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PROTECTIVE EFFECTS OF GRAPE SEED EXTRACT (VITIS VINIFERA) AGAINST CISPLATIN- INDUCED LIVER DAMAGE IN RABBITS. (HISTOLOGICAL AND HISTOPATHOLOGICAL STUDY)

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ABSTRACT

Cisplatin is a chemotherapeutic drug used in the treatment of various cancer types. It can be considered a double-edged sword. It is a potent anticancer agent at the same time has toxic effect on various tissues as it causes hepatotoxicity. It was determined to cause liver toxicity and to weaken the antioxidant defense systems in liver. A decrease in antioxidant enzymes resulted from cisplatin induced tissue toxicity. Moreover, the development of therapies to prevent the appearance of cisplatin- induced tissue toxicities has focused on administration of antioxidants along with cisplatin treatment. The grape seeds exhibit a broad spectrum of pharmacological properties against oxidative stress. Therefore, the aim of present study was designed to investigate the protective effects of grape seed extract on the liver damage in cisplatin treated rabbits. Twenty seven healthy rabbits of local mixed breed were selected, divided into three equal groups, the first group (CON group) served as control and received a single intraperitoneal injection of normal saline solution once per week for 6 weeks; the second group (CIS group) were treated with therapeutic dose of cisplatin intraperitoneally once per week for six weeks; the third group (CIS+GSE group) served as protective group and was concomitantly treated with grape seeds extract by oral gavages and cisplatin injection intraperitoneally once per week for six successive weeks. Administration of grape seeds extract by oral gavages starting from the first day of the experiment for 6 consecutive days before and 6 consecutive days after the cisplatin injection and continued daily for 6 weeks.

The histopathological findings present in current study showed that cisplatin toxicity produced significant structural changes in the liver of CIS group, cisplatin treated group, in the form of prominent disorganized architecture and loss of liver lobulation, distortion of the arrangement of

parenchyma of the liver, loss of radial arrangement of sinusoids from the central vein of the liver, Dilatation and congestion of central veins, sinusoids and portal traids, degenerative changes in hepatocytes and pyknotic nuclei indicating necrosis. Marked vacuolar degeneration mainly hydropic ballooning degeneration and hyper activation of Kupffer cells and inflammatory cellular infiltrations were also observed. This finding reflected the cytotoxic effects of cisplatin in hepatic tissue. While, in the sections from the liver of animals received therapeutic dose of cisplatin and treated by grape seed extract, group (CIS+GSE) clearly showed that there was a significant alleviated hepatotoxicity, since histopathological changes were markedly less pronounced

compared to animals treated with cisplatin alone. Therefore, and based on our findings, the grape seed extract contributes significantly to the improvement of histological alterations in rabbit liver caused by cisplatin.

KEYWORDS

Cisplatin, toxicity, grape seeds extract, liver, rabbits.

INTRODUCTION

The liver is one of the largest organs in the human body and the chief site for intense metabolism and excretion [1]. It plays a major role in detoxification and excretion of many endogenous and exogenous compounds; any injury to it or impairment of its functions may lead to many
implications on one's health [2]. Hepatic damage is associated with distortion of these metabolic functions. Liver damage is associated with cellular necrosis and fibrosis [3].
The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins [4].
Cisplatin is a well-known platinum-based anticancer agent that has a high effective rate in treating various cancers. It was discovered by Michel Peyrone in 1844, and its chemical composition was first described by Alfred Werner in 1893. It is widely used to treat various solid tumors, including head and neck, cervical, ovarian, prostate, testicular and colorectal cancers [5,6].
Generally, cisplatin interacts with purine bases of DNA in the cancer cells, preventing it from replicating and halting its biological function, eventually suppressing the growth of these malignant cells [7]. Cisplatin can be considered a double-edged sword. It is a potent anticancer agent, at the same time has toxic effect on various tissues as it causes ototoxicity (75–100%), nephrotoxicity (72%) [8], hepatotoxicity (36%)[9] and cardiotoxicity (6%)[10].
Cisplatin toxicity has also been associated with the excess production of reactive oxygen species (ROS), and a concomitant reduction in the antioxidant defense system. Oxidative stress is one of the most important side effects limiting the use of cisplatin [11]. Although cisplatin (CIS) is a highly effective anticancer drug, hepatotoxicity is one of the most common adverse effects associated with its use. Recently, reactive oxygen species (ROS) and inflammation are suggested to be key factors in the pathophysiology of CIS-induced acute liver damage [12]. Hepatotoxicity has been demonstrated in the patients who received low doses of cisplatin which probably due to cumulative effect in the liver cause massive hepatic toxicity, including dissolution of hepatic cords, focal inflammatory lesions and necrosis [13].
Dietary intervention is one of the anticipation ways for prevention of anti-cancer-related toxicity to minimize the harmful effects of cisplatin. In the clinical findings, some natural compounds showed potential in treating hepatotoxicity caused by chemotherapeutic drugs among cancer patients [14].
Herbal antioxidants have attracted the researchers due to its potential and efficacy against drug- induced liver toxicity. These are the potential sources for new therapeutic agents that could be used in the prevention of hepatic injuries. Natural products rich in flavonoids or polyphenols, have been now established as powerful hepatoprotective agents in experimental liver-injury cell and animal models [15]. Flavonoids are one of the most researched polyphenols to reduce cisplatin-induced hepatotoxicity. The mechanism of hepatoprotection by these compounds generally exerts multiple effects. Although they show hepatoprotection due to antioxidant effect and anti-inflammatory [16].
Grape seeds extract (GSE) is having abundant source of polyphenols. Polyphenols and flavonoids specifically

proanthocyanidins present in the GSE have been shown remarkable interest based on positive reports of their antioxidant properties and ability to serve as free radical scavengers [17].

Grape seed polyphenols have a higher antioxidant activity as compared to other well-known antioxidants (such as vitamin C, vitamin E, and β-carotene). Beside their antioxidant activity, it also contains some enzymes that catalyze the release of histamine during inflammation and allergies [18]. Polyphenols of grape seed extract have long been recognized to possess many

properties, including antioxidant, anti-inflammatory, anticarcinogenic, platelet aggregation inhibiting, and metal chelating properties [19].

Yamakoshi et al., 2002, showed that grape seed extracts are non-toxic to rats [20].

Phenolic compounds present in GSE exhibits anticancer and cell cycle modulation activity. These phenolic compounds show cytotoxic activity against tumor cells without affecting the normal healthy cells [21].

The present study aims to explore the possible protective effect of grape seeds extract (GSE) on cisplatin - induced hepatotoxicity in rabbits.

MATERIALS AND METHODS

The present experiment was conducted on twenty-seven healthy rabbits, 4-5 months old, of local mixed breed, weighing between 1.5 – 2.0 kg; they were kept under controlled laboratory conditions for one week for acclimatization of animals to the laboratory environment with

an alternating cycle of 12 h light and dark. The animals were allowed unrestricted access to food and water ad libitum. The rabbits were routinely observed for food consumption, fecal characteristics and any clinical signs might be appeared. Accordingly, the rabbits were randomly divided into three groups, each comprising of nine rabbits. The concentration of cisplatin dose and grape seeds extract was selected based on previous studies [22,23, 24]

The first group (CON group) served as control group and received a single intraperitoneal injection of 1ml normal saline solution (once per week for 6 weeks) for the duration of the experiment to simulate the effect of injection.

The second group (CIS group) were treated with a single (7mg/ kg of body weight) therapeutic dose of cisplatin intraperitoneally once per week for six weeks. The cisplatin dosage was selected on the basis of its effectiveness in inducing hepatotoxicity [25].

The third group (CIS+GSE group) served as protective group and was treated with grape seeds extract (dissolved in water). In this group, the animals received a dose of 250 mg/kg body weight [26] of grape seed extract by oral gavages, starting from the first day of the experiment for 6 consecutive days before and 6 consecutive days after the cisplatin injection and continued daily for 6 weeks. In addition, this group was treated with a single (7 mg / kg of body weight) therapeutic dose of cisplatin intraperitoneally once per week for six weeks.

