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# RESEARCH ARTICLE

## EFFECT OF GARLIC EXTRACT (ALLIUM SATIVUM) ON HEPATOTOXICITY INDUCED BY GEMCITABINE IN RABBITS (HISTOLOGICAL AND HISTOPATHOLOGICAL STUDY)

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**ABSTRACT**

Gemcitabine (pyrimidine analog 2′, 2′-difluorodeoxycytidine) is a nucleoside analog that has been used as a chemotherapeutic drug for more than 15 years. It was classified as an antimetabolite with many brand names including Gemzar. This drug cannot differentiate between normal cell and malignant cell, so it produces its toxic effect on both cells as the toxic impacts transfer from the tumor cells to the normal cells. Hepatotoxicity is the less well-known aspect of gemcitabine treatment, and there is little information about the underlying mechanism. In this broad context of interest in the potential hepatotoxicity of gemcitabine. Garlic (Allium sativum) has been used as a folk medicine and flavoring agent since ancient time. Accumulating evidences have demonstrated that garlic have numerous beneficial effects for healthy including anti-oxidation, anti-inflammation and anti-cancer. There is limited information on the possible protective effect of garlic extract on gemcitabine induced-hepatotoxicity. Therefore, aim of current study to demonstrate the effect of garlic extract *(Allium Sativum)* on hepatotoxicity induced by gemcitabine in rabbits. Twenty seven adult, healthy male and female local rabbits were used in this work, divided randomly into three equal groups; group (I), animals of control group received normal saline intraperitoneally injection once per week for six weeks; group (II), animals of treated group received 25 mg/kg body weight intraperitoneally injection of gemcitabine once per week for six successive weeks; group (III), served as protective group and was concomitantly treated with garlic extract by oral gavages in a dose of 250 mg/kg body weight and gemcitabine in a dose of 25 mg/kg body weight intraperitoneally injection once per week for six successive weeks. The results of the present investigation showed that gemcitabine toxicity produced significant disturbance in the normal architecture with evidence of structural changes in the liver of group II, gemcitabine treated group. The hepatotoxicity manifested by severely congested blood sinusoids, distortion of hepatocytes and focal necrosis. In addition, some hepatocytes were dissociated from hepatic cords, indicating liver injury. Presence of necrotic and hemorrhagic spots between the liver cells, vacuolation of the cytoplasm of pericentral hepatocytes had been also noticed. From the findings of our present study on domestic rabbits can be considered the liver as a target for gemcitabine, which expose to this compound can cause histological damage on hepatic tissue. Whereas, sections from the liver of animals received therapeutic dose of gemcitabine and treated with garlic extract, group (III), were showed that the severe hepatic lesions induced by gemcitabine were significantly decreased by the treatment with garlic extract. There were no obvious pathological changes, nearly normal liver histology was observed, except mild to moderate congestion and some hemorrhagic spots in central veins. The hepatocytes were appeared similar to normal. Based on our present study that indicated that the garlic extract possesses protective ability against - gemcitabine induced liver injury and might be an effective alternative medicine against acute oxidative liver toxicity. Therefore, the consumption of garlic may provide some kind of protection from cancer development.

## INTRODUCTION

The liver is the largest organ, accounting for approximately 2% to 3% of average body weight. The liver, with its multiple functions, is one of the most important organs.

It plays an active role in metabolism as it secretes bile that breaks down fats in the small intestine during digestion, stores and releases glucose and synthesizes different types of proteins.

In addition, the liver converts harmful ammonia into urea, processes haemoglobin, clears bilirubin, fights infections and detoxifies medicines and other toxic chemicals[1]. Due to the increasing numbers of cancer patients and breaking this out, medical science presents new drugs to market every day, but in spite of having high performance, most treatment of these drugs have side effects and are toxic to other cells and tissues. Among the most effective anti-cancer drugs which treat a variety of malignant tumors is gemcitabine. Previous studies on the effects of toxic side effects in other tissues have been observed during treatment. Application of anticancer drugs causes toxic impacts on healthy cells alongside on tumor cells due to its toxic metabolites[2]. Among the pyrimidine analogs, gemcitabine (2′,2′-difluorodeoxycytidine, dFdC**;** Gemzar®) is one the most widely used drugs in clinical oncology and ranked the third anticancer agent prescribed worldwide[3]. Gemcitabine is classified as an antimetabolite and is believed to act by inhibition of DNA synthesis in rapidly dividing cells[4]. The anticancer and antineoplastic drugs are known to interfere with DNA and its original substances, so they inhibit DNA synthesis and cause irreversible damage to it. Usually these drugs have unpleasant effects on the normal tissues as the toxic impacts transfer from the contiguous tumor cells to them by cellular diffusion or by membrane nucleoside transporters[5,6].

Mergental *et al*., 2005 reported that the gemcitabine has toxic effects on the livers of laboratory rats leading to; necrosis of hepatic cells and dense lymphatic filtration that extend to the parenchymal cells surrounded the distorted portal spaces with dense coexistence of inflammatory cells and damaged bile ductules[7]. Hailan *et al*., 2018 were conducted to demonstrate the cytotoxicity, apoptosis and hepatic damage induced by gemcitabine in laboratory mice[8]. Literatures have shown very strong evidence that consumption of fruits and vegetables can protect against a wide variety of cancers. Most fruits and vegetables are relatively have dietary fibers, rich sources of micronutrients and non-nutrient substances called phytochemicals. These phytochemicals are thought to contribute for their significant protective effect upon some of the most important diseases such as cardiovascular disease, cancer and many more [9]. Recent studies also attempted to revealed that the consumption of vegetables form the allium's family such as onion, garlic and leeks will inhibits stomach, colorectal, and prostate cancers[10,11]. Garlic (Allium sativum) is among the oldest of all cultivated plants. It has been used as a medicinal agent for thousands of years. It is a remarkable plant, which has multiple beneficial effects[12]. It is a widely consumed spice in the world and provides a useful source of new therapeutics[13]. A number of studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations including fresh garlic extract, aged garlic and garlic oil. The chemopreventive activity has been attributed to the presence of organosulfur compounds in garlic[14].

