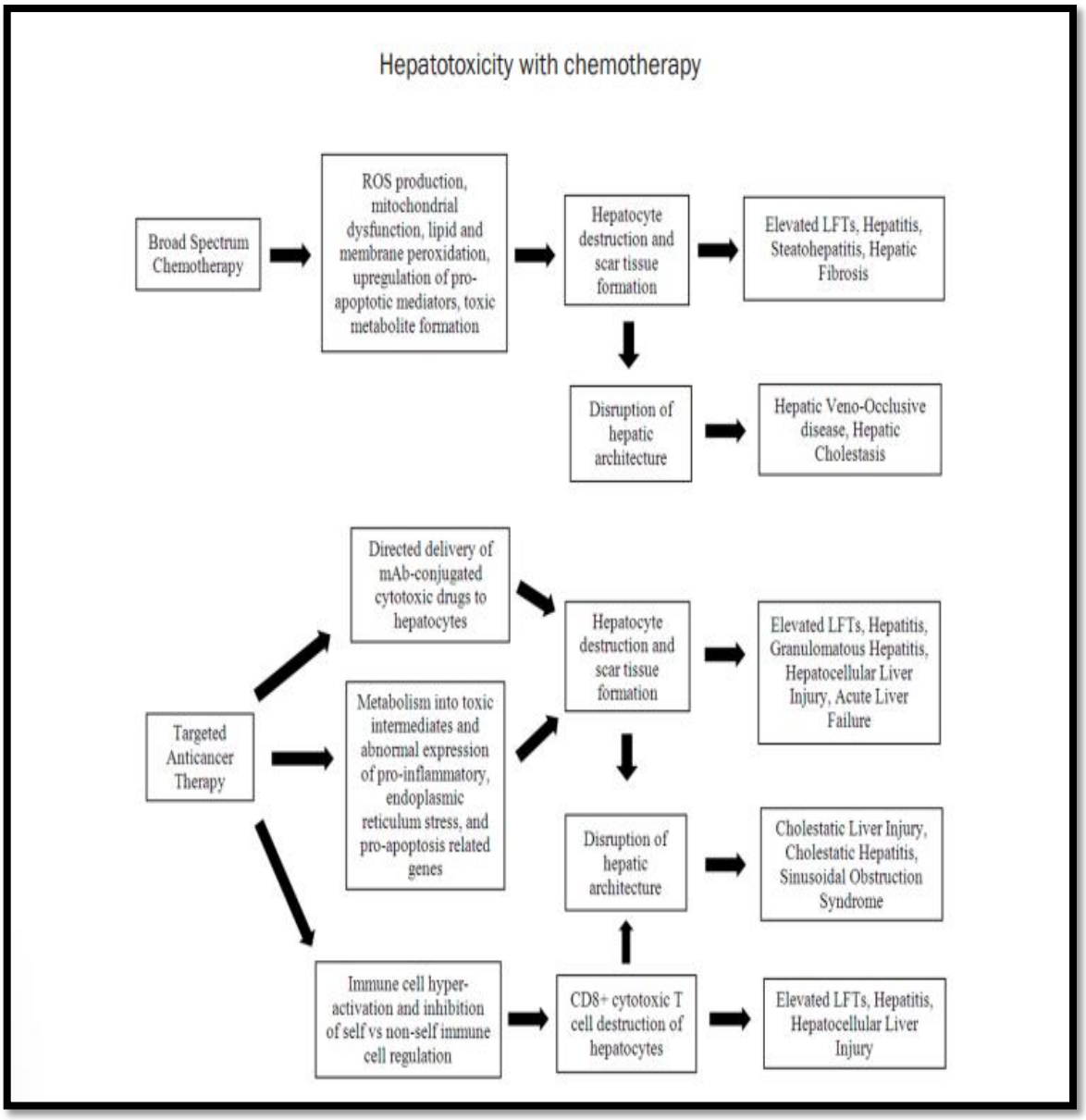

Figure (2-1): General mechanisms of anticancer treatment hepatotoxicity (Mudd & Guddati, 2021).

A wide variety of drugs exhibit hepatotoxicity that must be monitored for including, but not limited to, drugs such as acetaminophen, amiodarone, amoxicillin-clavulanate, and the statins. Additionally, conventional chemotherapeutic agents have well characterized hepatotoxic effects with some of the most common identified agents including methotrexate, irinotecan, and oxaliplatin (Björnsson, 2016).

Hepatotoxicity related to administration of chemotherapeutic agents includes elevations of liver function tests (LFTs), drug-induced hepatitis, veno-occlusive disease, steatohepatitis, as well as potential chronic manifestations such as fibrosis and liver failure. Typically, chemotherapy induced hepatotoxic can be properly managed via close

monitoring for elevations in LFTs that are suggestive of liver injury and dose reductions, with discontinuation of the offending agent if LFT recovery to normal levels is refractory to dose reduction (King & Perry, 2001).

Further complications to treatment strategy and management results from the complex nature of cancer patients, as many present with additional conditions including previous liver injury. In the cases in which liver function is already compromised, care must be taken to properly adjust drug dosages and to tailor therapy regimens correspondingly (Periáñez-Párraga *et al*., 2012).

Classes of chemotherapies that cause hepatotoxicity include the antitumor antibiotics, alkylating agents, platinum agents, antimetabolites, antimicrotubular agents, and topoisomerase inhibitor. These targeted agents include anti-HER-2 therapies, targeted small molecule inhibitors such as VEGF and tyrosine kinase inhibitors, immune checkpoint inhibitors (ICI), and chimeric antigen receptor T (CAR-T) cells (Mudd & Guddati, 2021).

2.6. Cytarabine

Cytarabine, commonly known as Ara-C, is a chemotherapy agent used primarily for the treatment of hematological cancers, such as non-Hodgkin lymphoma and acute myeloid leukemia. This agent was first discovered in the 1960s in Europe, and it was not approved for use in the US until nine years later, in 1969 (Houshmand *et al*., 2020). Figure (2-2) structure of cytarabine.

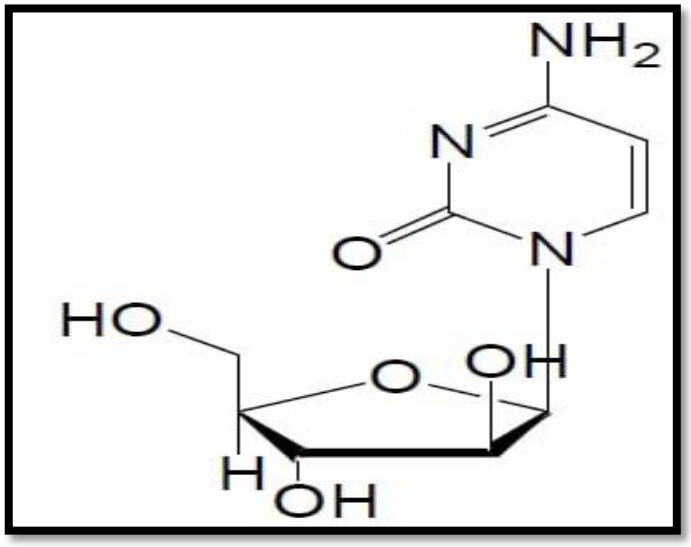

Figure (2-2): Structure of cytarabine(El-Subbagh & Al-Badr (2009)

Ara-C is used in standard induction regimens and consolidations/maintenance therapy after remission (Lombardo & Nichols, 2009). The drug "is phosphorylated to nucleotide" form, 1-β-Darabinofuranosyluracil (Ara-U) by the pymidine nucleoside deaminse (cytidine aminohydrolase), which damages DNA during the S phase of the cell cycle (Guerrero-Bustamante *et al*., 2021). It also inhibits both DNA and RNA polymerases and nucleotide reductase enzymes which are needed for DNA synthesis. All cells rapidly dividing , cancerous or normal, which need DNA replication for mitosis, are the most affected by the drug**.** Ara-C have shown that it is "active only against dividing cells, and does not cause the death of nondividing cell in vitro, even at extremely high concentrations (up to 2500 ug/ul)" **(**Lombardo & Nichols, 2009).

2.6.1. Cytarabine's Cellular Metabolism and Clinical Pharmacology

Ara-C is administered in the body through continuous intravenous (i.v.) infusion. The drug is metabolized in the liver, kidney, GI mucosa, and granulocytes. The drug, when administered intravenously, has an initial distribution half-life of about ten minutes. During the initial half life, the majority of the drug is metabolized in the kidney, liver and gastrointestinal tract into its inactive metabolite, uracil arabinoside (Chamberlin & Nichols, 2009). The secondary elimination half-life is longer, and lasts one to three hours. The majority of the dose administered is excreted via the kidney within one day.

This chemo agent is also very toxic to the human body, as well as to mouse models. Some toxic effects of the drug are: leucopenia, thrombocytopenia, anemia, GI tract abnormalities, fever, conjunctivitis, and pneumonitis (Raj *et al*., 2021).

Due to its hydrophilic properties, cytarabine requires transport into the cells and subsequent intracellular metabolic activation through sequential phosphorylation up to the cytotoxic triphosphate active form, which is incorporated into DNA, as false precursor in place of deoxycytidine triphosphate. This results in inhibition of DNA polymerase, chain termination and stalling DNA and RNA synthesis with the consequent blockage of the cell cycle from G1 to the S phase and neoplastic cell death (Kufe and Spriggs, 1985; Galmarini *et al.,* 2001).

2.6.2. Mechanism of Cytarabine

Cytarabine enters cells via nucleoside transport proteins, the most important one being the equilibrative inhibitorsensitive (es) receptor (ABC). Once inside the cell, cytarabine requires activation for its cytotoxic effects. The first metabolic step is the conversion of

cytarabine to cytarabine monophosphate by the enzyme deoxycytidine kinase (DCK). Cytarabine is subsequently phosphorylated to cytarabine diphosphate and cytarabine triphosphate, respectively. Cytarabine triphosphate is a potent inhibitor of DNA polymerases, which, in turn, interferes with DNA chain elongation, DNA synthesis, and DNA repair. In addition, cytarabine is incorporated directly into DNA and functions as a DNA chain terminator, interfering with chain elongation. Catabolism of cytarabine involves two key enzymes, cytidine deaminase (CDA) and deoxycytidyne monophosphate deaminase (DCMD). These breakdown enzymes convert cytarabine and cytarabine monophosphate into the inactive metabolites, uracil arabinoside and arabinosyluracil monophosphate, respectively. Other catabolic enzymes that may affect cytarabine metabolism include pyrophosphatase, 5-nucleotidase. The balance between intracellular activation and degradation is critical in determining the amount of drug that is ultimately converted to cytarabine triphosphate and, thus, its subsequent cytotoxic and antitumor activity.

The integral drug uptake depends on the proper balance of the nucleoside transporters and drug efflux proteins presented on cellular membranes. Therefore, the drug accumulation may be substantially reduced when the expression of hENT1 transporter is deficient, or the activity of ABC drug efflux transporter proteins is elevated. Cytarabine influx into the cells is strongly correlated with the cell surface abundance of hENT1 transporters, (Gati *et al.,* 1997) so that these membrane proteins are pharmacological determinants for drug bioavailability and response to treatment (Zhang *et al.,* 2007),

2.6.3. Pharmacokinetics of Cytarabine

The absorption of cytarabine is not effective when taken orally due to high first-pass metabolism. When administered subcutaneously, intrathecally, or intravenously, it has a high bioavailability. It has a low plasma protein binding capacity and thus a high volume of distribution. It can cross the blood-brain barrier and hence has an off-label use in primary CNS lymphomas. Cytarabine metabolism primarily occurs in the liver. It has an active metabolite- azacytidine triphosphate; and an inactive metabolite- uracil arabinoside, which is cleared by the kidney (Faruqi & Tadi, 2020).

2.7. L-carnitine:

L-carnitine is a water-soluble compound that humans may obtain both by food ingestion and endogenous synthesis from 6-N-trimethyl-lysine,Most L-carnitine is intracellular, being present predominantly in liver, skeletal muscle, heart and kidney. The

organic cation transporter-2 facilitates L-carnitine uptake inside cells. It has been estimated that the total L-carnitine content in the human body is about 300 mg/kg (Gnoni et al.,2020).Approximately 98% of L-carnitine is intracellular, existing either as free L-carnitine or as several species of acyl-carnitine esters, predominantly in the muscle and liver (Adeva-Andany *et al*., 2017). L-carnitine is generally believed to transport long-chain acyl groups from fatty acids into the mitochondrial matrix for ATP generation via the citric acid cycle. Based on Warburg´s theory that most cancer cells mainly depend on glycolysis for ATP generation, L-carnitine treatment could lead to disturbance of cellular metabolism and cytotoxicity in cancer cells (Huang *et al.,* 2012).