For histological analysis, specimens were taken immediately at the end of the experiment period. The collected samples of the liver, included in this study, were ran through paraffin embedding technique to get paraffin blocks. Histological serial sections were cut from the liver of each group. Serial sections 5 µm thickness were cut and mounted on glass slide, and then stained with ordinary Hematoxylin and Eosin (H and E) stain [27].

RESULTS

Clinical observations:

All the following observations were seen, 60 to 120 minutes after injection of the treated rabbits with therapeutic dose of Cisplatin, (CIS group):

The animals exhibited excessive sleeping, diarrhea, abdominal swelling, loss in ability to taste food, dry mouth, dark urine, fearless behavior, decrease movement, rapid breathing, 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 cisplatin for the desired duration.

While treatment with grape seeds extract (CIS + GSE group) ameliorated all above-mentioned clinical signs but they did not return to normalcy. No clinical signs were seen in control (CON group). There was no mortality among the animals of both control (CON group) and grape seed extract+Cisplatin (CIS+GSE group).

Histological and histopathological findings:

Control animals' group (CON):

Light microscopic examination of paraffin section of liver of control animals group (CON) stained with H& E., showed the normal liver parenchyma was organized as thousands of small hepatic lobules mingled with each other due to few or absent connective tissue septa between them. Each lobule comprised of the hepatocytes that arranged in irregular cell plates radiating from the central vein. There were blood spaces sinusoids interspersed between those hepatocytes.

In corners of each lobule there were connective tissue spaces, the portal space, occupied branches of portal vein, hepatic artery and bile ductule (the portal triads) (Fig.1a).

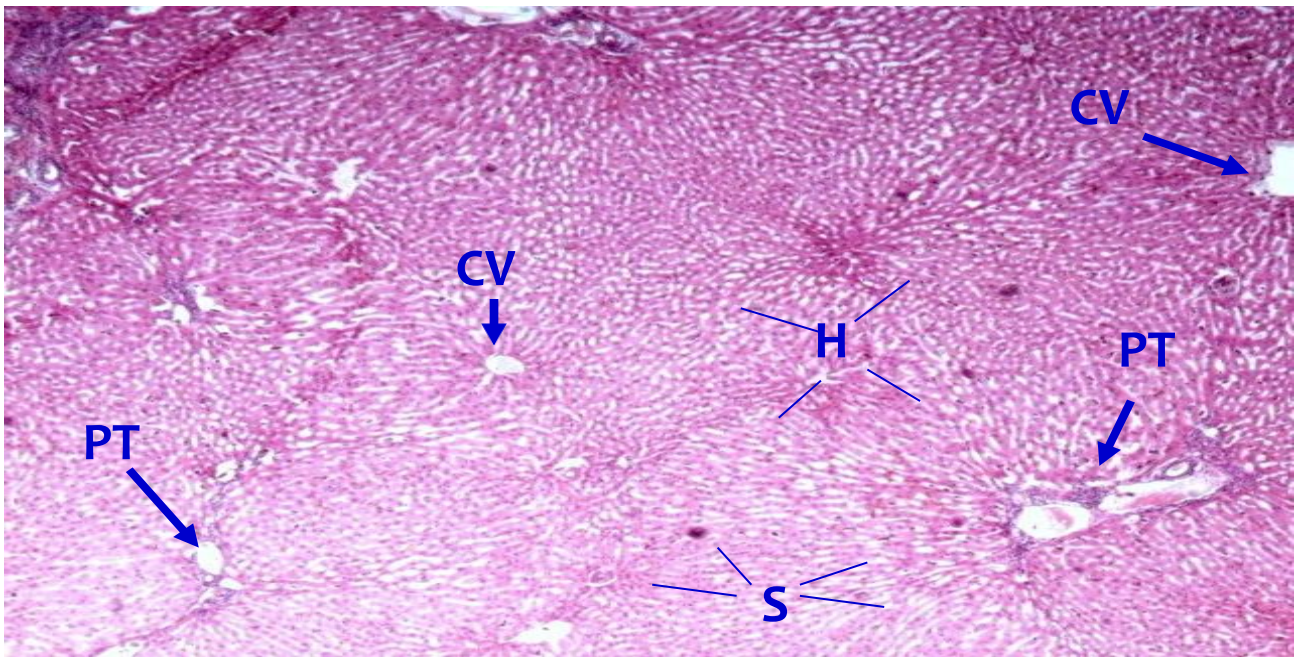

Figure 1a: *Photomicrograph illustrates the normal structure of rabbit liver of control (CON) group. Note, normal liver parenchyma was organized as multi-hepatic lobules. Central vein (CV), portal traids (PT), hepatocytes (H) and blood sinusoids (S). H&E stain. X10.*

The hepatocytes were appeared polygonal in shape with acidophilic cytoplasm and prominent large nuclei. Binucleated hepatocytes were sometimes present. The hepatocytes were separated by vascular sinusoids that emerge from the peripheral branches of the portal vein and hepatic artery and converge on the lobule's central vein. The sinusoids are lined by two other functionally important cells are; fenestrated liver sinusoidal endothelial cells and Kupffer cells (specialized stellate macrophages) interspersed onto the sinusoid's endothelium (Fig.1b).

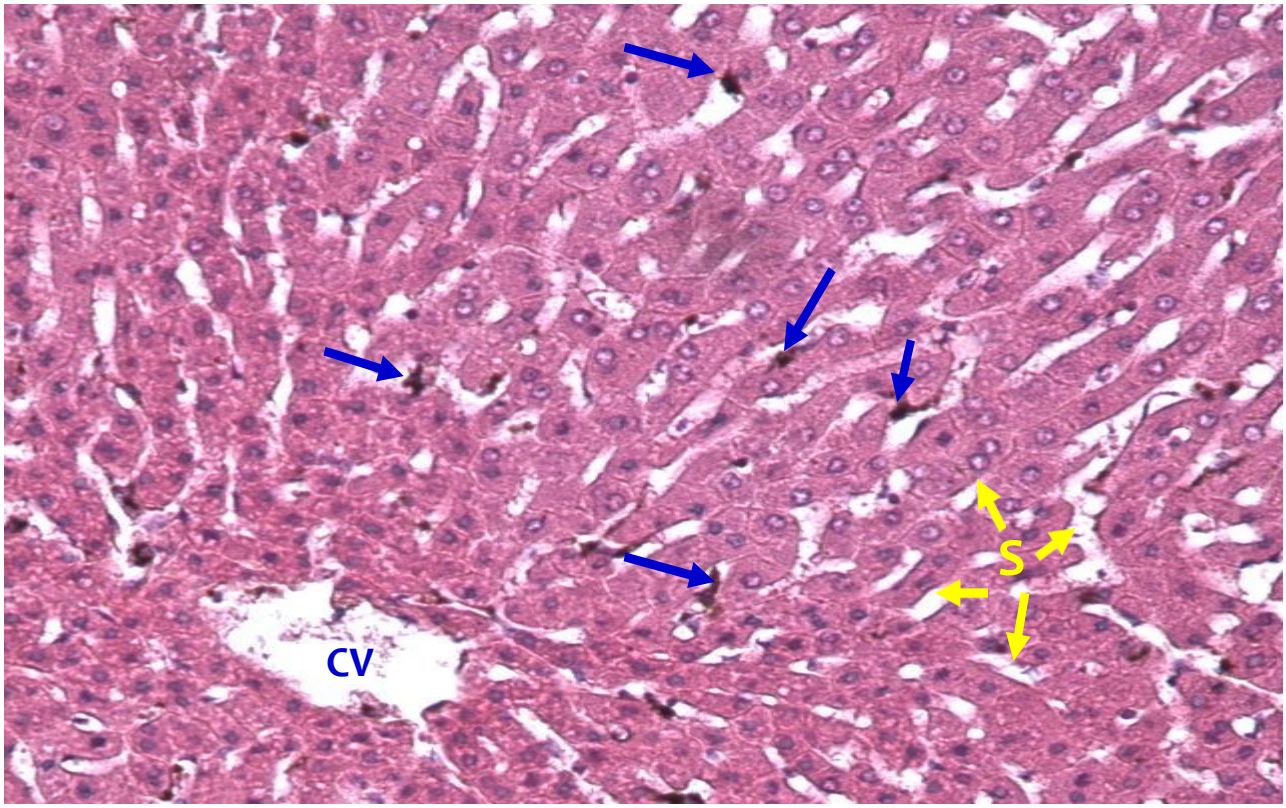

Figure 1b: *Photomicrograph illustrates the normal structure of rabbit liver of control (CON) group. Note, hepatocytes were polygonal in shape with acidophilic cytoplasm and prominent large nuclei. Sinusoids (S), central vein (CV), Kupffer cells (arrow) H&E stain. X20.*

High magnification section in normal rabbit liver of control (CON) group revealed that the hepatocytes were polyhedral cells with large deep staining one or two central nuclei. The central vein represented the center of each individual lobule was lined with flat endothelial cells (Fig.1c).