Substantial studies have shown that garlic and its bioactive constituents exhibit antioxidant, anti-inflammatory, antibacterial, antifungal, antithrombotic, antiartherosclerotic, immunomodulatory, cardiovascular protective, antihypertensive, anticancer, hepatoprotective, digestive system protective, anti-diabetic, anti-obesity, neuroprotective, and renal protective properties[15.16]. Garlic seems to detoxify chemical carcinogens and prevent carcinogenesis and can also directly inhibit the growth of cancer cells. A number of population studies demonstrated a relationship between excess garlic intake and reduction in received risks of pancreas, colon, stomach, esophagus and breast cancers [17]. Garlic and its active components can prevent and manage different cancers. These anticancer mechanisms include the regulation of carcinogen metabolism, inhibition of cell growth and proliferation, induction of apoptosis, suppression of angiogenesis, and inhibition of invasion and migration. Garlic can diminish the negative effects of anticancer therapies[16]. Previous studies and researches have been found that most cytotoxic chemotherapeutic drugs have an effect on hepatic tissues. There is paucity of literature regarding the effect of gemcitabine on hepatic tissues. Its effects on their cells have not been reported in literature to the best of our knowledge which motivates us to study the histopathological picture of the gemcitabine influences on these tissues. The expanding prevalence and effectiveness of natural products in the treatment of various disorders led the investigate in countering drug-induced liver toxicity.

## MATERIALS AND METHODS

Twenty seven adult, healthy male and female local rabbits were used, it's approximately the same age and their body weight ranged 1.5 to 2 kg. Animals were housed under optimal environmental condition in the lab of histology department of faculty of medicine, Benghazi University. The rabbits were routinely observed for food consumption, fecal characteristics and any clinical signs might be appeared. The rabbits divided into three equal number of groups (Nine animals each group) group I, group II and group III. The concentration of gemcitabine dose and garlic extract was selected based on previous studies [18,19].

**Group (I):** Animals of control group received normal saline injection, 1ml intraperitoneally once per week for six weeks to simulate the effect of injection.

**Group (II):** Animals of treated group received single therapeutic dose injection of gemcitabine alone in a dose of **25**mg/kg body weight intraperitoneally once per week for six successive weeks [18].

**Group (III):** Animals of this group received a dose of **250** mg/kg body weight [19] of garlic extract by oral gavages, starting from the first day of the experiment for 6 consecutive days before and 6 consecutive days after the gemcitabine injection and continued daily for 6 weeks. In addition, this group was treated with a single (**25** mg / kg of body weight) therapeutic dose of gemcitabine intraperitoneally once per week for six weeks. For histological analysis, after six weeks of experiment, the animals of the experimental and control groups were anaesthetized by ether inhalation, laparotomized, the liver samples were removed and divided into segments. Segments from all groups were placed in 10% formal saline for 48 hours for histopathological assessment. After a period of 48 hours fixation, tissue processing was done by using paraffin technique, the slides were stained with Hematoxylin and Eosin (H&E). Then sections were examined under a light microscope[20].

## RESULTS

**Clinical observations:** The following observations were noticed 60 minutes after injection in the rabbits of group (II) which were injected with therapeutic dose of Gemcitabine:

Excessive sleep, loss of appetite, fearless behavior, decrease movement, rapid breathing, occasional trembling, diarrhea and spasms and finally we were noticed some animals death after second and \ or third doses of drug. The dead animals were replaced by additional rabbits, injecting the same dose of gemcitabine for the desired duration. Mild to moderate clinical signs were noticed in rabbits of group (III), such as loss of appetite, animals were appeared lazy and fearless behavior. No clinical signs were seen in control group (I). There was no mortality among the animals of control group (I) and group (III) (garlic extract+ Gemcitabine).

**Histological and histopathological findings**

**Control group (I):** Cross section in the normal liver of domestic rabbit, control group (I), was consisting of several lobules without boundary lines due to few or absent connective tissue septa between them. Each lobule was composed of the epithelial cells grouped in irregular plates (the hepatocytes) with intervening sinusoids which drain into central vein. At the angles of each hepatic lobule, there was a dense connective tissue spaces (portal spaces), were occupied the portal triads, which was containing branches of hepatic artery, portal vein and bile duct (Fig.1a). The hepatocytes were polyhedral cells with acidophilic cytoplasm and prominent central round nuclei. Binucleated hepatocytes were present in the liver parenchyma. The central vein which was present in the center of each hepatic lobule, was lined by flat endothelial cells and surrounded by small amounts of connective tissue fibers. The sinusoids carry blood from the edges of the lobule to the central vein. The sinusoids had walls formed by a discontinuous lining of fenestrated endothelial cells that lack a basal membrane (Fig.1b).