2.7.1. Synthesis

L-carnitine is synthesized from the substrate 6-N-trimethyl-lysine. Lysine residues in some proteins undergo N-methylation using S-adenosylmethionine as methyl donor, forming 6-N-trimethyl-lysine residues, as in figure (2-3). It is generally assumed that 6-N-trimethyl-lysine is generated by degradation of proteins and converted to L-carnitine in four enzymatic steps, namely hydroxylation at carbon 3, aldol cleavage, oxidation of the aldehyde to 4-butyrobetaine and hydroxylation of 4-butyrobetaine at carbon 3 (Maas *et al*., 2020). The enzyme trimethyl-lysine dioxygenase (trimethyl-lysine 3-hydroxylase) catalyzes the hydroxylation of trimethyl-lysine at carbon 3 to yield 3-hydroxy-trimethyl-lysine. During this reaction, 2-oxoglutarate (a-ketoglutarate) is converted into succinate, and carbon dioxide is released as in figure (2-3). The human gene encoding trimethyl-lysine dioxygenase (TMLHE) maps to Xp28 (Wang *et al.*, 2019).

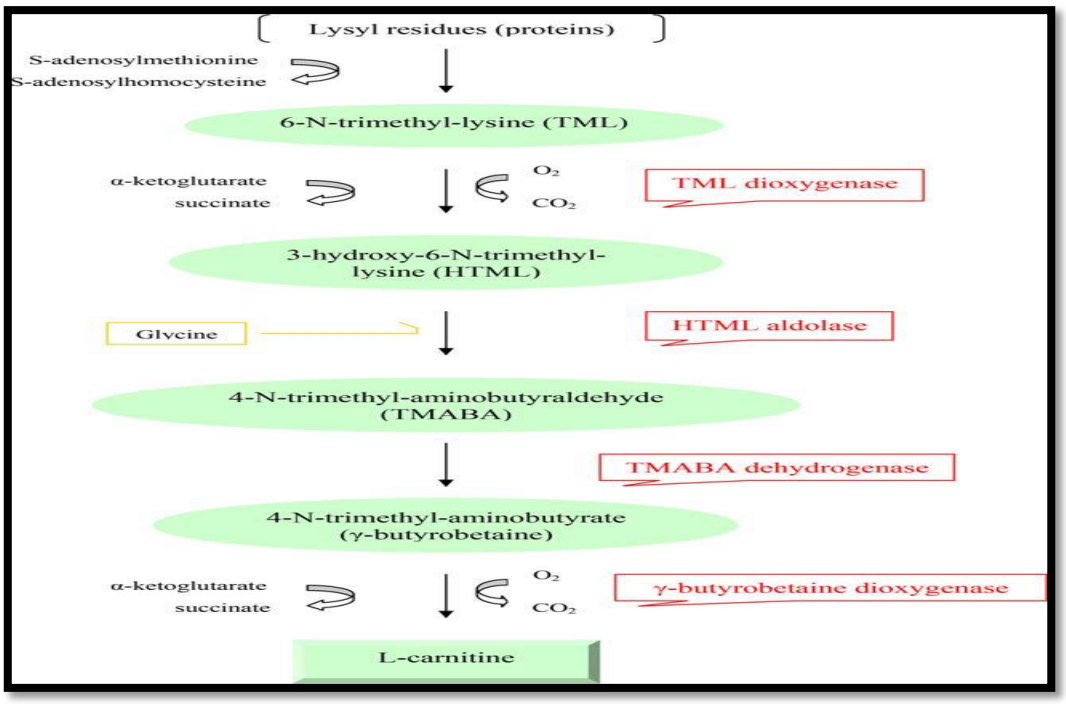

Figure (2-3): Pathway of L-carnitine synthesis (Adeva-Andany *et al.*, 2017)

2.7.2. Role of L-carnitine in the Body:

2.7.2.1 Transport of Long-Chain Fatty Acids into the Mitochondrial Matrix:

The essential function of carnitine is to transport long-chain fatty acids (LCFAs) from the cytoplasm to the mitochondrial matrix for subsequent degradation by β-oxidation, known as "carnitine shuttle" (Modanloo & Shokrzadeh., 2019). LCFA activation occurs in the cytosol, but the enzymes required to catalyze LCFA oxidation exist in the mitochondrial matrix (Merkel *et al.*, 2000). In this process, LCFA must be first activated into lipoyl-CoA via acyl-CoA synthetase (ACS) (Yan *et al.*, 2015) Then, lipoyl-CoA is transported into the mitochondria. Since the inner mitochondrial membrane is impermeable to lipoyl-CoA , the entry of lipoyl-CoA relies on a shuttle system, which requires carnitine (Merkel *et al.*, 2000).

This process occurs in three main steps:

First, CoA must be transferred from lipoyl-CoA to the hydroxyl group of carnitine to form lipoyl-carnitine. This transesterification is catalyzed by carnitine palmitoyl transferase I (CPT I) in the outer membrane, Second, the lipoyl-carnitine ester enters the matrix by facilitated diffusion through carnitine-acylcarnitine translocase (CACT) located in the inner mitochondrial membrane,In the final step, lipoyl-CoA is

enzymatically transferred from carnitine to intramitochondrial CoA by carnitine palmitoyl transferase II (CPT II). This isozyme, located on the inner face of the inner mitochondrial membrane, regenerates lipoyl-CoA and releases free carnitine into the matrix, Carnitine enters the intermembrane space again via CACT (Li & Zhao., 2021) as shown in figure (2-6).

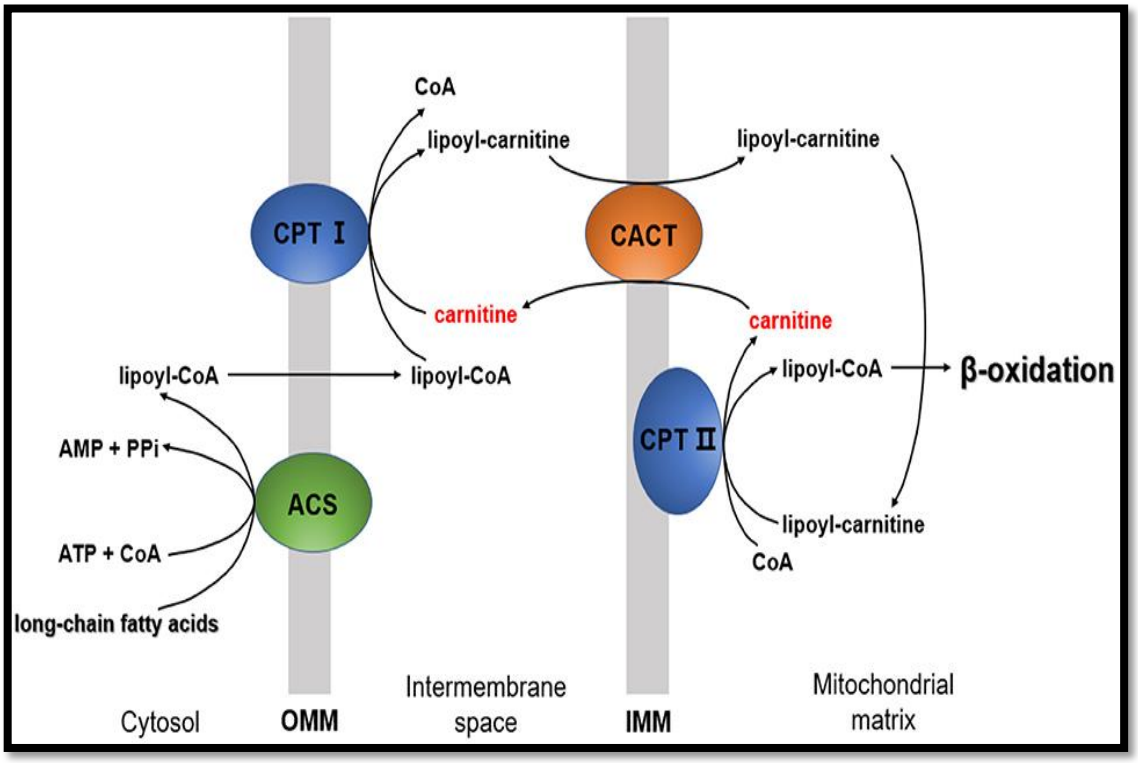

Figure (2-4): The mechanism by which long-chain fatty acids enter the mitochondria. CPT I, carnitine palmitoyltransferase I; CPT II, carnitine palmitoyltransferase II; ACS, acyl-CoA synthetase; CACT, carnitine-acylcarnitine translocase; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane (Li & Zhao., 2021).

2.7.2.2. Regulation of Acetyl-CoA/CoA Ratio:

Carnitine can buffer excess acetyl-CoA in the mitochondria via the formation of acetyl-carnitine (Rinaldo *et al.,* 2008), which requires the presence of carnitine acyltransferase and carnitine acylcarnitine translocase. Acetyl-CoA is either metabolized through the tricarboxylic acid cycle (TCA cycle) or exported as acetyl-carnitine by carnitine. When there is persistent excess or underutilization of certain fatty acids, non-metabolizable acyl-CoAs accumulate. In such situations, carnitine acts as a receiver for these acyl groups by removing them from the tissues and excreting them in the urine (Steiber *et al.,* 2004), or they get separated from carnitine and reused (Savic *et al.,* 2020). Carnitine regulation of acetyl-CoA/CoA reduces the

inhibition of many intramitochondrial enzymes involved in glucose and amino acid catabolism (Tanphaichitr and Leelahagul, 1993).

2.7.2.3. Inter-organellar Acyl Transfer:

Long-chain fatty acids (LCFA) and branched chain fatty acids are oxidized in peroxisomes. In contrast to mitochondrial β-oxidation, incomplete peroxisomal oxidation of fatty acids yields acetyl-CoA and shortened medium-chain acyl-CoAs. In order to completely oxidize these substances into CO_2, the products of peroxisome fatty acid oxidation must be transported to the mitochondria (Steiber *et al.*, 2004).

Since CoA and CoA esters cannot penetrate the cell membrane, they must be converted into their respective carnitine esters by catalase and carnitine octyltransferase (COT) in peroxisomes. Therefore, the carnitine esters are transported from peroxisomes to mitochondria through peroxisome and mitochondrial carnitine-acylcarnitine translocase (CACT), then reconverted into CoA esters by mitochondrial CPT II in the mitochondrial matrix (Wanders *et al.*, 2016). These are then oxidized into CO_2 and H_2O through mitochondrial β-oxidation, TCA cycle, and electron transfer.

2.7.2.4. Reduction of Oxidative Stress:

Carnitine has several protective effects on oxidative stress. These include direct scavenging of free radicals, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide dismutase and hydrogen peroxide, and metal chelation to catalyze free radical formation, such as Fe^{2+}; inhibition of reactive oxygen species-producing enzymes such as xanthine oxidase (XO) and nicotinamide adenine dinucleotide phosphate oxidase (NOX); upregulation of antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) glutathione peroxidase (GPx), heme oxygenase, endothelial nitric oxide synthase, and other protective proteins (Modanloo and Shokrzadeh , 2019).

2.7.3. Effect of L-carnitine on Liver:

L-carnitine has been proposed for treatment of various kinds of disease, including liver injury. Also shown that L-carnitine administration can ameliorate or prevent liver damage of various etiologies (Hanai *et al.,* 2020).

Animal studies showed that dietary supplementation with L-carnitine could prevent hepatitis and subsequent hepatocellular carcinoma (Chang *et al.*, 2005; Al-Rejaie *et al.,* 2009).

In addition, some experimental and clinical data suggested that early intravenous supplementation with L-carnitine could improve survival in severe valproic acid - induced hepatotoxicity (Sato *et al.,* 2020).

Reactive oxygen species (ROS) are considered to be involved in liver damage induced by several conditions such as alcohol abuse, fibrosis/cirrhosis of various etiologies, hepatocellular carcinoma (HCC), ischemia/reperfusion (I/R) liver injury, paracetamol overdose, and viral hepatitis (Muriel, 2009).

L-carnitine protected liver cell membranes against oxidative modifications in ethanol-intoxicated rats through its ability to scavenge free radicals, therefore antioxidant activity of L-carnitine may make it plays a role in the treatment of liver diseases (Dobrzyńska *et al.,* 2010).