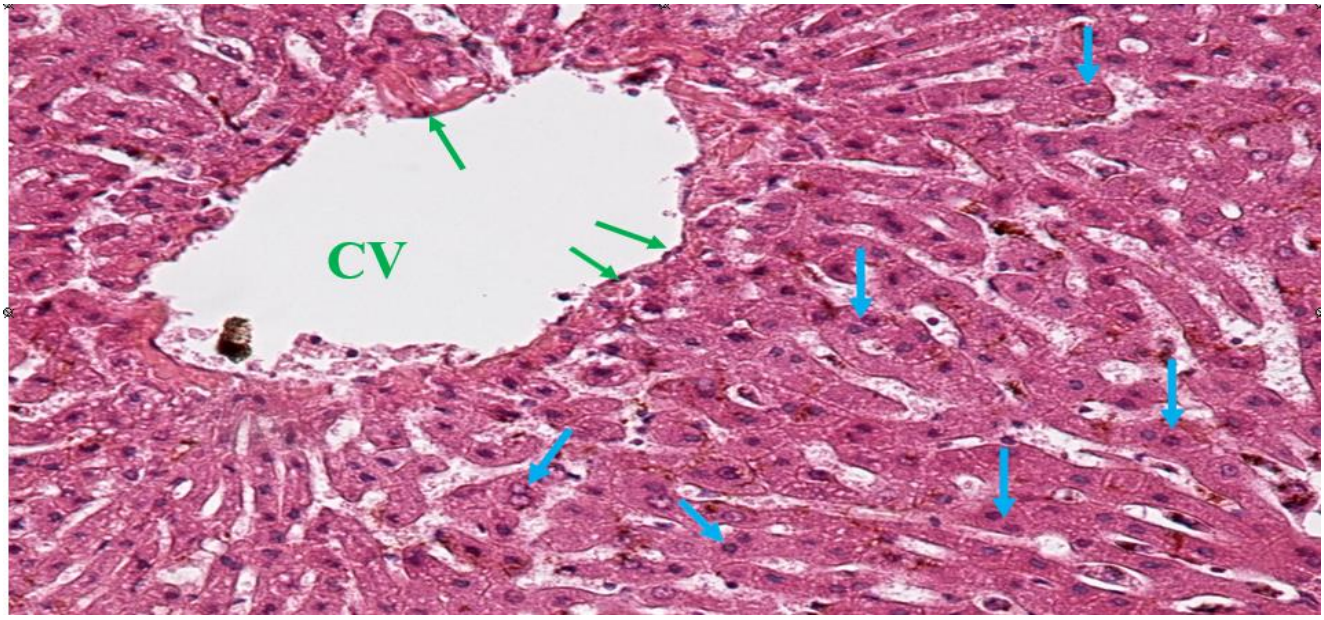

Figure 1c: *High magnification photomicrograph illustrates the normal structure of rabbit liver of control (CON) group. Notes, hepatocytes were polyhedral cells with large deep staining one or two central nuclei (blue arrow). The central vein (CV) was lined with flat endothelial cells (green arrow).H&E stain. X40.*

TREATED ANIMALS GROUP (CIS):

Histopathological examination of sections of liver treated by therapeutic dose of Cisplatin (CIS) group when compared to the control (CON) group were showed distortion of the arrangement of parenchyma of the liver, loss of radial arrangement of sinusoids from the central vein of the liver. Many hepatic cells were damaged and lost their characteristic appearance. Prominent disorganized architecture and loss of liver lobulation (Fig. 2).

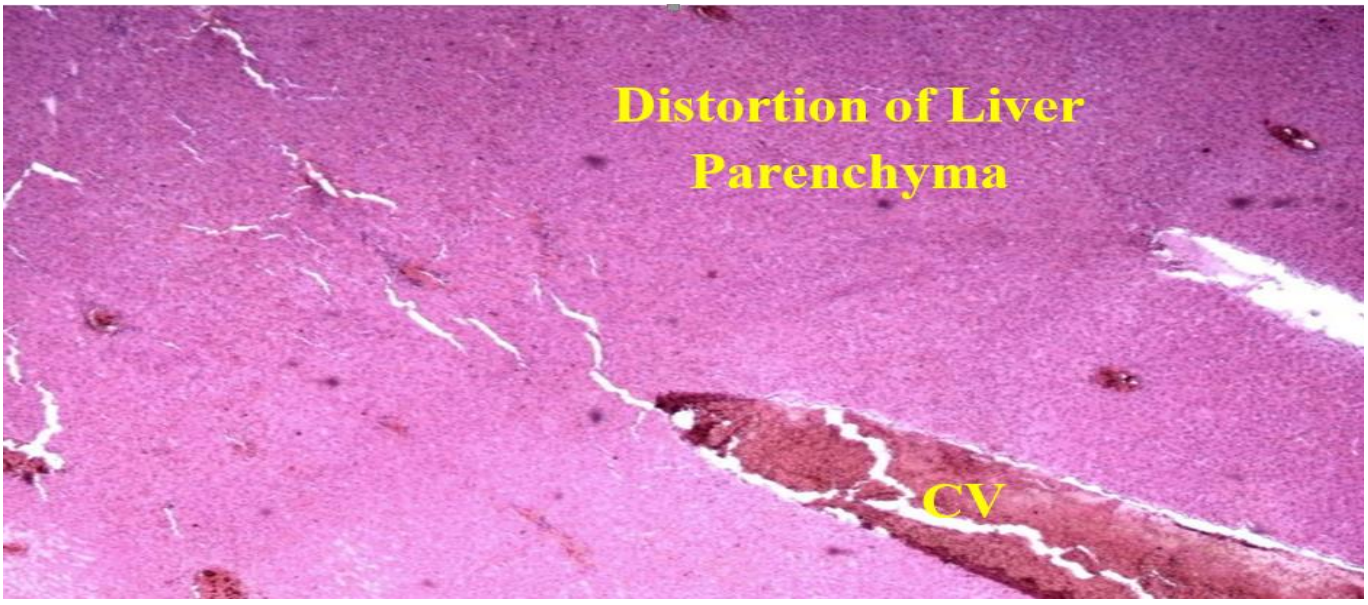

Figure 2: *Photomicrograph illustrates structure of rabbit liver treated with therapeutic dose of cisplatin of (CIS)*

group. Note, distortion of the arrangement of parenchyma of the liver. Prominentdisorganized architecture and loss of liver lobulation. Central vein (CV). H&E stain. X10.

There was severe structural damage in the liver. Most of hepatocytes appeared fused together and lose their organization. Dilatation and congestion of central veins, sinusoids and portal traids also had been observed (Fig. 3).