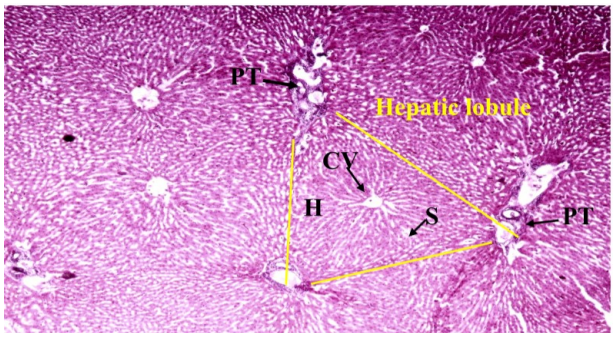


**Figure1a: Photomicrograph illustrates the normal structure of rabbit liver of group (I). Note, central vein (CV), hepatocytes (H), blood sinusoids (S) and the portal triads (PT). H and E. X10**

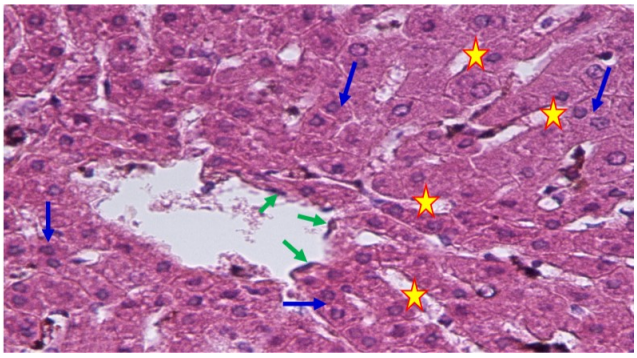

**Figure1b: Photomicrograph illustrates the normal structure of rabbit liver of group (I). Note, polyhedral hepatocytes (blue arrow), central vein lined by flat endothelial cells ( green arrow). And sinusoidal capillaries (stars) H and E. X20.**

**Treated group (II):** Sections from the liver of treated animals with gemcitabine, group (II) revealed disturbance in the normal architecture with evidence of structural changes than those in the control group (I). The group (II) that receiving gemcitabine showed hepatotoxicity manifested by severely congested blood sinusoids, distortion of hepatocytes and focal necrosis. In addition, some hepatocytes were dissociated from hepatic cords, indicating liver injury (Fig.2).

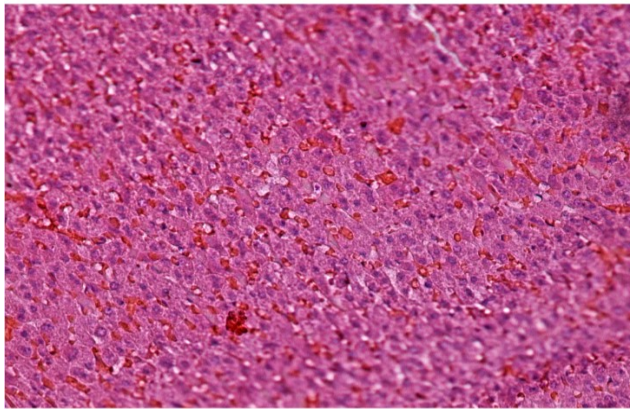

**Figure 2: Photomicrograph illustrates cross section of liver treated with gemcitabine of group (II). Note, severely congested blood sinusoids, distortion of hepatocytes and focal necrosis. H&E. X10**

In some sections, there was disorganization of hepatic cords, dilatation in the central veins with degeneration of endothelium cells and cellular injury around the veins. Inflammatory cells infiltration around the central veins. Severe congestive manifestations and dispersed hemorrhaging between the liver cells and expansion of blood sinusoids (Fig.3).

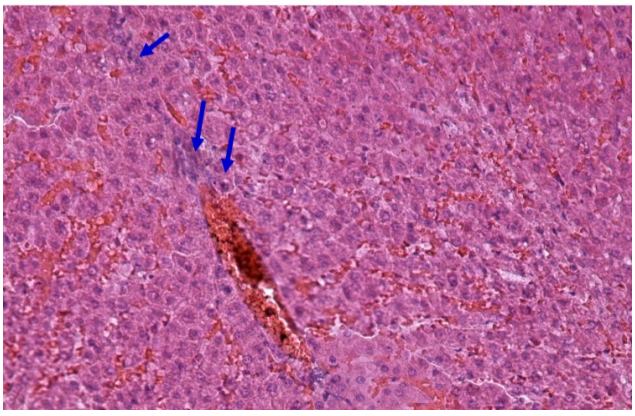

**Figure 3: Photomicrograph illustrates cross section of liver treated with gemcitabine of group (II). Note, severe congestive manifestations and dispersed hemorrhaging between the liver cells and expansion of blood sinusoids. Inflammatory cells infiltration around the central veins (arrow). H&E. X20**

Necrotic spots and presence of hemorrhagic spots between the liver cells, vacuolation of the cytoplasm of pericentral hepatocytes, diffuse hepatocellular vacuolar degeneration and necrosis, and the sinusoid filled with red blood cells, had been noticed (Fig.4). Other sections, showed acute cell bloating as well as necrotic areas were also frequently observed. Severe hydropic degeneration exhibited by vacuolar degeneration in the hepatocytes surrounding the central vein. The hepatocytes showed pyknotic nuclei and karyolysis with irregular nuclear membrane.