Chapter Three: Methodology

3.1. Methodology

3.1.1. Equipment and Instruments:

Equipment and Instruments used in this study shown in table (3-1)

Table (3-1): The equipment and instruments which were used in this study

NO.	Equipment & Instruments	Company	Country
1.	Analytical sensitive balance	Sartorius	Germany
2.	Balance for animals	Shimadu company	Japan
3.	Centrifuge	Hettich Roto fix11	Japan
4.	Digital camera	Toup Cam	China
5.	Electronic Balance	Metter company	Switzerland
6.	ELIZA reader	Bio Kit	USA
7	ELIZA washer	Bio Kit	USA
8.	Freezer	Hitachi	Japan
9.	gel tube	AFMA-Dispo	Japan
10.	Incubator	BINDER	Germany
11.	Latex gloves	Great glove	Malaysia
12.	Light microscope	Leica	China
13.	Plan tube	AFMA- Dispo	Japan
14.	Spectrophotometer	Labomed	UK
15.	Sterile syringes 5ml	PROTON	Malaysia

16.	**Water bath**	**K.F.T.Lab. Equipment**	**Italy**

3.1.2. Chemicals:

Table (3.2)_ The chemicals were used in this study and their sources:

No.	Chemicals	company	Country
1.	ACE Colorimetric. Kit	GMBH	Germany
2.	ALT (GPT) Colorimetric. Kit	Giesse Diagnostics	Italia
3.	AST (GOT) Colorimetric. Kit	Giesse Diagnosti	Italia
4.	CAT	laboratorio ct	Italy
5.	Chloroform	Noorbrok	England
6.	Cytarabine drug (chemotherapy)	SOLARBIO	China
7.	DMSO	LOBA	Chemie
8.	Eosin-Hematoxilin Stain	Merck	Germany
9.	Ethanol	Merck	Germany
10.	Formalin 10 %	TEDIA Company	USA
11.	GGT Colorimetric. Kit	Giesse Diagnosti	italia
12.	L carnitin	Basic Nutition	UK
13.	MDA	laboratorio ct	Italy
14.	Normal saline	Labort	India
15.	Paraffin Wax	Merck	Germany
16.	SOD	laboratorio ct	Italy

17.	Xylole	Scharlau	Spain

3.2. Preparation of Drug Cytarabine :

Cytarabine, commonly known as Ara-C, is a chemotherapy agent used primarily for the treatment of hematological cancers, such as non-Hodgkin lymphoma and acute myeloid leukemia **Cytosar-U®** , were intraperitoneally Injection (Benkessou.,2019). The activistic ingredient, a white crystalline powder with a molecular formula of C9H13N3O5 and a molecular weight of 243.22 g/mole**. cytarabine** 25mg/kg was initially dissolved in10ml distilled water, the cytarabine solution was prepared immediate before use (Namoju & Chilaka 2021).

3.3. Preparation Drug L-carnitine:

L-carnitine powder was dissolved in normal saline, the L-carnitine solution was prepared immediate before use. L-Carnitine (50 mg/kg/bw), and (300 mg/kg/bw) for intraperitoneally injection for 4 weeks (Masoumi-Ardakani *et al.*, 2020).

3.4. Experimental Animals:

The experiment was carried at the laboratory animal's facilities college of Veterinary Medicine College / University of Kerbala. Twenty-four rats (*albino rats*) were used in this study with an average Wight between 280-300 g. These animals were kept in suitable environmental condition with a temperature of around 25-28 C°, relative humility 40٪ - 60٪ conditions room with a 12:12 h/light/dark cycle (Meyer *et al.*, 1982). The animals were housed in aplastic cage with diameters of 50×35×15 cm. The food given was pellets. The animals were kept for at last 15 days for acclimatization before experiment began.

3.5. Experimental Design:

Twenty-four adult male rats were randomly divided into four groups comprising six animals for each group as the following: -

*** First group: (G1)**: Six male rats were injected **normal saline** (0.1 ml, daily, intraperitoneally injection) and served as the control.

*** Second group: (G2)**: Six male rats were injected single concentration of **Cytarabine (25 mg/kg/bw)** intraperitoneally injection (Namoju and Chilaka, 2020).

*** Third group: (G3)**: Six male rats were injected single concentration of **Cytarabinee (25 mg/kg/bw)** intraperitoneally and **L-Carnitine (50 mg/kg/bw)** for intraperitoneally injection for 4 weeks (Masoumi-Ardakani *et al.*, 2020).

*** Fourth group: (G4)**: Six male rats were injected a concentration of **Cytarabinee (25 mg/kg/bw)** intraperitoneally and **L-Carnitine (300 mg/kg/bw)** for intraperitoneally injection for 4 weeks (Masoumi-Ardakani *et al.*, 2020).

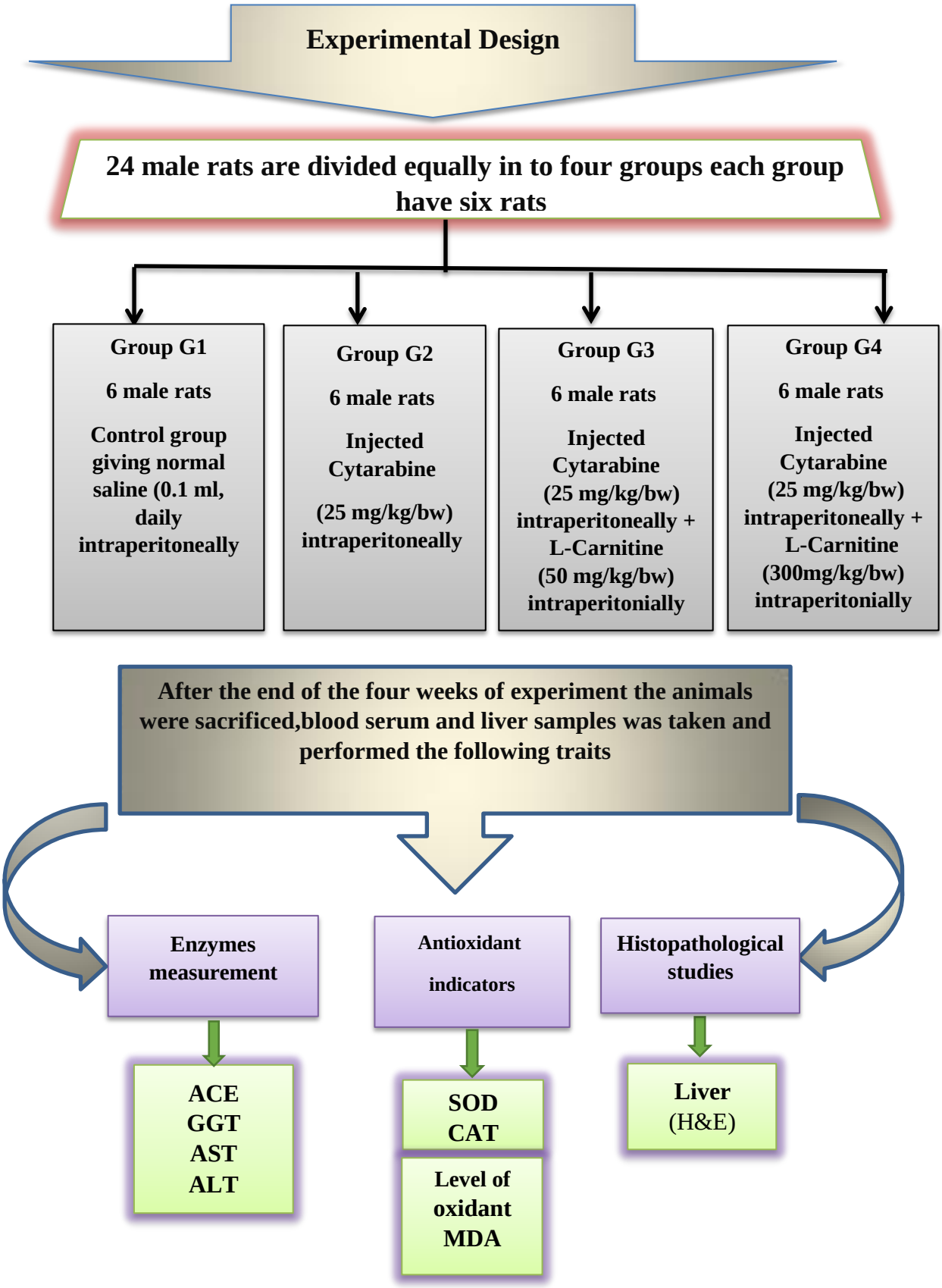

Figure (3-1): Experimental Design

3.6. Preparation and Collection of the Samples:

At the end of the experiment, the animals that were (anesthetized by chloroform inhalation). The chest and abdomen were open by thoracotomy and laparotomy. Followed by collection of blood to perform biochemical and take the liver for histopathological study.

3.6.1. Blood Sample:

Experimental animals (rats) get anaesthetized by putting them in covered jar include cotton rinsed with chloroform to be sedated for the next step which is blood via cardiac puncture in sterile syringes by needle prick in the heart draining 5 ml of blood carefully;

The blood sample (2 ml) set in gel tube without anticoagulant ,it is left about half hour at room temperature for properly agglutinated.

Then it would be separated at centrifuge at 3000 rpm for fifteen minutes to get the serum apart in Eppendorf tube and kept frozen at -20 °C.

3.6.2. Liver Samples

Liver was to be removed and the organs were being fixed in to 10% of formalin for histological examination.

3.7. Methods:

3.7.1. Antioxidant Induces Assessment

3.7.1.1. Determination of Serum Superoxide Dismutase Concentration :

Superoxide dismutase (SOD) activity determination according to Marklund and Marklund, (1974), as shown in Appendix I

3.7.1.2. Determination of Serum Catalayse Concentration:

Hadwan and Abed, (2016). Data supporting the spectrophotometric method for the estimation of catalase activity, as shown in Appendix II.

3.7.1.3. Determination of Serum Malondialdehyde Concentration:

Malondialdehyhe was estimated by Thiobarbituric acid (TBA) assay method of (Buege and Aust, 1978) on spectrophotometer, as shown in Appendix III

3.8. Enzymes Measurements

3.8.1. Angiotensin Converting Enzyme :

Angiotensin converting enzyme (ACE) was estimated by Angiotensin I Converting Enzyme 1 ELISA Kit (Angiotensin I Converting Enzyme Peptidyl-Dipeptidase A), according to Yoshiji *et al.*, (2001), as shown in Appendix IV.

3.8.2. Gama Glutamayl Transferase :

Gama Glutamayl Transferase (GGT) measurements according to Bodewes *et al*., (2015), as shows in Appendix V.

3.8.3. Serum Aspartate Aminotransferase Activity :

Aspartate aminotransferase (AST) concentration was determined by using a special AST Kit (Bio systems, Spain) **(**Schumann *et al.*, 2010)**,** as shows in Appendix VI.

3.8.4. Serum Alanine Aminotransferase Activity :

Alanine aminotransferase concentration was determined by using a special ALT Kit (Bio systems, Spain) (Schumann *et al.*, 2010), as shown in Appendix VII.

3.9. Histological Study:

The animals' anatomy in the laboratory and the organs of rats were dissected (liver) rapidly excised for histological study and rinsed in normal saline then fixed by immersing deeply in a large volume of 10% formalin at least ten times the volume of the tissue as rapidly as was feasible there by keeping postmortem changes at a minimum. The samples were put in a labeled container contain 10% formalin, and shaking of the container gently several times to make certain that the fluid reached all surface and that the pieces were not sticking to the bottom or sides (A shank of glass wool placed in the container will aid in keeping the tissue free of the bottom) according to luna (1968) as shown in Appendix VIII.

3.10. 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 (Green and Salkind, 2016).

Chapter Four: Results and Analysis

4. Results and Analysis

4.1. Effect of cytarabine with 50 & 300 mg/kg/BW L-carnitine on serum liver enzymes in adult male rats:

4.1.1. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum Alanine aminotransferas of adult male rats:

The main value of serum Alanine aminotransferas (ALT) shown a significant increase ($p \leq 0.05$) in G2 group when compared with G1, G3 and G4 groups. While there is no significant ($p \geq 0.05$) difference between G1 and G3 groups. On the other hand, G4 shows a significant ($p \leq 0.05$) decrease when compared to the other groups.

The main value of serum ALT **(U/L)** was (104±7.697, 134.66±13.08, 105.66±5.89, 94.33±3.5) for groups control, cytarabine with 50 & 300 mg/kg/bw L-carnitine respectively (LSD=7.997), figure (4-1).