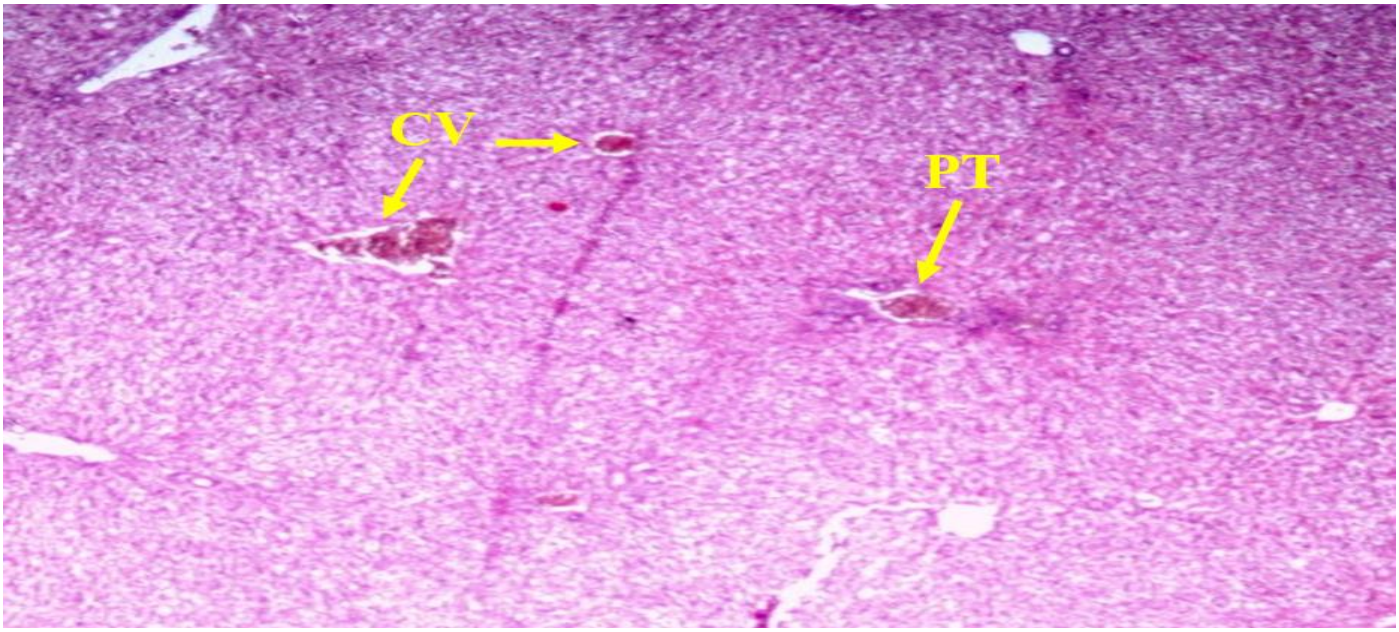

Figure 3: *Photomicrograph illustrates structure of rabbit liver treated with therapeutic dose of cisplatin of (CIS) group. Note, most of hepatocytes appeared fused together and lose their organization. Dilatation and congestion of central vein (CV) and portal traids (PT) H&E stain. X10.*

In some sections of liver of (CIS) group noted severe congestion and more distended of central vein and surrounding sinusoids with massive hemorrhage extending to the nearby cells. Distortion of hepatocytes and focal necrosis. These necrotic cells appeared homogenous structurless with degenerated nuclei (Fig. 4a & b).

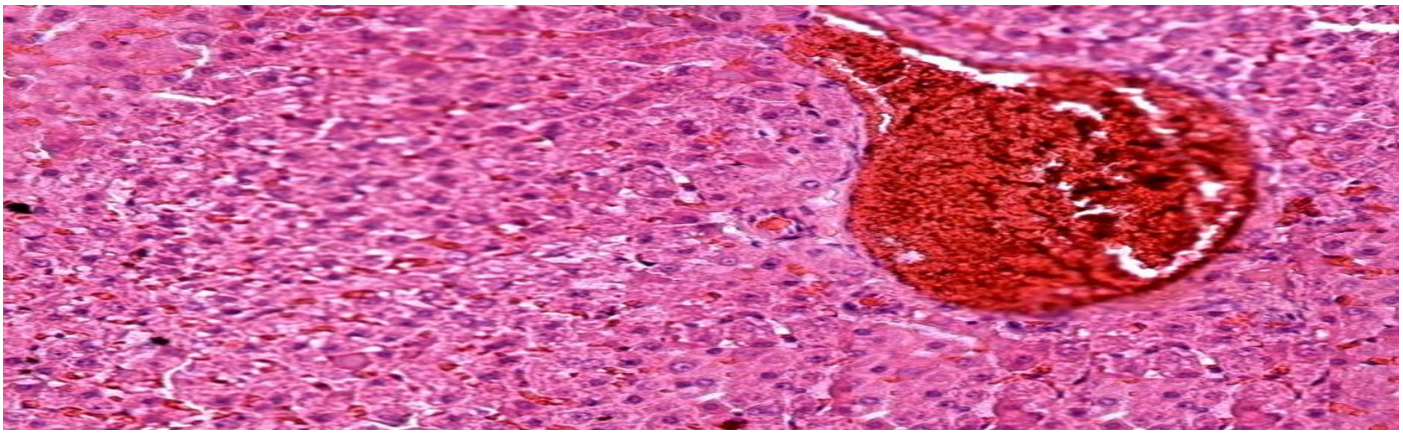

Figure 4a: *Photomicrograph illustrates structure of rabbit liver treated with therapeutic dose of cisplatin of (CIS) group. Note, severe congestion of central vein and surrounding sinusoids. H&E stain. X40.*

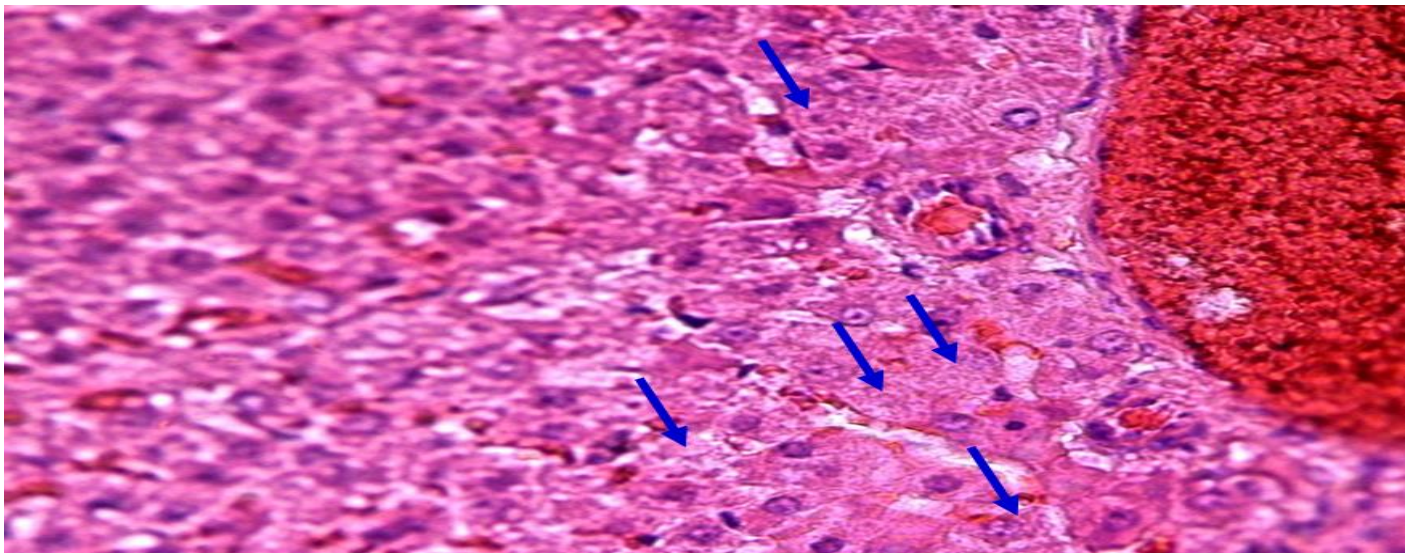

Figure 4b: *High magnification photomicrograph illustrates structure of rabbit liver treated with therapeutic dose of Cisplatin of (CIS) group. Note, severe congestion and distended of central vein and sinusoids. Distortion of hepatocytes and focal necrosis (arrow). H&E stain. X100.*

Marked necrosis of hepatocytes, that appeared deeply acidophilic, and some with pyknotic nuclei when compared with the control (CON) group. The hepatocytes appeared large with light and foamy cytoplasm filled with numerous vacuole-like spaces. Many hepatic cells were damaged and lost their characteristic appearance. Others showed severe cytoplasmic vacuolation which was so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass were left (Fig.5).