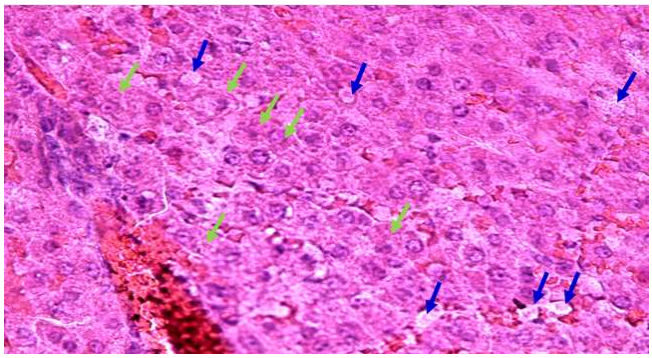

**Figure 4: High magnification photomicrograph illustrates cross section of liver treated with gemcitabine of group (II). Note, vacuolation of the cytoplasm of hepatocytes (blue arrow), diffuse hepatocellular vacuolar degeneration (green arrow) and the sinusoid filled with red blood cells. H&E. X40**

Histopathological alterations were also including perivascular severe focal mononuclear cellular infiltration around the central vein, associated with membrane changes of endothelial lining cells manifesting periportal fibrosis (Fig.5). Focusing on the portal space occupying the portal traids of liver of rabbits treated by therapeutic dose of gemcitabine, were showing severe dilatation in portal vein, hepatic artery and bile ductule with severely congestive manifestations. Accumulation of fibrosis also noticed within the surrounding connective tissue and inflammatory cells infiltration **(Fig.6).**

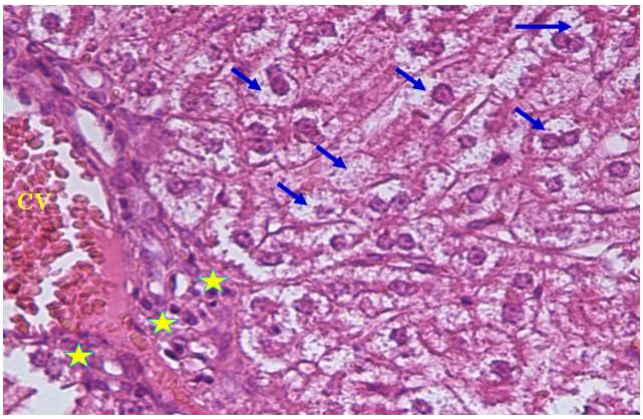


**Figure 5: High magnification photomicrograph illustrates cross section of liver treated with gemcitabine of group (II). Note, vacuolar degeneration( arrow) in the hepatocytes surrounding the congested central vein(CV). Severe focal mononuclear cellular infiltration (stars). H&E. X40**

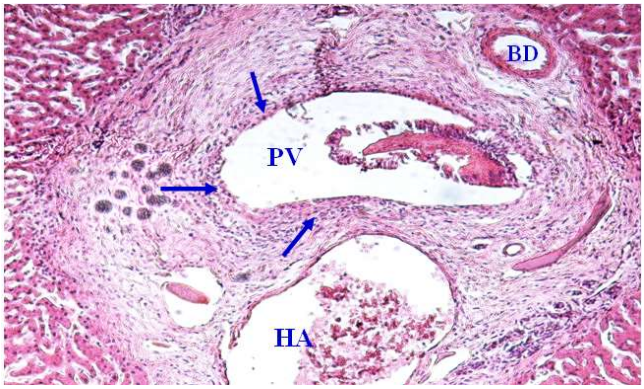


**Figure 6: High magnification photomicrograph illustrates cross section in the portal space of liver treated with gemcitabine of group (II). Note, severe dilatation and congestion in portal vein (PV), hepatic artery (HA) and bile ductule (BD). Accumulation of fibrosis and inflammatory cells infiltration (arrow). H&E. X40.**

**Protective group (III):** Sections from the liver of animals received therapeutic dose of gemcitabine and treated by garlic extract, group (III), the liver histological alterations were performance less intense compared to gemcitabine -treated rabbits of group (II). Microscopic evaluations showed that the severe hepatic lesions induced by gemcitabine were significantly decreased by the treatment with garlic extract. There were no obvious pathological changes, nearly normal liver histology was observed, except mild to moderate congestion and some hemorrhagic spots in central veins. The hepatocytes were appeared similar to normal. Slightly dilated blood sinusoids had been noticed (Fig.7).

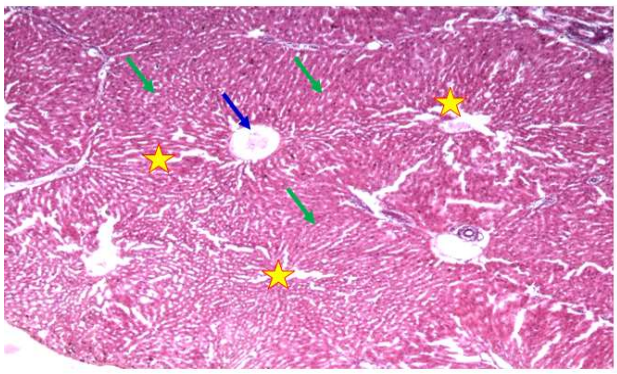

**Figure 7. Photomicrograph illustrates cross section of the liver of group (III). Note, mild to moderate congestion in central veins (blue arrow). The hepatocytes were appeared similar to normal (green arrow). Slightly dilated blood sinusoids (stars). H&E. X10**

There were marked decreases in degenerative changes of the hepatocytes and mild congestion of blood vessels. Some degenerative changes in some hepatocytes located around the central vein. Others were appeared nearly normal polyhedral hepatocytes. The blood sinusoids also had variable appearance, which some were slightly congested and others appear similar to normal **(Fig.8).**