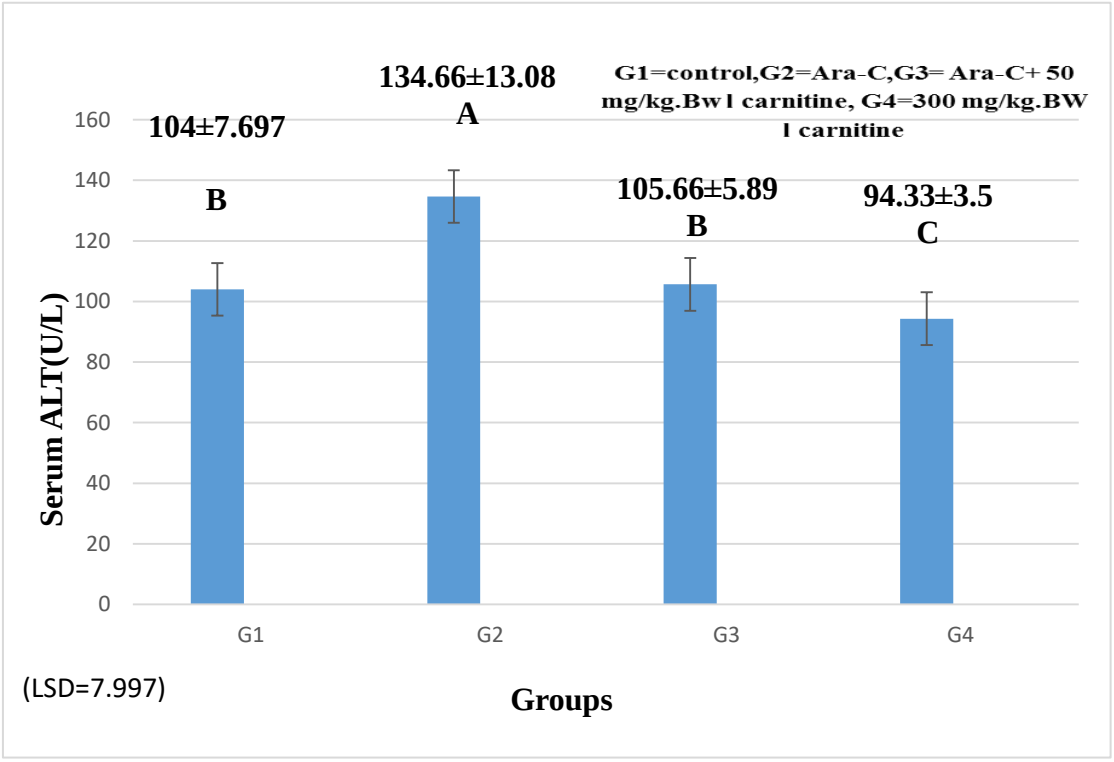

Figure (4-1): Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum ALT(**U/L).**

Capital letters indicate the difference between the groups.

4.1.2. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum Gamma-glutamyl Transferase of adult male rats:

The main value of serum GGT shows a significant increase ($p \leq 0.05$) in G2 group when compared with G1, G3 and G4 groups. While there is no significant ($p \geq 0.05$) difference between G1, G3, G4 groups.

The main value of GGT **(ng/ml)** was (2.66±0.72, 5.6±1.79, 3.66±1.80, 3±1.93) for groups control, cytarabine with 50 & 300 mg/kg/bw L-carnitine respectively (LSD=1.939), figure (4-2).

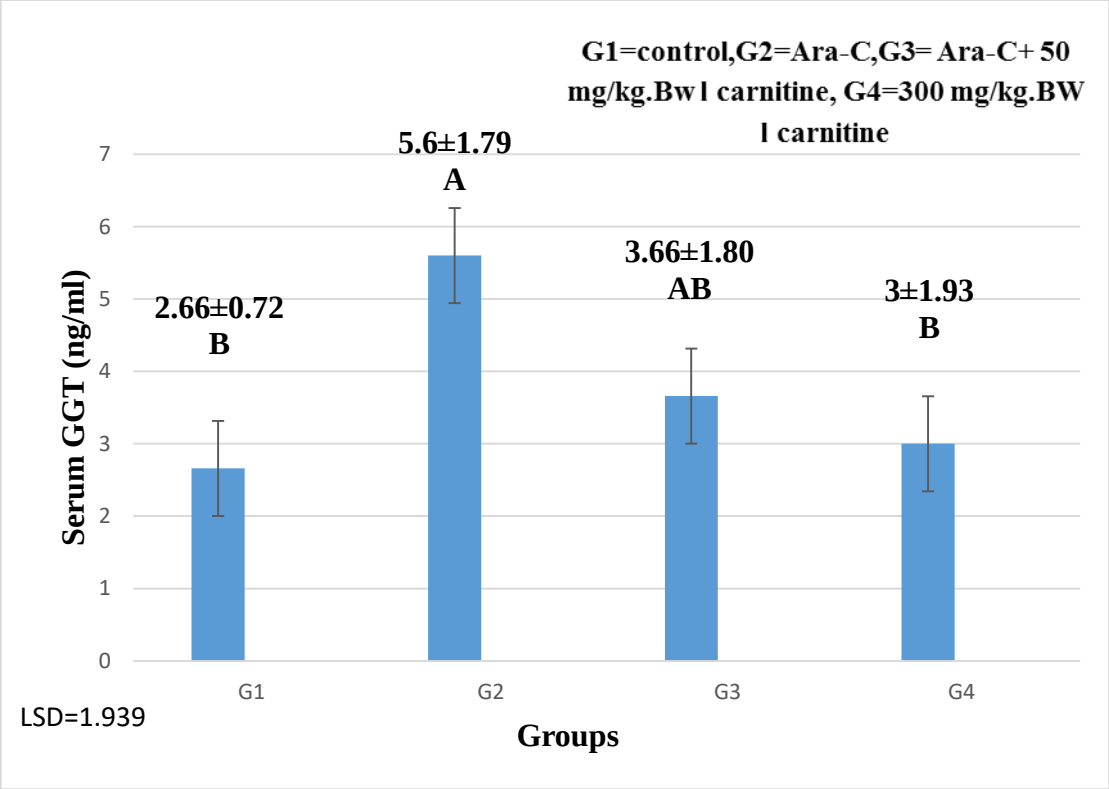

Figure (4-2): Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum GGT(ng/ml).

Capital letters indicate the difference between the groups.

4.1.3. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum Angiotensin Converting Enzyme of adult male rats:

The main value of serum Angiotensin Converting Enzyme (ACE) shows a significant increase ($p \leq 0.05$) in G2 group when compared with G1, G3 and G4 groups. While there is no significant ($p \geq 0.05$) between G3, G4 groups. Also there is a significant ($p \leq 0.05$) decrease in G1 group when compared to the others groups.

The main value of ACE (nmol/mL) was (2.3±0.99, 5.77±0.96, 3.86±0.7, 3.23±0.87) for groups control, cytarabine with 50 & 300 mg/kg/bw L-carnitine respectively (LSD=0.857), figure (4-3).

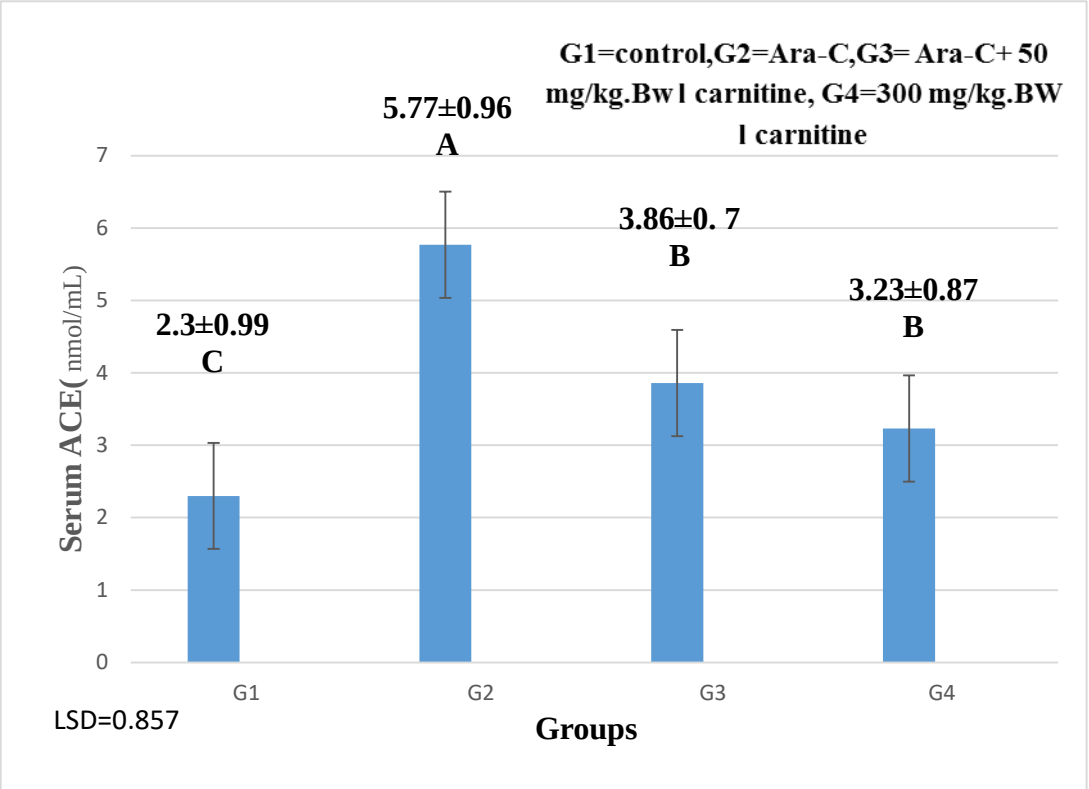

Figure (4-3): Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum ACE(nmol/mL).

Capital letters indicate the difference between the groups.

4.1.4. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum aspartate aminotransferase in adult male rats:

The main value of serum aspartate aminotransferase (AST) shows a significant increase ($p \leq 0.05$) in G2 group when compared with G1, G3 and G4 groups. While there is no significant ($p \geq 0.05$) between G3 and G4 groups. also G1 show a significant decrease ($p \leq 0.05$) when compared to G2 and G3. It shows no significant ($p \geq 0.05$) difference when compared to G4.

The main value of AST (**U/L)** was (39±7.29, 52±3.87, 45.33±3.16, 42±2.44) for groups control, cytarabine with 50 & 300 mg/kg/Bw L-carnitine respectively (LSD=4.406), figure (4-4).

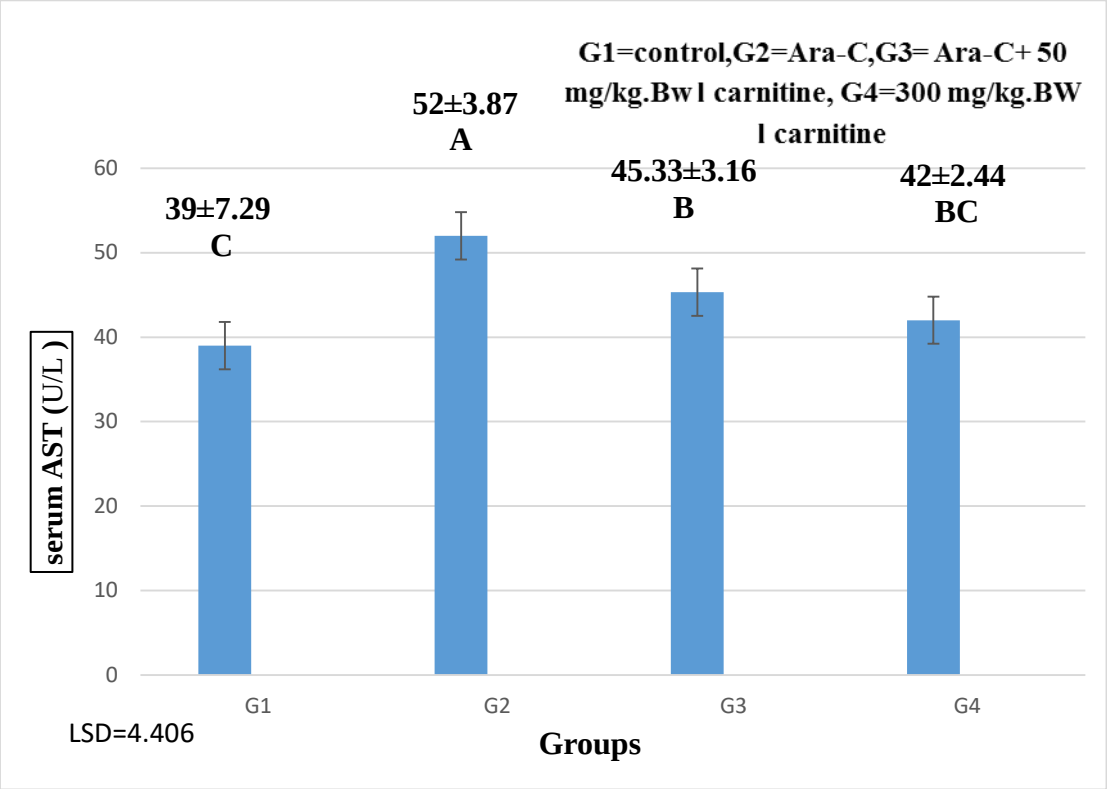

Figure (4-4): Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum AST(**U/L)**

Capital letters indicate the difference between the groups.

4.2. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum antioxidant of adult male rats:

4.2.1. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum catalase of adult male rats:

The main value of serum catalase **(**CAT) shows a significant ($p \leq 0.05$) increases in G4 group when compared with G1 and G2 groups. While there is no significant ($p \geq 0.05$) deference between G4 and G3 groups. The is a significant increase ($p \leq 0.05$) in G1 Group when compared to G2.

The main value of CAT(**U/L)** was (92.23±16.09, 63.04±25.88, 112.33±32.83, 119±15.12) for groups control, cytarabine with 50 & 300 mg/kg/bw L-carnitine respectively (LSD=21.770), figure (4-5).