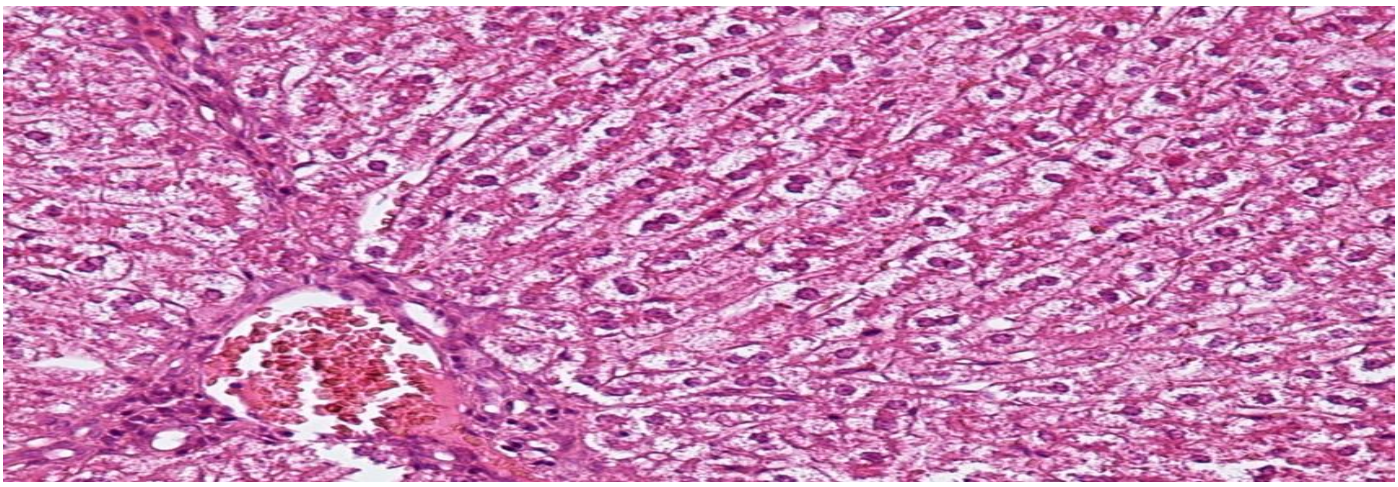

Figure 5: *Photomicrograph illustrates structure of rabbit liver treated with therapeutic dose of cisplatin of (CIS) group. Note, hepatocytes appeared large with light and foamy cytoplasm filled with numerous vacuole-like spaces. H&E.X20.*

Marked vacuolar degeneration mainly hydropic ballooning degeneration and hyper activation of Kupffer cells were observed. There were severe diltations and congestions of centeral veins. Some areas showed multifocal to diffuse type of coagulative necrosis with inflammatory cells infiltrations (Fig. 6).

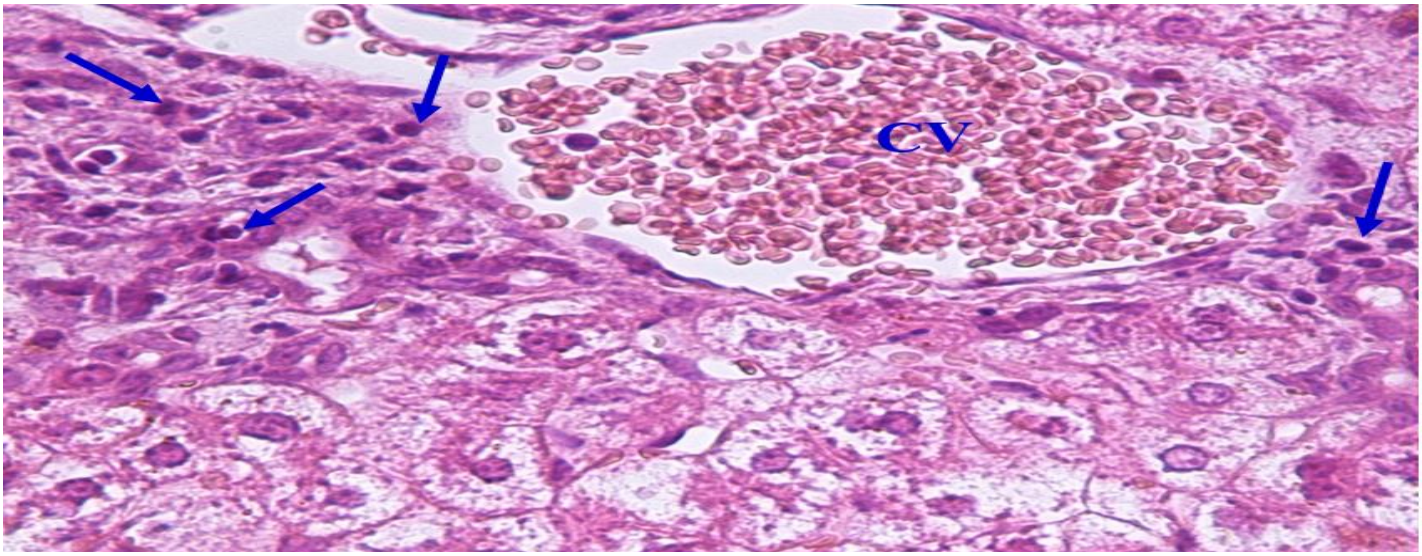

Figure 6: *Photomicrograph illustrates cross section of rabbit liver of group (CIS). Note, marked vacuolar degeneration, hyper activation of Kupffer cells and inflammatory cells infiltrations (arrow). Diltation and congestion of centeral vein (CV). H&E. X40.*

The portal traids of liver sections treated by cisplatin of (CIS) group, were showed congested and dilated portal vein and hepatic artery. The bile ductules were seen enlarged and hyperplasic and degeneration of their lining epithelium (Fig.7a).

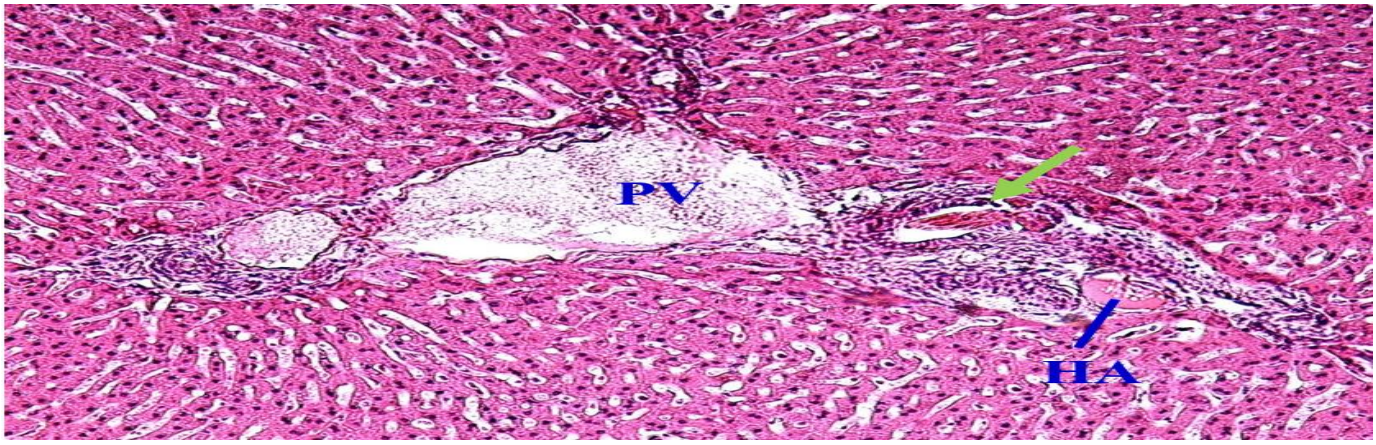

Figure 7a: *Photomicrograph illustrates cross section in portal area of rabbit liver of group (CIS). Note, distension*

and severe congestion of portal vein (PV) and hepatic artery (HA). The bile ductules were seen enlarged and hyperplasic (green arrow). H&E. X20.

Extensive congestion within the bile ductules was also seen. Mild periductal fibrosis around bile duct in the portal area were observed. Also, there were focal degenerative and necrotic changes along with focal mononuclear cellular infiltration (Fig. 7b).