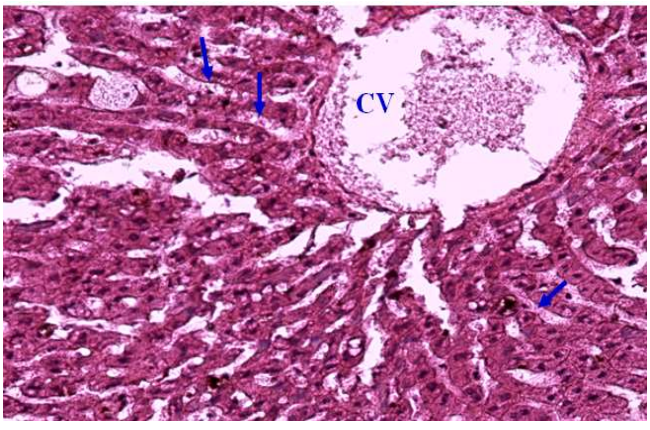


**Figure 8. Photomicrograph illustrates cross section of the liver of group (III). Note, mild congestion of central vein (CV), some sinusoids was slightly congested (arrow) and others appear similar to normal. H&E. X20**

High magnification in cross section of the liver received therapeutic dose of gemcitabine and treated by garlic extract of group (III), demonstrated no cytoplasmic vacuolation was present within hepatocytes, whereas mild degree of pyknotic nucleus had been noticed. The central vein and blood sinusoids exhibited some hemorrhagic spots (Fig.9).

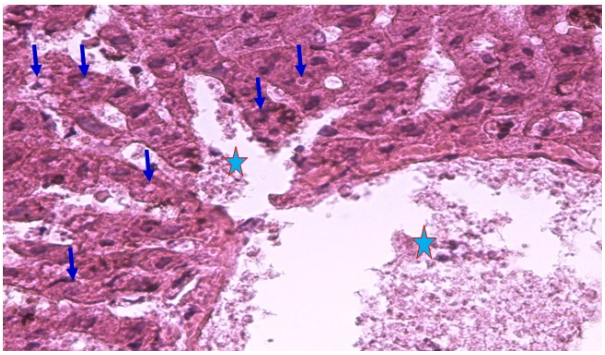

**Figure 9. High magnification photomicrograph illustrates cross section of the liver of group (III). Note, no cytoplasmic vacuolation. Mild degree of pyknotic nucleus (arrow) was present within hepatocytes. The central vein and blood sinusoids exhibited some hemorrhagic spots (stars). H&E. X40**

No prominent morphologic changes were seen in the hepatocytes architecture. The cytoplasm of the hepatocytes showed moderate basophilia and their nuclei appeared dark rounded located centrally. Lobular organization of the liver was preserved. Passive hyperaemia within central veins with dilatation of hepatic sinusoids was observed. Some proliferation of Kupffer cells noticed within the sinusoids (Fig.10).

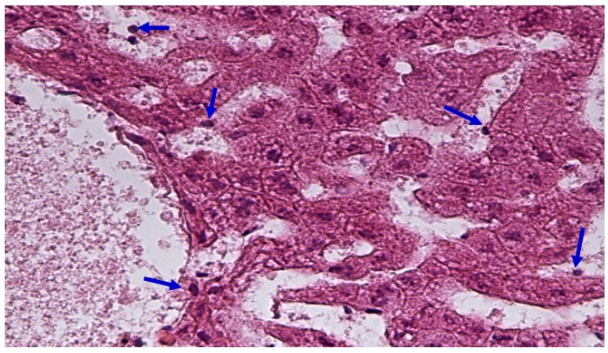

**Figure 10. High magnification photomicrograph illustrates cross section of the liver of group (III). Note, cytoplasm of the hepatocytes showed moderate basophilia and their nuclei appeared dark rounded located centrally. Passive hyperaemia within central vein. Some proliferation of Kupffer cells noticed within the sinusoids (arrow). H&E. X40**

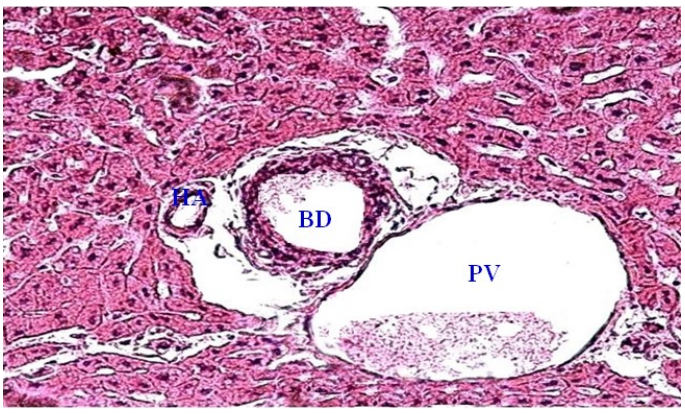


**Figure 11. High magnification photomicrograph illustrates cross section in the portal space of the liver of group (III). Note, mild to moderate congestion in portal vein (PV), hepatic artery (HA) and bile ductile (BD).H&E. X40**

Compared to sections of liver treated with gemcitabine group (II), the portal triads within their connective tissue spaces of liver of animals received gemcitabine and then treated with garlic extract group (III) were demonstrated mild to moderate congestion in portal vein, hepatic artery and bile ductile. These results were indicated that the garlic extract improved of the liver cells, central vein structure and the portal region (Fig.11).