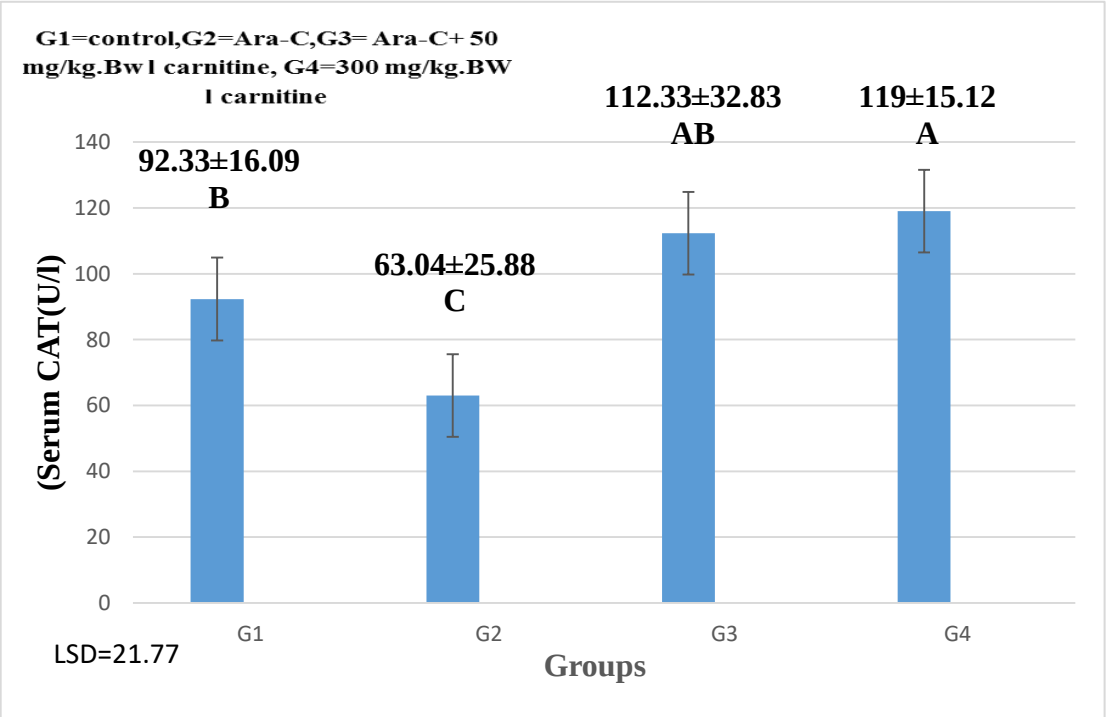

Figure (4-5): Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum CAT(**U/L)**

Capital letters indicate the difference between the groups.

4.2.2. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum superoxide dismutase of adult male rats:

The main value of serum superoxide dismutase **(**SOD) shows a significant ($p \leq 0.05$) increases in G4 group when compared with G1and G2 groups. The is no significant ($p \geq 0.05$) deference between G4 and G3 groups. On the other hand, G1 shows a significant ($p \leq 0.05$) increases when compared to G2.

The main value of SOD(**U/L)** was (51.74±5.60, 40.26±5.53, 55.34±10.84, 61.34±6.76) for groups control, cytarabine with 50 & 300 mg/kg/bw L-carnitine respectively (LSD=7.405), figure (4-6).

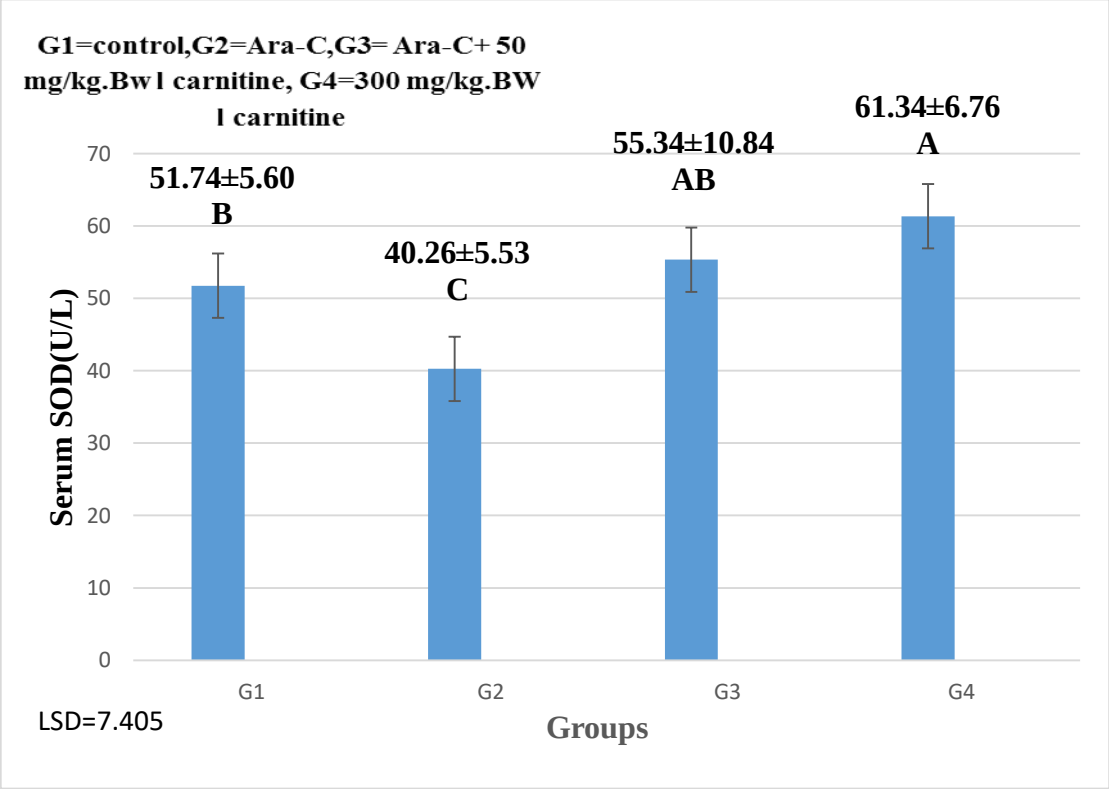

Figure (4-6): Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum SOD(**U/L)**

Capital letters indicate the difference between the groups.

4.3. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum level of malondialdehyde (MDA) of adult male rats:

The main value of serum malondialdehyde (MDA) shows a significant increase ($p \leq 0.05$) in G2 group when compared with G1 and G4 groups. The is no significant ($p \geq 0.05$) difference between G2 and G3 groups. On the other hand, there is no significant ($p \geq 0.05$) between G1, G4 groups.

The main value of serum MDA(**U/L)** was (2.41±1.016, 4.80±1.74, 4.73 ±1.91, 2.46±0.81) for groups control, cytarabine with 50 & 300 mg/kg/Bw L-carnitine respectively (LSD=1.484), figure (4-7).

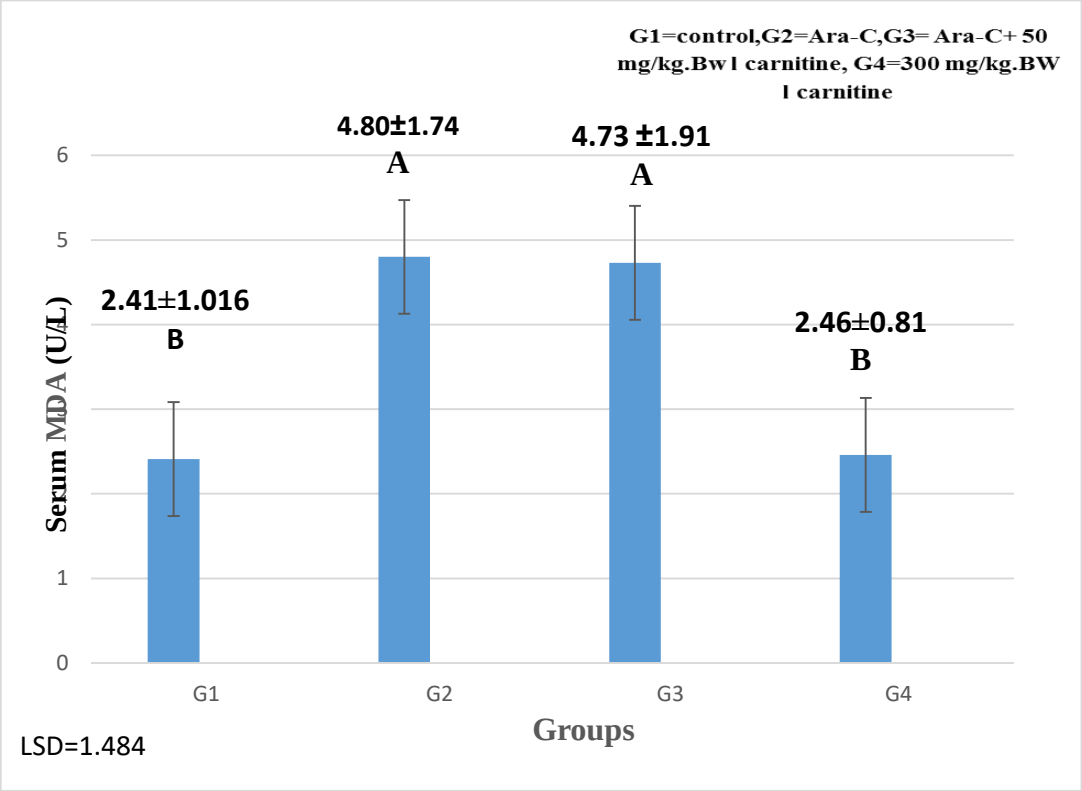

Figure (4-7): Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum MDA(**U/L)**

Capital letters indicate the difference between the groups.

4.4. Histological Study:

4.4.1. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on liver histology.

The liver of the control animals group showed normal histology (figure 4-8) stained with (H&E,10X), and was revealed normal hepatic architecture and normal hepatocytes cords with slight congestion of the hepatic central vein.

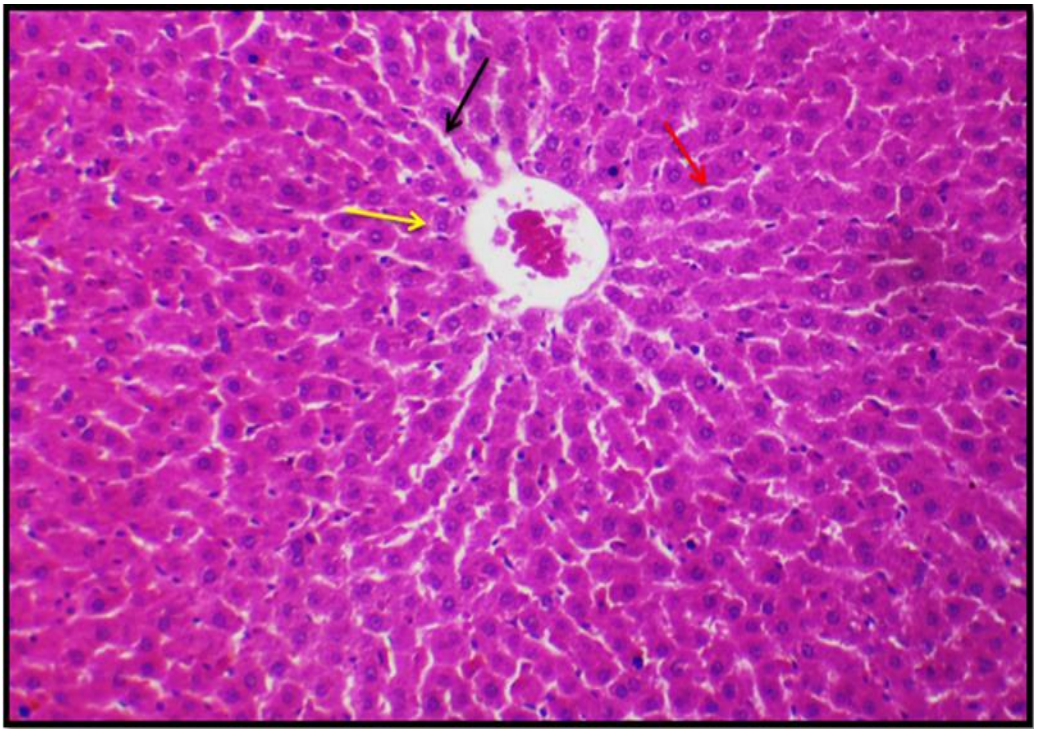

Figure (4-8): histological section of liver in Control group showed the normal histological architecture of hepatic tissue, normal hepatocytes cords (black arrow) radiating around central vein (yellow arrow), significant rounded nuclei with granular cytoplasm (red arrow), (H&E, 10X).