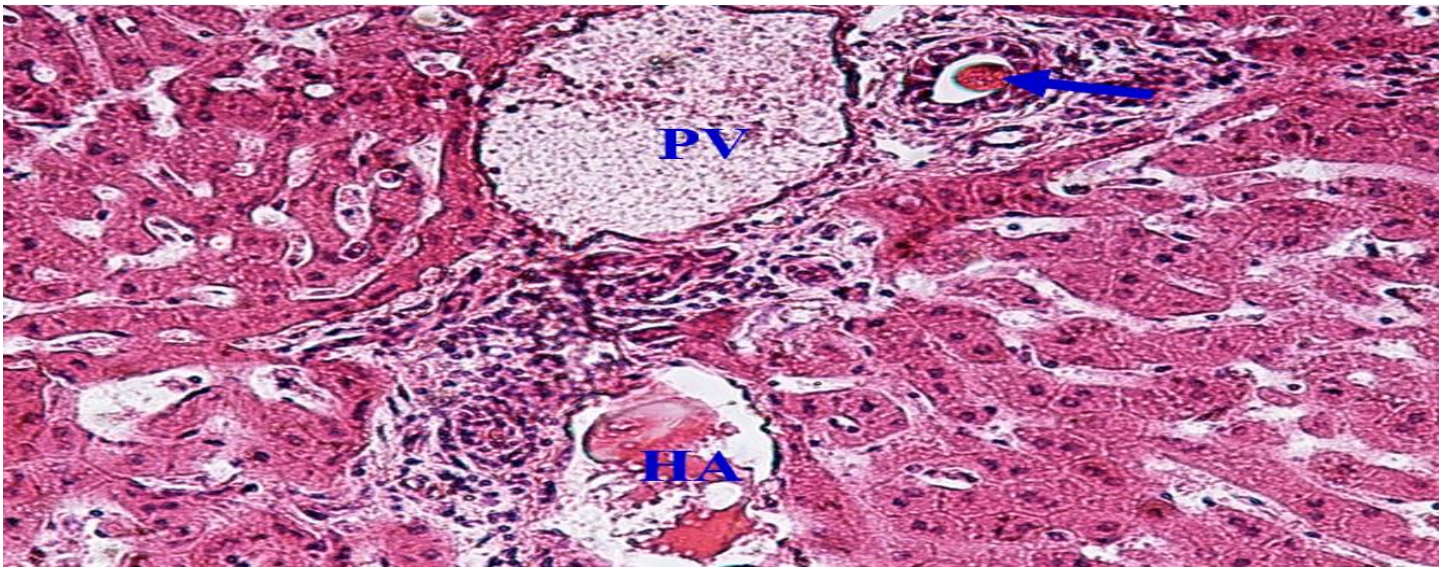

Figure 7b: *Photomicrograph illustrates cross section in portal area of rabbit liver of group (CIS). Note, extensive congestion within the bile ductules (arrow). Mild periductal fibrosis around bile ductules in the portal area. Portal vein (PV), hepatic artery (HA) H&E. X40*

Protective animals group (CIS+GSE):

Microscopic examinations showed that the severe hepatic lesions induced by Cisplatin were significantly decreased by the treatment with grape seeds extract, nearly normal liver histology in sections of liver received therapeutic dose of Cisplatin and treated by grape seeds extract (GSE) group was observed.The central vein was appeared more or less normal. The hepatocytes regained their normal organization and architecture. Moderate dilatations of blood sinusoids were also noticed. Mild congested in portal area was also observed (Fig.8).

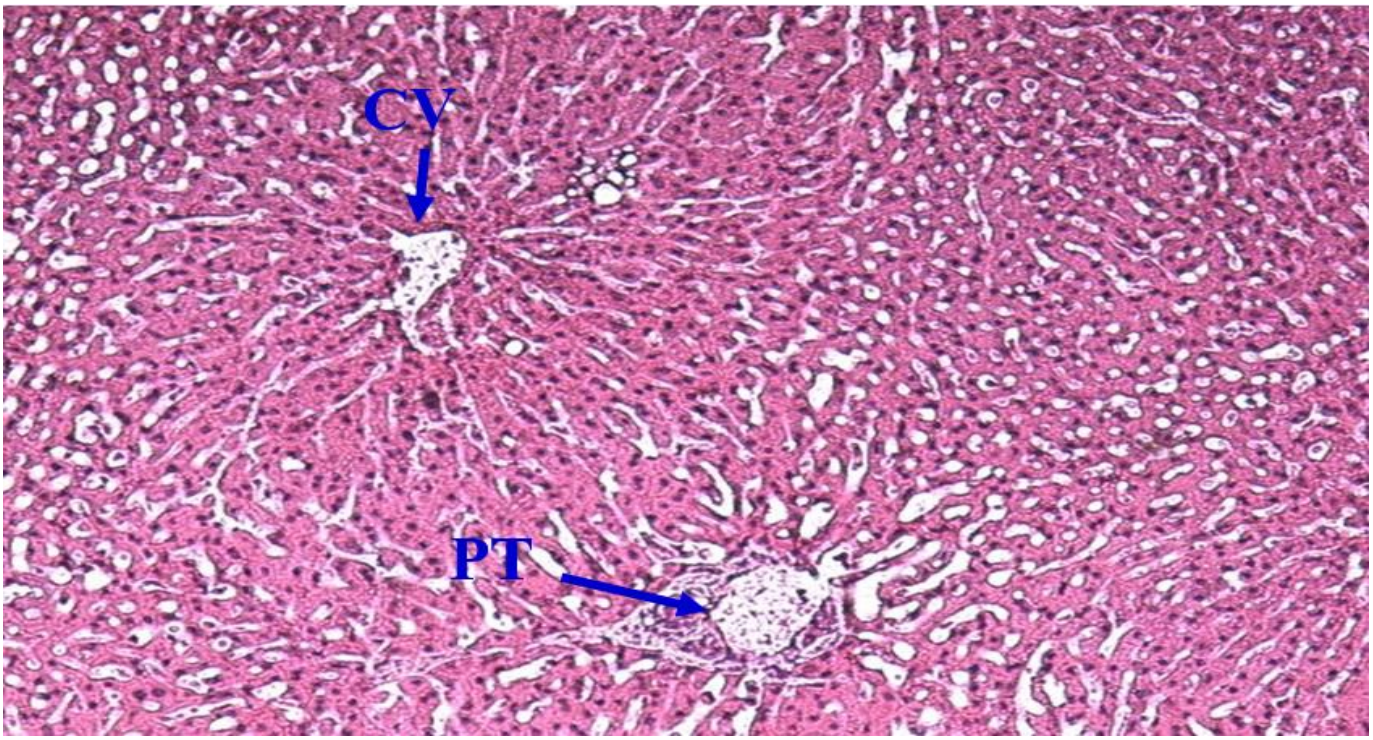

Figure 8: *Photomicrograph illustrates cross section of rabbit liver of group (CIS+GSE). Note, the central vein was appeared more or less normal. The hepatocytes regained their normal organization and architecture and moderate dilatations of blood sinusoids. H&E. X20*

Some sections of liver of (CIS+GSE) were demonstrated that the hepatocytes were comprised of moderate basophilic cytoplasm and their nuclei appeared dark rounded located centrally. The central veins still appeared dilated and congested. Passive hyperaemia were noticed within their lumen (Fig. 9).