# DISCUSSION

Gemcitabine is one of the most advised worldwide cytotoxic medicine, classified as an antimetabolite. It acts by inhibiting DNA synthesis and begins intracellular activation through deoxycytidine kinase [21,22,23]. This drug cannot differentiate between normal cell and malignant cell, so it produces its toxic effect on both cells but the normal cells can repair the injury and retain their normal function while the malignant cell will be destructed and all the adverse reactions of gemcitabine are due to this process [24]. It is metabolized in liver and excreted by kidney so it is recommended to be given with attention in patients with renal and hepatic insufficiency[25]. Gemcitabine exerts its antitumor activity via multiple mechanisms of action. Specifically, it undergoes intracellular phosphorylation to the active metabolites gemcitabine diphosphate and gemcitabine triphosphate, which inhibit polymerases enzymes of DNA with cessation of its synthesis, so this leads to inhibition of duplication of DNA in the cellular cycle at S-phase leading to programmed cell death [26]. Liver injury caused by toxic chemicals and certain drugs, especially anticancer drugs, is a common toxicological problem. The damage pathogenesis is ranging from inflammation to apoptosis [27]. In this broad context of interest in the potential hepatotoxicity of gemcitabine, Mergental, 2005 reported that gemcitabine has toxic effects on the livers of rats as causing necrosis of hepatic cells and dense lymphatic filtration that extend to the parenchymal cells surrounded the distorted portal spaces with dense coexistence of inflammatory cells and damaged bile ductules[7]. On the other hand, Kamer *et al*., 2003 said that gemcitabine usage is safe under certain conditions and its side effects as weight loss is resulted from diarrhea [28]. Moreover, Hailan *et al*., 2018 reported that elevations in the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the sera, meanwhile, are an indication of gemcitabine-induced changes in liver tissues [8]. In the present study, rabbits that received intrapretonial therapeutic dose of gemcitabine (group II) were showed a significant clinical signs, such as diarrhea, decrease in the amount of feed consumed and loss of skeletal muscles compared to the control group, which could in turn be ascribed to drug induced toxicity, psychological pressures and the necrotizing effects of the drug on the digestive system [29]. Furthermore, the drug also affects the mucous lining of the gastro-intestinal tract [28]. Our observations are in agreement with the findings of previous researchers; Germoush, 2009[29]; Rickenbacher *et al*., 2011[30]; Hailana *et al*., 2018 [8].

In the present work the histological descriptions for the slides stained by Haematoxylin and Eosin stain of control group (group I) were noticed that the liver of the adult rabbits were consisting of several lobules without boundary lines due to few or absent connective tissue septa between them. Each lobule was composed of the epithelial cells grouped in irregular plates (the hepatocytes) with intervening sinusoids which drain into central vein. At the angles of each hepatic lobule, there was a

dense connective tissue spaces (portal spaces), were occupied the portal triads, which was containing branches of hepatic artery, portal vein and bile duct. The hepatocytes were polyhedral cells with acidophilic cytoplasm and prominent central round nuclei. Binucleated hepatocytes were present in the liver parenchyma. The central vein which was present in the center of each hepatic lobule was lined by flat endothelial cells. The sinusoids had walls formed by a discontinuous lining of fenestrated endothelial cells that lack a basal membrane. These findings were corroborate with the previous investigators; Al- Abdel- Maaboud *et al*., 2003 [31] **;** Hamdany 2019 [32] and Al-samawy *et al*., 2022 [33]. The results of the present investigation showed that gemcitabine toxicity produced significant disturbance in the normal architecture with evidence of structural changes in the liver of group II, gemcitabine treated group, than those in the control group I. The hepatotoxicity manifested by severely congested blood sinusoids, distortion of hepatocytes and focal necrosis. In addition, some hepatocytes were dissociated from hepatic cords, indicating liver injury. These findings were consistent with Abdullah *et al*., 2022 in male rat who reported that liver tissue showing congestion and dilation of the portal vein, lymphocytic infiltration around the portal vein, necrotic spots, and congestive manifestations with dispersed hemorrhaging between the liver cells and expansion of blood sinusoids.

They found that gemcitabine lead to DNA fragmentation due to cessation of DNA synthesis, in addition to stimulation of apoptotic process in the tissue[18]. Necrotic spots and presence of hemorrhagic spots between the liver cells, vacuolation of the cytoplasm of pericentral hepatocytes, diffuse hepatocellular vacuolar degeneration and necrosis. The hepatocytes showed pyknotic nuclei and karyolysis with irregular nuclear membrane was also frequently observed in this current study. Similar kinds of alterations are also observed after use of many anticancer drugs especially reported with Methotrexate, Abha Jain *et al*., 2005[34] ; Cisplatin, Doxorubicin and Fluorouracil, El- Sayyad *et al*., 2009 [35]; Cyclosporine, Akbulut *et al*., 2015 [36]; Cyclophosphamide, Bhat *et al*., 2018 [37]. Our findings were clear indicator of cellular leakage and loss of functional integrity resulting from liver damage. Destruction of hepatocellular architecture associated with inflammatory infiltration was comparable to many previous studies. The thinning of cell plates is explained by the compression of the hepatocytes in the cords adjacent to the dilated and congested sinusoids where the initial toxic effects probably occurred [38].

The hepatocellular destruction together with features of nuclear degeneration and apoptosis like karyolysis and nuclear pyknosis and vacuolations occurred due to a direct effect of drugs [39].