The histopathological examination from the second group of the liver tissue treated with cytarabine revealed that there was sever 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 4-9) stained with (H&E,10X), as compared to the normal histological structure of the liver (Figure 4-8).

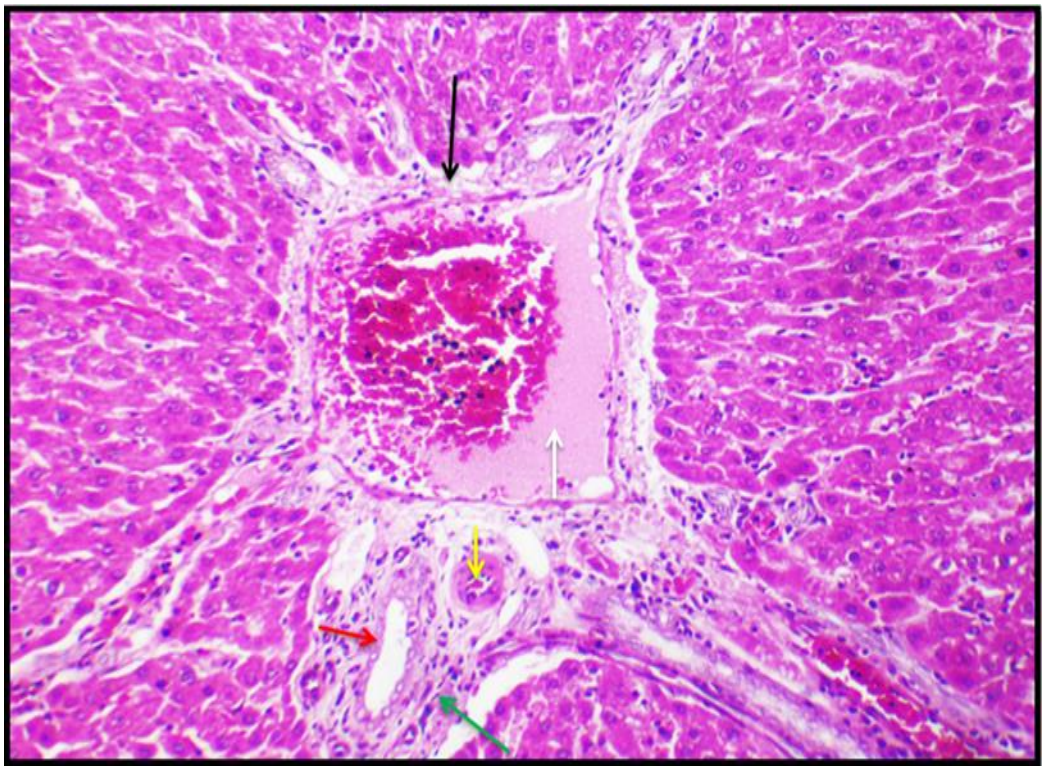

Figure (4-9): histopathological section of liver: Cytarabine treated group showed sever congestion of portal vein (black arrow) with exudation (white arrow), remarkable heavy infiltration of inflammatory cells in portal area (green arrow) with proliferation of biliary ductule (yellow arrow) and hyperplasia of portal arteriole endothelia (red arrow), (H&E, 10X).

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 4-10) stained with (H&E,10X).

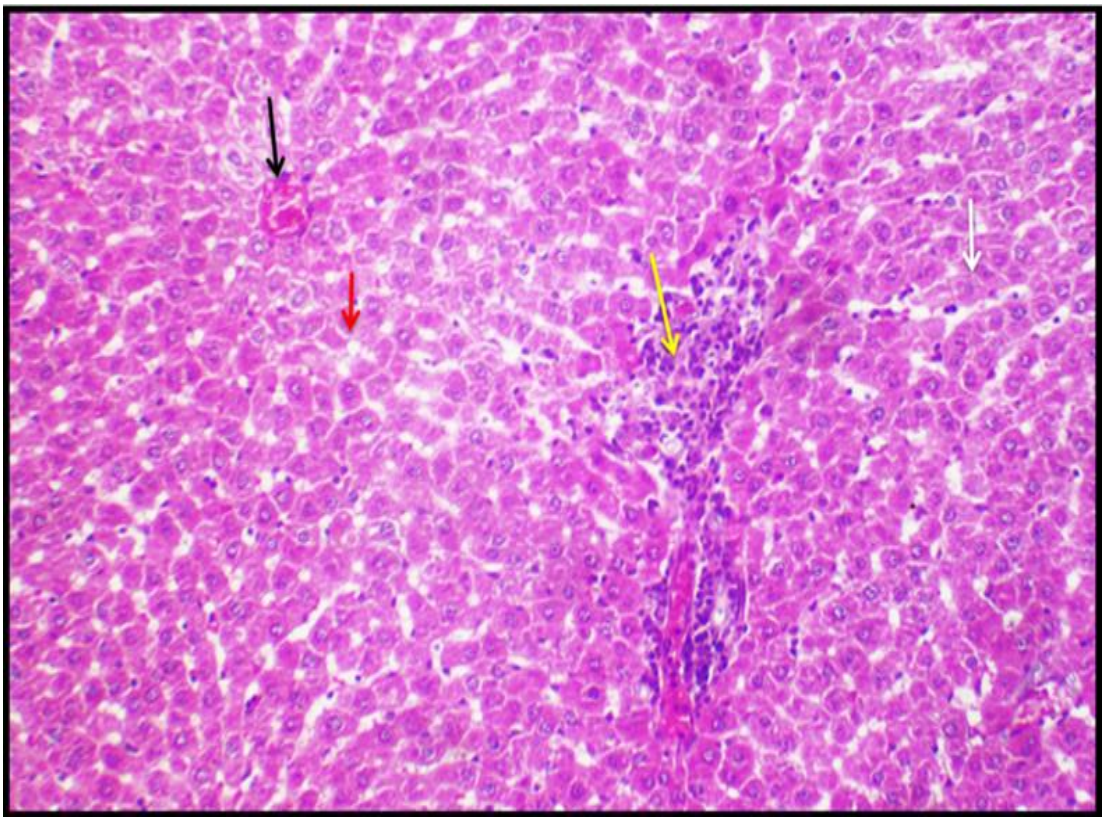

Figure (4-10): histopathological section of liver: Cytarabine + L-carnitine 50 mg/kg/Bw treated group animal showed moderate congestion of central vein (black arrow), congestion in portal area with moderate inflammatory cells infiltration (yellow arrow), remarkable degeneration changes of hepatocytes (white arrow) with keeping the normal arrangements in cords around central vein and some vacillation of hepatocytes (red arrow), (H&E, 10X).

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 4-11) stained with (H&E,10X).

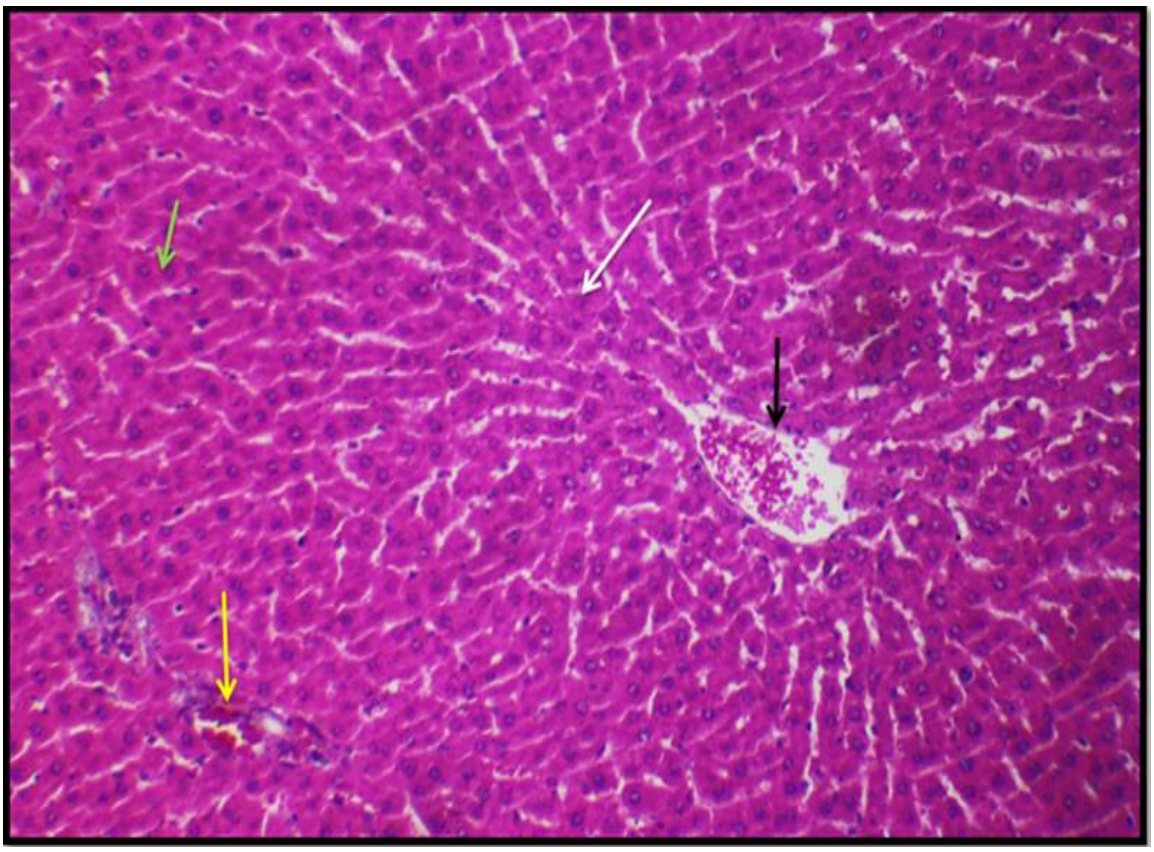

Figure (4-11): histopathological section of liver section: Cytarabine + L-carnitine 300 mg/kg/Bw treated group animal revealed mild congestion of central vein (black arrow) with hepatocytes reverse their regular arrangements (white arrow), remarkable subsides hepatic inflammation (green arrow) with improved to normal portal area (yellow arrow), (H&E, 10X).

Chapter Five: Discussion

5. Discussion

5.1. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum liver enzymes in adult male rats:

In the current study showed a significant differences in cytarabine, treated group as compared with other groups, after intrapertonial administration of cytarabine, showed significant increase in serum liver enzymes activities ALT, AST, GGT, and ACE. The results were consistent with those obtained by Herzig *et al.,* (1983), who studied cytosine arabinoside therapy for refractory leukemia (transient elevations in transaminase, alkaline phosphatase) when they reported that acute leukemia complicated by hyperbilirubinemia due to high dose cytosine arabinoside therapy. Another result was obtained by Pizzuto *et al.,* (1983) who, reported that cytosine arabinoside induced liver damage.

This data agreement with Lala *et al.* (2022), who, found the liver enzymes (ALT, AST, GGT, and alkaline phosphatase) were elevated. The findings agreement with Gustafsson *et al.,* (1998), who discovered hepatic dysfunction and jaundice following high-dose cytosine arabinoside,.also, Tanaka *et al.,* (2007) obtained a similar result when they investigated low-dose cytarabine-induced hepatic and renal dysfunction in a patient with myelodysplastic syndrome.

Sun *et al.,* (2009) confirmed these results, discovering that serious liver damage and pathological changes in the liver were able to alleviate: For starters, the number of white blood cells in the peripheral blood was significantly lower, and there were fewer transplanted K562 leukemia cells.

This results is obtained due to the ability of cytarabine to cause liver damage and liver necrotic and rises the liver enzyme value and decrease the liver ability to syntheses antioxidant (Floyd and Kerr, 2016).

The result of the effect of L-carnitine was a decrease in the plasma concentration of intracellular serum enzymes (ALT, AST, GGT, ACE) and MDA when compared to that in the animals of the control with cytarabine-induced acute drug damage (Dudina *et al.,* 2018).

L-carnitine plays an important role hepatic cells like transporting FAs to the mitochondrial matrix for β-oxidation, regulating acetyl-CoA/CoA, exporting acetyl-

and chain-shortened acyl products from peroxisomes, and reducing oxidative stress. L-carnitine supplementation can normalize or reduce serum levels of liver enzymes, decrease the incidence and severity of NAFLD, and improve both the lipid profile and mitochondrial function (Li & Zhao.,2021).

Farther more, L-carnitine supplementation increased the percentage of hepatic cells in S phase, which was accompanied with the decrease of cells in G2/M phase. These results indicate that L-carnitine can increase cell proliferation, which is consistent with previous findings by (Zhang *et al*., 2020).

5.2. Effect of cytarabine with 50 & 300 mg/kg/Bw L-carnitine on serum antioxidant and malondialdehyde of adult male rats:

The antioxidant ability of the liver was obtained by increasing the activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), while decreasing the contents of malondialdehyde (MDA) in the liver.