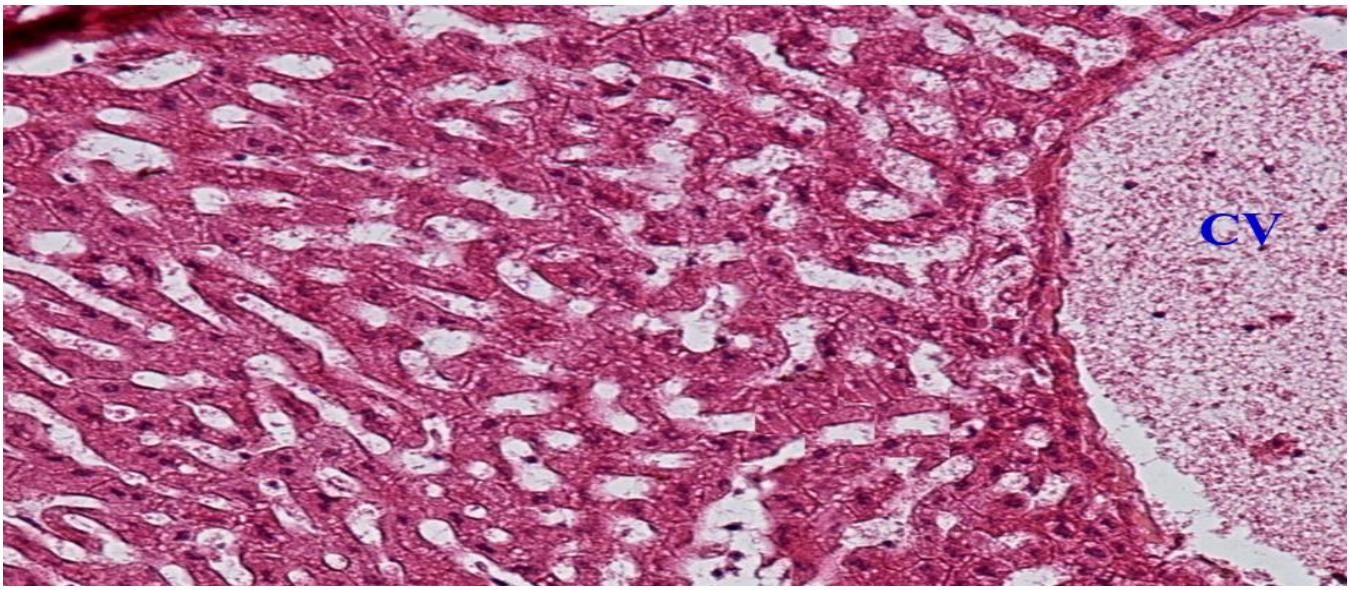

Figure9: *Photomicrograph illustrates cross section of rabbit liver of group (CIS+GSE).Note, hepatocytes had moderate basophilic cytoplasm and their nuclei appeared dark rounded located centrally. The central veins dilated and congested (CV) H&E. X40.*

High magnification of these sections of liver of (CIS+GSE) were explained that there were no cytoplasmic vacuolation, necrotic hepatocytes or marked degeneration of hepatic cords and there was no loss of hepatic tissue structural pattern. The blood sinusoidal spaces were appeared similar to normal. Some proliferation of Kupffer cells noticed within the blood sinusoids (Fig. 10).

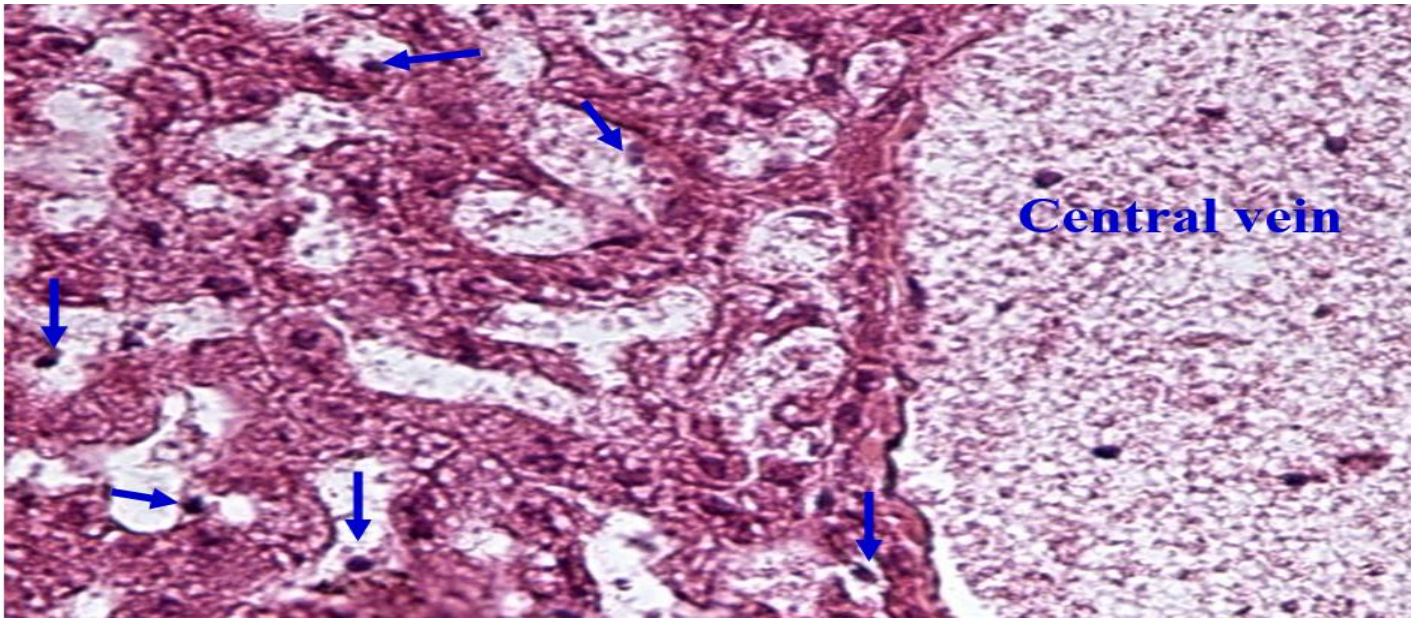

Figure 10: *High magnification photomicrograph illustrates cross section of rabbit liver of group (CIS+GSE). Note, no cytoplasmic vacuolation, necrotic hepatocytes or marked degeneration of hepatic cords. Some proliferation of Kupffer cells noticed within the blood sinusoids (arrow). H&E. X100.*

The portal traids of liver sections received therapeutic dose of Cisplatin and treated by grape seeds extract (CIS+GSE) group were reveled mild to moderate congestion in portal vein, hepatic artery and bile ductules (Fig. 11).

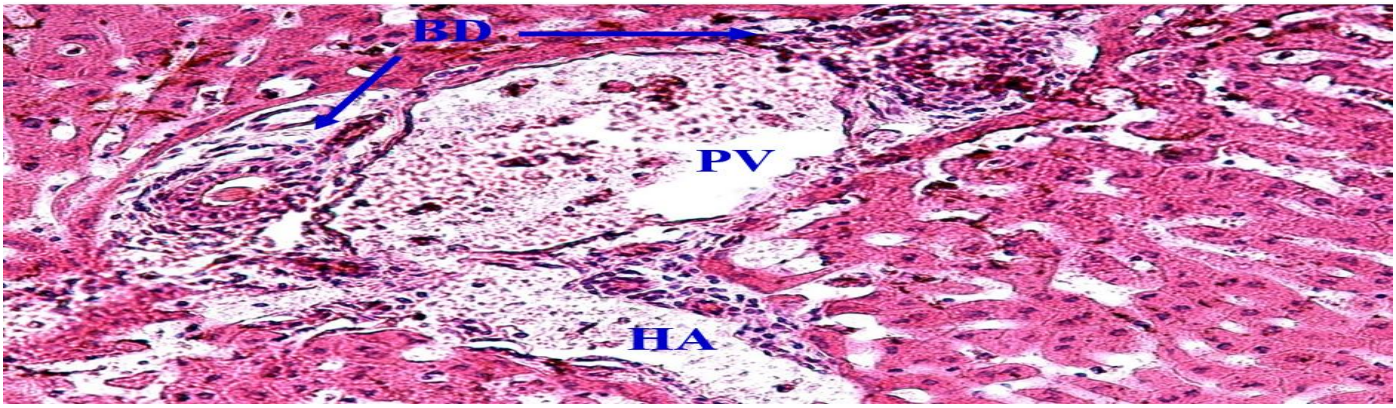

Figure11: *High magnification photomicrograph illustrates cross section in the portal space of the liver of group (CIS+GSE). Note, mild to moderate congestion in portal vein (PV), hepatic artery (HA) and bile ductule (BD).H&E. X40.*

The histopathological examinations were confirmed these results showing the improvement in the signs of hepatic degeneration and inflammatory cellular infiltration when were used the grape seeds extract during chemotherapeutic treatment.

DISCUSSION

Cisplatin (cDDP, cis-diaminedichloroplatinum II) is one of the most common and potent antineoplastic agents used against a broad range of malignancies including testicular, ovarian, cervical, bladder, head, neck as well as the lung. Despite its beneficial application as an antineoplastic agent, cisplatin causes several toxicities that limit its clinical use including nephrotoxicity, hepatotoxicity, cardiotoxicity, neurotoxicity, and ototoxicity [28,29,30].