In the present study, severe hydropic degeneration exhibited by vacuolar degeneration in the hepatocytes surrounding the central vein was clearly noticed. Interpretation of vacuolar formation following chemical treatments has been subjected to wide speculation by many investigators; Robbins and Angell, 1976; Albasha and Azab, 2014, were regarded such vacuolation to represent primary morphologic response to many forms of cell injury. They also attributed it to the noxious effects of treatment on the cell membranes, both structurally and functionally, causing marked disturbances in its permeability system. This presumably leads to enhanced imbibition of water into the cells. When it sufficiently accumulates in the cells, such intracellular water produced clear cytoplasmic vacuoles indication the occurrence of the pathologic symptoms commonly referred to as hydropic degeneration [40,41]. Toxic effects of drugs can cause degenerative changes in the hepatocytes, including their necrosis. The mechanisms explaining drug-induced liver injury include mitochondrial damage and oxidative stress[42]. Hryciuk *et al*., 2018 reported that four patients suffered from toxicity after gemcitabine administration seen as liver cell necrosis, microvascular degeneration, intracellular cholestasis, and focal necrosis[43]. Mascherona *et al*., 2020 reported that on the liver biopsy, there were histological findings of mild-to-moderate portal hepatitis, eosinophilia, bile duct injury, and mild perisinusoidal fibrosis. The liver biopsy and the improvement in symptoms and liver values after gemcitabine discontinuation confirm the drug-induced liver injury[44]. Cells exposed to toxic chemicals experience morphological or structural anomalies that are characteristic of the occurrence of apoptosis. These include changes in plasma membrane integrity, shrinkage and rounding of cells, chromatin condensation, disruption of the nuclear envelope and DNA fragmentation[45]. Hailan *et al*., 2018 were showed that in the gemcitabine treated liver sections the presence of liver injury in the form of diffused centrilobular congestions, hemorrhage, and the appearance of apoptotic/necrotic areas. The presence of pyknotic cells is an indication of apoptosis. The increased rate of the incidence of apoptosis in gemcitabine-treated animal tissues as a result of inhibition or disruption of cells' DNA synthesis may be the mechanism by which this drug induces toxicity in the animal body[8]. Apoptosis is a common feature of hepatotoxicity induced by many chemicals; it may precede necrosis, as in the hepatotoxicity induced by thioacetamide[46]**,** or it may occur concurrently with necrosis as in hepatotoxicity associated with acetaminophen[47]. Apoptosis is form of cell death, serves as a process by body which eradicates damaged cells. The two main apoptotic pathways are triggered by the agents used in anti-cancer therapies. The intrinsic pathway is carried by DNA damaging cells, oxidative stress and the extrinsic pathway is carried after binding of specific ligands to the corresponding receptors[48]. Although apoptosis and necrosis are quite different from one another morphologically, as well as in their mechanism of occurrence, there are still few similarities between them. Incessant apoptosis leads to leakage of cellular contents into the blood stream and the intercellular spaces, if accompanied by necrosis[49]. This fact explains what was observed during our study. Exposure to certain drugs and toxins leading to liver injury results in both structural and functional impairment of the hepatocytes by the free radicals, reactive oxygen or nitrogen species released either by Kupffer cells or endothelial cells, resulting in cellular apoptosis. Although the exact mechanism of action of gemcitabine-induced liver damage is unknown[50]. From the findings of our present study on domestic rabbits can be considered the liver as a target for gemcitabine, which expose to this compound can cause histological damage on hepatic tissue. The incidence of apoptosis in gemcitabine-treated animal tissues as a result of inhibition or disruption of cells' DNA synthesis may be the mechanism by which this drug induces toxicity in the animal body. Sections from the liver of animals received therapeutic dose of gemcitabine and treated by garlic extract, group (III) were showing the liver histological alterations were performance less intense compared to gemcitabine -treated rabbits of group (II). Microscopic evaluations showed that the severe hepatic lesions induced by gemcitabine were significantly decreased by the treatment with garlic extract. Garlic (Allium sativum L. family Liliaceae) is a widely consumed, ingredient in foods with its health benefits. It is known for its unique taste and odor along with immune-

boosting functions in the body and is considered as one of the important medicinal plants and provides a useful source of new therapeutics[51]. The chemopreventive activity has been attributed to the presence of organosulfur compounds (OSCs) in garlic. How this is achieved is not fully understood, but several modes of action have been proposed. These include its effect on drug metabolizing enzymes, antioxidant properties and tumor growth inhibition. Most of these studies were carried out in the animal models[52]. Organosulfur compounds (OSCs) derivative garlic act as chemotherapeutic agents to prevent carcinogens. It also prevents antibacterial, anti-inflammatory and anti-fungal activity[53]. These organosulphur compounds (OSCs) incite the activity of enzyme and formation of adducts in several tissues. The anti-cancer property of garlic derived organosulphur compounds (OSCs) is based on the pre apoptosis properties[53]. Upreti *et al*. 2008 mentioned that the cytotoxic chemotherapy drugs- induced apoptosis is carried by mitochondrial pathway and releases cytochrome c from the outer membrane and due to mitochondrial dysfunction may be generated by the disruption of oxidative energy production within the hepatocytes[54]. Mitochondrial membrane permeabilization can lead to apoptosis; a rupture in mitochondrial membrane can lead to ATP depletion and subsequent necrosis. The mitochondrial enzymes have been called as one of the major cellular generators of reactive oxygen spaces (ROS) and one of the important antioxidant enzyme is catalase which is scavenged H2O2[55] The anticancer mechanisms of action of the garlic-derived organosulphur compounds (OSCs) include altering mitochondrial permeability, inhibiting angiogenesis, enhancing antioxidative and proapoptotic properties, and regulating cell proliferation. All these effects of garlic's sulfur-compounds have been demonstrated in various human cancers[56]. It has been shown that garlic and its major components can ameliorate the toxicity of different agents in brain, kidney, blood, liver, spleen, pancreas, heart, reproductive system in part through radical scavenging, antioxidant effect, reducing lipid peroxidation, anti-inflammatory, cytoprotective activities, increase protein synthesis in damaged tissues and suppressing apoptosis[57].