In the study found that adding of cytarabine, showed decrease in serum CAT, SOD and increase in MDA levels.

The process of lipid peroxidation is closely related to hepatotoxicity, which causes cell death primarily due to oxidative stress, which is caused by an alteration in the intracellular pro-oxidant to antioxidant ratio in favor of pro-oxidants. Lipid peroxy radicals cause increased cell membrane permeability, decreased cell membrane fluidity, membrane protein inactivation, and mitochondrial membrane polarity loss. (Sies,1997).

In the present study, the level of liver MDA in the cytarabine treated group was significantly higher compared with their levels in the controls. Increasing MDA levels indicated that lipid peroxidation, mediated by ROS, was an important contributing factor in the development of Cytarabin-mediated tissue damage(Bilgin et al.,2020).

The rats treated with Ara-C have shown a dose-dependent and significant reduction in placental GSH, SOD, GPx, CAT and increase in MDA levels as compared to control rats and this agreement with (Esfahani *et al.,* 2012; Khan *et al.*, 2017; Fu *et al.,* 2019 and Namoju and Chilaka, 2020).

Cellular antioxidant defense systems are insufficient to keep ROS levels below a dangerous threshold, oxidative stress ensues. This can be caused by excessive ROS

generation, antioxidant defense failure, or both. (Yildirim *et al.,* 2007). Studies suggest that L-carnitine may play an important role in oxidative/antioxidative balance and has an antiperoxidative effect on several tissues (Cayr *et al.,* 2009; Pehlivan *et al.,* 2009).

L-carnitine is produced from both dietary sources (75%) and endogenous biosynthesis (25%) in the human body (Siktar *et al.,* 2011). Endogenous L-carnitine is synthesized from lysine and methionine primarily in the liver, as well as in the kidneys and brain tissue (Rebouche and Engel, 1980).

On the other hand, L-carnitine 50 mg/kg/BW and 300 mg/kg/bw show a reduced in the ALT, ACE and increase in SOD and CAT where it was found that there is an improvement in the performance of the liver, and this is attributed to the ability of the L-carnitine to improve the physiological condition of the liver, which leads to reducing would the harm resulting from us give cytarabine and thus improve the patient's condition (Li and Zhao, 2021).

Previous researches have shown that L-carnitine has a key role in the oxidative/antioxidative balance as well as the transport of long-chain fatty acids into mitochondria in biological systems (Al-Majed, 2007; Bayraktar *et al.,* 2008).

However, the effects of L-carnitine on certain antioxidant enzymes that detoxify H2O2 in water, such as GPX, CAT, and MPO, have been independently observed in a few studies (Cayir *et al.,* 2009; Cetinkaya *et al.,* 2006).

Cayir *et al*. (2009) investigated the effects of 500 mg/kg (IP) L-carnitine on cisplatin-induced oxidative damage in rat liver and kidney tissues. Their findings revealed that L-carnitine produced considerable protective action in the liver and kidney by lowering MDA levels and increasing GPX activity, implying that this molecule has an antioxidant effect.

Cetinkaya *et al.* (2006) investigated the effects of L-carnitine at a dose of 500 mg/kg (IP) on the oxidant/antioxidant status in acetic acid-induced colitis, finding that L-carnitine administration to the acetic acid-treated rats significantly reduced MDA levels and MPO activity, while CAT activity increased in colon tissue. Unlike Cetinkaya's findings, two separate dosages of given L-carnitine significantly increased MPO activity in liver tissue in our study.

Furthermore, treating hyperthyroid rats for 10 days with both low-dose (100 mg/kg) and high-dose (500 mg/kg) L-carnitine resulted in a significant increase in antioxidant enzyme activities in the liver tissue as compared to levels in the second group of rats, the high-dose L-carnitine group (Hyper+LC500) had higher L-carnitine-induced increases in MPO, GPX, and CAT activities than the low-dose group (Hyper+LC100), but the differences between the groups were not statistically significant (Yildirim *et al.,* 2013).

Derin *et al.* (2004) found that pretreatment with L-carnitine (100 mg/kg, IP) increased tissue catalase activity and protected the gastric mucosa from ischemia-reperfusion injury by decreasing lipid peroxidation via its lipid peroxidation-decreasing activity, which protected the gastric mucosa from ischemia-reperfusion injury.

Previous researches have found that reactive oxygen species (ROS) play a role in xenobiotic kidney and liver toxicity, by raising the activity of the cytochrome P450 enzymes NADPH oxidase, xanthine oxidase, and adenosine deaminase, cytarabine promotes the intracellular synthesis of oxygen and nitrogen species in the liver. However, pretreatment with L-carnitine significantly prevented cytarabine–induced lipid peroxidation in the liver tissues, implicating an antioxidant effect from this molecule. This was probably due to less damage having occurred from oxygen-free radicals (Tozan *et al.,* 2007; Sohn *et al.,* 2008).

Reactive oxygen species readily attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction. The destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for the viability of cells, even tissues. Enzymatic (catalase, superoxide dismutase), since lipid peroxidation is a self-propagating chain-reaction, the initial oxidation of only a few lipid molecules can result in significant tissue damage (Mylonas and Kouretas 1999; Chairuangkitti *et al.*, 2013; Christiansen *et al.*, 2015).

The result of study agreement with finding of Hamza *et al.* (2019), L-carnitine prevented ASP-induced liver damage, the co-treatment of LC showed different improvement mechanisms against ASP-induced liver impairment. So, the intake of ASP should be regulated and taken with L-carnitine when it is consumed in different

foods or drinks to decrease its oxidative stress, histopathology, and genotoxicity of liver.

Studies have reported that dietary L-carnitine supplementation inhibited lipid peroxidation and improved fish resistance to oxidative stress by increasing the activities of antioxidant enzymes, which may be attributed to L-carnitine supplementation promoting lipid hydrolysis, improving cholesterol transport and scavenging excessive ROS. (Mohseni and Ozorio, 2014; Sabzi *et al.*, 2017; Zhou *et al.*, 2020; Chen *et al.*, 2022).

reactive oxygen species(ROS) such as superoxide radical and these various oxidants can promote toxicity by protein oxidation and enzyme inactivation and by damage to cell membranes via lipid peroxidation and production of reactive lipid aldehydes, such as malondialdehyde and 4-hydroxynonenal (Ademowo *et al.*, 2017).

Severe impairment of mitochondrial fatty acid B-oxidation causes microvesicular steatosis, characterized by accumulation of tiny lipid vesicles in the cytoplasm of hepatocytes. Finally, disruption of hepatic mitochondrial b-oxidation decreases delivery of hepatic ketone bodies and glucose to peripheral tissues. The resulting deficiency of energy substrates may cause renal failure, pancreatitis, coma, and death (Fromenty and Pessayre, 1995).

Drug-induced liver injury may manifest a cumulative effect, where in earlier harm may compound to cause impaired drug metabolism and increased toxicity.The hepatotoxicity of many drugs. Dysfunction of these vital cell organelles results in impairment of energy metabolism and an intracellular oxidant stress with excessive formation of reactive oxygen species and peroxynitrite (King and Perry, 2001).

As a result, proper monitoring and strategies such as discontinuation or dose-modification of pharmacologic agents are commonly required when hepatotoxicity occurs.

Research exploring the mechanism of methotrexate related hepatotoxicity identified the activation of inflammatory pathways and cytokines, upregulation of pro-apoptotic mediators, and reactive oxygen species (ROS) formation as contributing factors to liver damage (Mudd and Guddati, 2021).

5.3. Histopathological change in the liver

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 case a necrotic of the cells.

This results agreement with the previous study which showed that hepatic apoptosis and cell necrosis which can lead to acute or chronic hepatic failure (Liu *et al.,* 2018; Taqa and Alnema., 2020). Hepatotoxicity can result from damage to structures such as the liver sinusoids, vasculature, bile ducts, and direct damage to hepatocytes themselves (Mudd and Guddati, 2021).

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 (Jaeschke *et al.,* 1996; Jaeschke and Smith, 1997; Laskin and Laskin, 2001).

Jaeschke *et al.* (2002) 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 significant improvement in the liver histological section in the 50 and 300 mg/kg/bw L-carnitine.

L-carnitine is known to target the anti-oxidation mechanisms of cell damage in the body by serving as free radical scavengers and therefore promoting hepatoprotection (Pehlivan *et al.,* 2009).

Results of histopathological and electron microscopic examination proved the biochemical feedback and the improved L-carnitine effect on liver toxicity (Hamza *et al.,* 2019).

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 (Cetinkaya *et al.,* 2006).

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 (Cayir *et al.,* 2009).

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 (Mudd and Guddati, 2021).

Additionally, damaging to mitochondria, oxidative stress impairs mitochondrial -oxidation, which causes hepatocytes to accumulate fatty acids and causes hepatic damage such 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 (Jaeschke *et al.,* 1996; Jaeschke and Smith, 1997).

Chapter Six: Conclusions and Recommendations

6.1. Conclusions

1- The concentration of L-carnitine 300 mg/kg.BW ameliorating the hypatotoxic effect induced by cytarabine better than 50 mg/kg.BW.

2- Hepatoprotection effect of L- Carnitine 300 mg/kg.BW better than 50 mg/kg.Bw in hypertoxic effect induced by cytarabine by decrease liver enzyme.

3-The hepatoprtective effect of L- carnitine against cytarabine induce hepatotoxicity manifested by increase antioxidant/oxidant balance and decrease liver damage.

6.2. Recommendations

1-Conducting the experiment on other laboratory animals such as rabbits, hamsters…etc

2- Study the effect of L-carnitine with the same dose of cytarabine and study it effect on the kidneys ,brain and other organs.

4-Studying the benefits effect of L-carnitine in preserving DNA from the change that occurs as a result of giving a dose of cytarabine.

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Appendix

Appendices

Appendix I

Determination of Serum Concentration of (SOD)

SOD activity determination

Preparation

1. Tris buffer (pH 8.0): was prepared by dissolving 0.258 gm of tris and 0.111 gm of Ethylene diamine tetra acetic acid (EDTA) in dH2O and completing the volume to 100 ml.

2. Pyragallol solution (0.2 mM): was prepared by dissolving 0.0252 gm of pyragallol with 10 ml of HCl and completing the volume to 100 ml with dH2O.

Procedure

According to Marklund and Marklund, (1974), reaction mix is consisting of 50 µl crude enzyme extract with 2 ml of tris buffer and 0.5 ml of pyragallol (0.2 mM) which absorbs light at 420 nm. Control solution contains the same materials except for the enzyme extract that was replaced by dH2O. As a blank, dH2O was used. Single unit of enzyme is defined as the amount of enzyme that is capable of inhibiting 50% of pyragallol oxidation. SOD activity was calculated using the following equation (Ma *et al.,* 2009):

SOD activity (u/ml) = (Vp-Vs)/(Vp*0.5) *(Vt/Vs) *n

Vp=Auto oxidation rate of pyrogallol rate of pyrogallol (control)

Vs= Auto oxidation rate of sample (with enzyme)

Vt=Total reaction volume (ml)

Vs= volume of enzyme used for the assay (ml)

n= dilution fold of the SOD sample

0.5= factor for 50% inhibition

Appendix II

Determination of Serum Concentration of Catalase (CAT)

Procedure:

Catalase activity was assessed by incubating the enzymes ample in 1.0 ml substrate (65 mmol/ml hydrogen peroxide in 60 mmol/l sodium–potassium phosphatebuffer, pH 7.4) at 37 °C for three minutes. There action was stopped with

ammonium molybdate. Absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at374nm against the blank.

Reagents:

1. Sodium, potassium phosphate buffer (50 mM, pH 7.4): this buffer is prepared by dissolving 1.1g of Na2HPO4 and 0.27g of KH2PO4 in 100 ml distilled water.

2. H2O2 (20 mM) in 50 mmol/l sodium, potassium phosphate buffer: this solution is freshly diluted and standardized daily using a molar extinction coefficient of 43.6 M_1 cm_1 at 240 nm.

3. Ammonium molybdate (32.4mmol/l).

Calculation

The rate constant of a first-order reaction (k) equation is used to determine catalase activity:

t: time.

S°: absorbance of standard tube.

S: absorbance of test tube.

M: absorbance of control test (correction factor).

Vt: total volume of reagents in test tube. Vs: volume of serum.

Appendix III

Determination of Serum Malondialdehyde (MDA) Concentration (µ mol /L):

Malondialdehyde was estimated by Thiobarbituric acid (TBA) assay method of Buege & Aust, (1978) on spectrophotometer.

Principle:

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of Thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm.

Stock TCA – TBA – HCl

Reagent:

It was prepared by dissolving 15% W/V trichloroacetic acid and 0.375% W/V Thiobarbituric acid and 0.25N HCl to make 100 ml (2.1 ml of concentrated HCl in 100 ml). This solution was mildly heated to assist in the dissolution of TBA. Dissolved 15 gm TCA and 0.375 mg Thiobarbituric acid in 0.25 N HCl and volume was made up to 100 ml with 0.25 N HCl.

Procedure:

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added to eliminate centrifugation. This absorbance of pink colour was measured at 535 nm against blank which contained distilled water in place of serum. In blank 0.4 ml distilled water and 0.6 ml TCA-TBA-HCl reagent was mixed and boiled. Blank was always taken.

Calculation:

Extinction coefficient of MDA at 535 nm is = 1.56×105

MDA concentration = χ / 0.0624 nmol / ml.

Appendix IV

Determination of Serum Angiotensin-converting enzyme

Reagent Preparation:

Standards:

Add 1 mL Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.

Note:

If the standard tube concentration higher than the range of the kit, please dilute it and labeled as zero tube.

1. Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 mL of the Sample Dilution Buffer into each tube. Add 0.3 mL of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3 mL from 1^{st} tube to 2^{nd} tube and mix them thoroughly. Transfer 0.3 mL from 2^{nd} tube to 3^{rd} tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.

Note:

It is best to use Standard Solutions within 2 hours.

2. Preparation of Biotin-labeled Antibody Working Solution:

Prepare it within 1 hour before experiment. Calculate required total volume of the working solution: 0.1ml/well x quantity of wells. (Allow 0.1-0.2 mL more than the total volume.

1. Dilute the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 µL Biotin-labeled antibody into 99 µL Antibody Dilution Buffer).

Protocol:

Wash plate 2 times before adding Standard, Sample (diluted at least 1/2 with Sample Dilution Buffer) and Control (blank) wells.

1. Wash plate 2 times before adding Standard, Sample (diluted at least 1/2 with Sample Dilution Buffer) and Control (blank) wells.

2. Add 100 µL standard or sample to each well and incubate for 90 minutes at 37 °C.

3. Aspirate and wash plates 2 times. Add 100 µL Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37 °C.

4. Add 100 µL Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37 °C.

5. Aspirate and wash plates 3 times. Add 100 µL SABC Working Solution into each well and incubate for 30 minutes at 37 °C.

6. Add 100 µL SABC Working Solution into each well and incubate for 30 minutes at 37 °C.

7. Aspirate and wash plates 5 times.

8. Add 90 µL TMB Substrate Solution. Incubate 10-20 minutes at 37 °C.

9. Add 50 µL Stop Solution. Read at 450 nm immediately and calculation.

Appendix V

Gamma-Glutamyl Transferase (GGT)

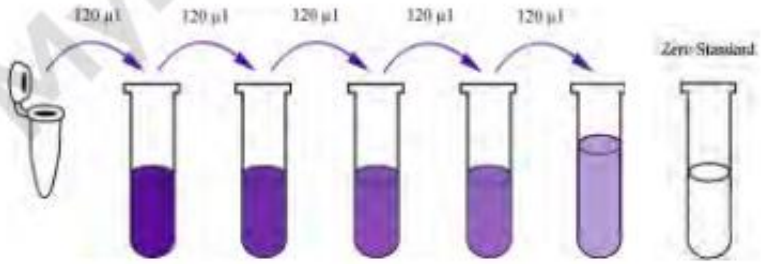
Procedure Test

Reagent Preparation

All reagents should be brought to room temperature before use. Standard Reconstitute the 120 µl of the standard (80 ng/ml) with 120 µl of standard diluent to generate a 40 ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by

serially diluting the standard stock solution (40 ng/ml) 1:2 with standard diluent to produce 20 ng/ml, 10 ng/ml,
5 ng/ml and 2.5ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

40ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
20ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
10ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
5ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
2.5ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
80ng/ml	40ng/ml	20ng/ml	10ng/ml	5ng/ml	2.5ng/ml

Wash Buffer Dilute 20 ml of Wash Buffer Concentrate 30x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Add 50 µl standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.

4. Add 40 µl sample to sample wells and then add 10 µl anti-GGT antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. Add 50 µl Stop Solution to each well, the blue color will change into yellow immediately.

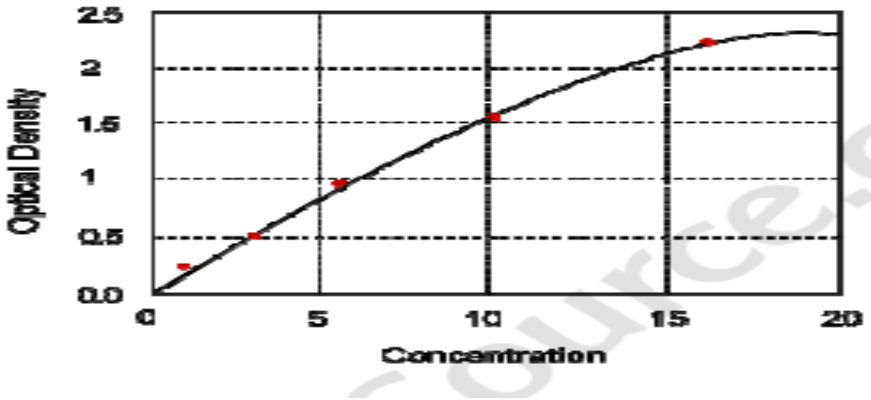
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution

Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.



Appendix VI

Estimation of Biochemical Liver Function Tests

A. Serum Aspartate Aminotransferase Activity (AST):

Aspartate + 2 – Oxoglutarate $\xrightarrow{AST}$ Oxalacetate + Glutamate

Oxalacetate + NADH + H^+ $\xrightarrow{MDH}$ Malate + NAD^+

Procedure Test:

Reaction temperature	37ºC	30°C
Working Reagent	1.0 mL	1.0 mL
Serum	50 µl	100 µl

1. Working reagent was prepared by pouring the contents of the Reagent (B) into the Reagent (A) bottle. And then mixed gently.
2. Working reagent and the instrument were brought to reaction temperature (30 or 37°C).
3. Working reagent and serum sample were pipetted into a tube:
4. The tube was mixed and inserted into the spectrophotometer (340 nm). After 1 minute, the initial absorbance at 1 minute intervals was recorded and thereafter the second absorbance for 3 minutes was recorded.
5. The difference between consecutive absorbance, and the average absorbance difference per minute (ΔA/min) were calculated.

Calculation

Serum AST (GOT) concentration is calculated using the following formula:

$$\Delta A/min \times \frac{Vt \times 106}{\varepsilon \times l \times VS} = U/L$$

The molar absorbance (ε) of NADH at 340 nm is 6300.

Appendices

The light path (l) is 1 cm, the total reaction volume (Vt) is 1.05 at 37°C and 1.1 at 30°C. The sample volume (Vs) is 0.05 at 37°C and 0.1 at 30°C, and 1 U/L are 0.0166 µkat/L

	37ºC	30°C
A/minΔ	×3333= U/L ×55.55= µkat/L	×1746= U/L ×29.1= µkat/L

Appendix VII

Alanine Aminotransferase (ALT) in Serum

Procedure Test:

Reaction temperature	37ºC	30°C
Working Reagent	1.0 mL	1.0 mL
Sample	50 µl	100 µl

1. Working reagent was prepared by pouring the contents of the Reagent B into the Reagent A bottle. And then mixed gently.

2. Working reagent and the instrument were brought to reaction temperature (30 or 37°C).

3. Working reagent and the samples of control and treated groups were pipetted into a tube.

4. The tube was mixed and inserted into the spectrophotometer (340 nm). After 1 minute, the initial absorbance at 1 minute intervals was recorded and thereafter the second absorbance for 3 minutes was recorded.

5. The difference between consecutive absorbance, and the average absorbance difference per minute (ΔA/min) were calculated.

Calculation

The ALT/GPT concentration in the sample is calculated using the following general formula:

$$\Delta A/min \times \frac{Vt \times 106}{\varepsilon \times l \times VS} = U/L$$

The molar absorbance (ε) of NADH at 340 nm is 6300.

The lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.05 at 37°C and 1.1 at 30°C. The sample volume (Vs) is 0.05 at 37°C and 0.1 at 30°C, and 1 U/L are 0.0166 μkat/L

	37ºC	30°C
A/minΔ	×3333= U/L ×55.55= μkat/L	×1746= U/L ×29.1= μkat/L

Appindx VIII

Histological Study:

The animals' anatomy in the laboratory and the organs of rats were dissected (liver) rapidly excised for histological study and rinsed in normal saline then fixed by immersing deeply in a large volume of 10% formalin at least ten times the volume of the tissue as rapidly as was feasible there by keeping postmortem changes at a minimum. The samples were put in a labeled container contain 10% formalin, and shaking of the container gently several times to make certain that the fluid reached all surface and that the pieces were not sticking to the bottom or sides (A shank of glass wool placed in the container will aid in keeping the tissue free of the bottom) according to Al-khalissi, (2014).

Histopathological Technique:

The liver was quickly removed and rapidly weighed then prepared for histological study according to Al-khalissi, (2014), with aid of the light microscope as the following steps:

1. Fixation

The specimen was fixated in the natural buffered formalin 10% for 24–48 hours.

2. Washing and Dehydration

After fixation the specimens were washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later. By dehydration the water had been completely extracted from fragments by bathing them successively in a graded series of ethanol and water (70%, 80%, 90%, and 100% ethanol).

3. Clearing

Bathing the dehydrated fragments in solvent (xylene) for 30–60 minutes; this step was repeated 3 times. As the tissues clearing, they generally became transparent.

4. Infiltration and Embedding

Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52–60 °C. The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin.

5. Sectioning

After hold from the oven the specimen let at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotary microtome and were sliced by the microtome, s steel blade into sections 5 micrometers thick. The sections were floated on water bath (50–55 °C), then transferred into glass slides coated with Mayers albumin as adhesive substance and left to dry.

6. Staining

The histological sections of the studied organs were stained with Hematoxylin– Eosin.

الخلاصة

أجريت هذه الدراسة لتقييم تأثير جرعتين من L-carnitine ضد تسمم الكبد بعد العلاج الكيميائي بالسيتارابين والتغيرات النسجية للكبد في ذكور الجرذان . أجرت الدراسة في جامعة كربلاء / كلية الطب البيطري و تم استخدام أربع وعشرون من ذكور الفئران البالغة في التجربة.

تم تقسم الجرذان بشكل عشوائي إلى أربع مجموعات كل مجموعة تحتوي على ست جرذان ، حقنت المجموعة الأولى من بمحلول فسلجي داخل الصفاق وتعيين كمجموعة تحكم (G1) ، والمجموعة الثانية تم حقنها بمادة السيتارابين (25 ملغ/كغ من وزن الجسم في داخل الصفاق وتم تعينها(G2) ، المجموعة الثالثة حقنت السيتارابين (25 ملغ/كغ/وزن) و (50 ملغ/كغ/وزن) L-carnitine (G3) ، المجموعة الرابعة من السيتارابين (25 ملغ/كغ/وزن و L-carnitine 300 ملغ لكل كلغ و تم تعيينها ك (G4)

أظهرت الدراسة أن استخدام L-carnitine يقلل من التأثير الضار للسيتارابين في الجسم عند مقارنته بالمجموعة التي تم حقنها فقط بمادة السيتارابين عند مستوى (p-value <0.05).حيث أظهرت مجموعة السيتارابين (G2) زيادة معنوية في أنشطة إنزيمات الكبد في مصل الألانين aminotransferase ؛ جاما غلوتامايل ترانسفيراز (GGT) ، وإنزيم (ACE) ،و (AST) ، كما لوحظ ان هناك انخفاض في مضادات الأكسدة سوبر ديسموتاز (SOD) وكاتلاز (CAT) أيضًا ، و زيادة مستوى مالونديالديهايد (MDA) بالمقارنة مع غيرها من المجاميع المختلقة ، أظهرت التجربة أيضًا وجود انخفاض كبير (قيمة p <0.05) في مستوى Alanine aminotransferase (ALT)، gama glutamayl transferase (GGT) ، و الإنزيم (ACE) و (AST،MDA)) وزيادة ديسموتاز الفائق الأكسدة (SOD) والكاتلاز (CAT) في مصل الدم في مجموعة (G4) و (G3).

في الختام ، فإن حقن 300 ملغ/كغم L-carnitine يخفف من اصابة الكبد نتيجة للتسمم الناتج عن حقن السيتارابين أفضل من 50 مجم / كجم L-carnitine ،وذلك لان الL-carnitine يلعب دورا مهما في خفض تركيز انزيمات الكبد في مصل الدم كما يساعد على تنظيم الجهد التاكسدي في الجسم.

الخلاصة

جامعة كربلاء

كلية الطب البيطري

فرع الفسلجة والكيمياء الحياتية والأدوية

التأثير الوقائي لل L_Cartine ضد سمية الكبد المستحدثه بالسترابين Cytrabine في ذكور الجرذان

رسالة مقدمة الى مجلس كلية الطب البيطري / جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في اختصاص علوم الطب البيطري/الفسلجة

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التاريخ الميلادي

2022

التاريخ الهجري

1444