Oxidative modification of DNA, proteins and lipids by reactive oxygen species (ROS) plays a role in disease, including inflammatory diseases and cancer. Garlic extract protects DNA against free radical--mediated damage and mutations, inhibits multistep carcinogenesis and defends against ionizing radiation and UV-induced damage and against liver toxicity caused by carbon tetrachloride (an industrial chemical) and acetaminophen, an analgesic[58]. In the current investigation, there were no obvious pathological changes in section of rabbit liver received gemcitabine and treated by garlic extract, group III, nearly normal liver histology was observed, except mild to moderate congestion with some hemorrhagic spots in central veins and slightly dilation of blood sinusoids. The hepatocytes were appeared similar to normal. Our observations were supported by other studies suggesting that garlic exerts protective effects against many chemical agent induced hepatotoxicity; Naji *et al*., 2017 [59]; Ahmed, 2018 [60]; Azab and Albasha, 2018 [61]; Abdel-Daim *et al*., 2020 [62]; Mousa *et al*., 2021 [63]. Abdella and Gad, 2008 proved that the diallyl disulfide compound, as the most important biological active organosulphur compound of garlic extract, revealed a significant protective effect against mutagenic, genotoxic, and DNA strand breaks and chromosomal damage induced by the mercuric chloride. In addition, they explained the role of garlic as antigenotoxic agent may be due to the presence of sulfhydryl groups which may provide protection against increasing free radical formation which induce DNA damage [64]. Furthermore, Ahmed, 2018 reported that the rats treated with aged black garlic (ABG) then cyclophosphamide (CP) showed significant improvement in DNA by decreasing the number of damaged and strongly damaged spots in the liver of rats. The author revealed that oral administration of aged black garlic before cyclophosphamide significantly decreased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and bilirubin when compared with the cyclophosphamide -treated group. It suggested that aged black garlic has antiapoptotic properties against cyclophosphamide - induced apoptosis by inhibition of oxidative stress[60]. Abdel-Naim *et al*., 2002 reported that pretreatment of rats with garlic oil protected the liver from the toxicity of ethanol and carbon tetrachloride by decreasing the levels of ALT and AST activities near to normal levels, and prevented liver histopathological changes which establishes the hepatoprotective potential of garlic extract. Hepato protection might be due to garlic oil effects against cellular leakage and protection of the integrity of the cell membrane in the liver[65]. These findings support our result that observed in histopathological pictures which was indicated that the garlic extract improved of the liver cells, central vein structure and the portal area. The cytoprotective effect of garlic extract might be due to increased regenerative capacity of epithelial liver cells, or an inflammatory response through a cytoprotective effect and reduction of the immune-mediated liver damage. Moreover, the garlic extract was shown to block the apoptosis generation [66]. In addition, many previous investigations reported that the effect of different potential antioxidant and antiapoptotic agents on chemotherapeutic drugs-induced changes in the activity of antioxidant enzymes in the liver has been investigated to determine the extent of tissue damage due to oxidative stress [57,58]. A combination of these antioxidants with chemotherapeutic drugs produced nearly normalized liver tissues with no significant degenerative changes accompanied with decrease in liver enzymes when compared with the chemotherapeutic drugs -treated alone. The improvement of liver architecture and enzymes might be attributed to the stabilizing effect of these antioxidants on the hepatocyte cell membrane, which prevents AST, ALT, and bilirubin leakage into the extracellular fluid. The reduction in the serum levels of the liver biomarkers could be considered as an index to the regenerating activity of the damaged hepatocytes [67,68]. Accumulating studies have revealed that several natural products, including garlic had hepatoprotective effects. Considering that, the garlic extract is one of the most important antiapoptotic and antioxidant agent, thus and based on our present study that indicated that the garlic extract possesses protective ability against - gemcitabine induced liver injury and might be an effective alternative medicine against acute oxidative liver toxicity. Therefore, consumption of garlic may provide some kind of protection from cancer development.

# CONCLUSION

The present work confirmed that the use of intrapritoneal dose equivalent to human therapeutic dose of gemcitabine may cause hepatotoxicity in domestic rabbits. This toxicity manifested by significant disturbance in the normal architecture with evidence of structural changes; distortion of

hepatocytes and focal necrosis, congestion and dilation of the portal vein and blood sinusoids, necrotic and hemorrhagic spots between the liver cells, and vacuolation of the cytoplasm of pericentral hepatocytes. These clear indicators of cellular leakage and loss of functional integrity resulting from liver damage. Histological observations can provide a direct, assessment of the severity of liver injury that can help guide clinical management. The increased rate of the incidence of apoptosis in gemcitabine-treated animal tissues as a result of inhibition or disruption of cells' DNA synthesis may be the mechanism by which this drug induces toxicity in the animal body. Concomitant administration of garlic extract with gemcitabine reduced the histopathological liver changes induced by gemcitabine alone. This effect is seems to be related to the anti apoptotic and antioxidant properties of garlic extracts, which can prevent the oxidative damage to DNA resulting in inhibition of apoptosis as confirmed by maintaining the histological architecture of the liver.

# RECOMMENDATION

According to present study, we advise to avoid using gemcitabine for a long duration, as it may cause adverse effects in the liver, and it is also recommended to consume garlic as an anti apoptotic and an antioxidant supplementary agent with this medicine.

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