Cisplatin exerts its action through the following mechanism. After entering the target cells, cisplatin binds to the cellular DNA to form a covalent complex. The drug causes reversible alkylation of guanine and adenine, and forms intra- and inter-strand cross-links in the DNA, thereby inhibiting elongation of DNA by DNA polymerase (inhibition of DNA transcription and replication). In addition, the formation of intra-strand cross-links results in changes in the conformation of the cells. These changes induce apoptosis and necrosis of the cancer cells, and underlie the antitumor effect of the drug [31]. Many experimental studies documented the common toxic side effects associated with cisplatin, including hepatotoxicity [32].

Several studies documented that the cisplatin toxicity is developed by the generation of reactive oxygen species (ROS) such as superoxide anion and hydroxyl radicals leading to damage of cells. Excess ROS induces cell arrest and apoptosis in both cancer cells and non-target normal cells [33]. Mora et al., 2003 added that a decrease in antioxidant enzymes resulted from cisplatin induced tissue toxicity [34].

Moreover, the development of therapies to prevent the appearance of cisplatin- induced tissue toxicities has focused on administration of antioxidants along with cisplatin treatment. Thus, many studies for protective effects against cisplatin induced tissue toxicities have been reported for extracts of natural products and dietary antioxidant [35].

Hepatotoxicity is the less well-known aspect of cisplatin treatment, and there is little information about the underlying mechanism. The major alterations reported in cisplatin toxicity are the occurrence of oxidative stress linked to the accumulation of reactive oxygen species and decreased efficiency of some antioxidant defense systems including antioxidant enzymes and non-enzymatic antioxidants such as reduced glutathione [36].

Several attempts have been conducted to ameliorate the hepatotoxic effect of cisplatin by using different antioxidant agents. There is limited information on the possible protective effect of grape seeds extract on cisplatin-hepatotoxicity. Most earlier studies were designed to administer the

extracts either before or after the cisplatin injection. However, in the present study, it was hypothesized that the administration of grape seeds extracts before and after cisplatin injection might ameliorate the toxic effect of cisplatin and/or accelerates the course of hepatic recovery.

In the current study, the clinical signs that were observed in group of animals treated intrapretonially by therapeutic dose of cisplatin (CIS group) as excessive sleeping, diarrhea, loss in ability to taste food and decrease movement, 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 [37]. Furthermore, the drug also affects the mucous lining of the gastro- intestinal tract and increased metabolic rate, which were considered side effects of the chemotherapy [38].

Our observations were corroborating the findings of previous researchers; Leite et al., 2009 [39]; Rickenbacher et al. 2011 [40]; Hesham and Ghobara, 2013 [41]; and Aboraya et al., 2022 [42].

In the present work, the histological descriptions for the slides stained by Haematoxylin and Eosin stain of control group (CON group) were noticed that the liver of the adult rabbits was formed of several lobules mingled with each other due to few or absent connective tissue septa between them, random irregular distribution of central veins within the hepatic lobules was observed.

The hepatocytes are polyhedral with smaller rounded nuclei arranged as irregular plates or cords. The portal triads lie at the angles of the hepatic lobules with dense connective tissue containing branches of hepatic artery, portal vein and bile duct. These findings are following other previous investigators; Al- Abdel- Maaboud et al., 2003[43]; Hamdany 2019[44]; and Al-samawy et al., 2022[45].

The results of the present investigation showed that cisplatin toxicity produced significant structural changes in the liver of group CIS, cisplatin treated group, in the form of prominent disorganized architecture and loss of liver lobulation, distortion of the arrangement of parenchyma of the liver, loss of radial arrangement of sinusoids from the central vein of the liver, Dilatation and congestion of central veins, sinusoids and portal traids, degenerative changes in hepatocytes and pyknotic nuclei indicating necrosis. Marked vacuolar degeneration mainly hydropic ballooning degeneration and hyper activation of Kupffer cells and inflammatory cellular infiltrations were observed. These findings agree with many authors who reported the toxicity of cisplatin on the liver; El-Sayyad et al. 2009 [46]; Kart et al., 2010 [47]; Karadeniz et al., 2011 [48]; Kandemir et al., 2012 [49]; Hesham and Ghobara, 2013 [41]; Mir et al., 2015 [50].

In contrast, Leite et al., 2009 stated that, concerning hepatotoxicity, no histopathological alteration was observed after treatment by cisplatin [39].

Cell necrosis and vacuolization induced by cisplatin toxicity as shown in the present work were described previously by other studies; El-Sayyad et al. 2009 [46]; Abdelmeguid et al., 2010 [51]; Attyah and Ismai, 2012 [52].

Robbins and Angell, 1976 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 [53].

In our study, cisplatin -treated hepatocytes of liver of group (CIS) were shown pyknotic nuclei, indicating apoptosis in the rabbit liver. Nasr, 2013 explained by using electron microscopic analysis of liver sections of cisplatin -treated rats, that there was degeneration of cellular organelles, including the mitochondria, and vesicular dilation of the rough endoplasmic reticulum, disturbed nuclear envelope, reduced amount of condensed heterochromatin on the nuclear envelope around the wide nuclear pores with an absence of nucleolus have been observed [54].

This result was reflected our work. It was evidenced that, the reactive oxygen resulting from oxidative stress in the tissue causes a significant alteration in the cellular structure resulting in tissue injury and cell damage.

Cisplatin which is a chemotherapeutic drug used in the treatment of various cancer types was determined to cause liver toxicity and to weaken the antioxidant defense systems in liver tissue and to increase oxidative stress. This is in accordance with Kandemir et al., 2012 who observed that intra-hepatic blood vessels, central and portal veins are congested and their lining epithelia are eroded. This is besides an inflammatory cellular infiltration [49]. These observations were more pronounced in our study on the livers of rabbits which were given therapeutic dose of cisplatin per week and for six weeks.

The histopathological alterations in the hepatic tissue could be explained by clear indications of cellular leakage and loss of functional integrity of the mitochondrial membrane resulting from liver damage, and due to mitochondrial dysfunction may be generated by the disruption of β-oxidation of lipids and oxidative energy production within the hepatocytes. Mitochondrial membrane

permeabilization can lead to apoptosis, a rupture in mitochondrial membrane can lead to ATP depletion and subsequent necrosis [51]. The mitochondrial enzymes have been called as one of the major cellular generators of ROS and one of the important antioxidant enzymes is catalase which is scavenged H_2O_2. Moreover, cisplatin treatment reduced the glutathione level and antioxidant enzyme activities, increased lipid peroxidation reduced cell viability, enhanced ROS generation and caused hepatic DNA fragmentation which ultimately leads to cellular necrosis [55].

The appearance of inflammatory cells in the hepatic tissue, due to cisplatin exposure, may suggest that the cisplatin could interact with proteins and enzymes of the hepatic interstitial tissue interfering with the antioxidant defense mechanism and leading to reactive oxygen species generation which in turn may imitate an inflammatory response [56].

The histopathological findings present in our study were supported by the biochemical parameters that were indicated by previous studies who mentioned that the oxidative stress was implicated in the pathogenesis of liver injury induced by cisplatin. Therapeutic doses of cisplatin demonstrated liver function impairment as a result of increased levels of enzymatic biomarkers including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine and malonaldehyde (MDA), whereas the activity of serum albumin and calcium levels were decreased after cisplatin injection [57,58]. The increases in these enzymatic biomarkers activities in liver after cisplatin exposure may supports adaptation of liver associated deleterious alterations in the liver of rabbits. It suggests that required ATP for detoxification mechanism or DNA repair mechanism against to DNA damage by induced cisplatin toxicity increased.

Recent trends in controlling and treating diseases tend to favor natural antioxidant compounds rather than synthetic ones. The human diet, which contains a large number of natural compounds, is essential in protecting

the body against the development of diseases. Grape is one of the well-known plants with remarkable anti-carcinogenic and antioxidant properties [59]. Grape seeds extract is considered to be bioactive compounds because they influence physiological and cellular processes and, therefore, can have beneficial effects on health [60].

The results of the current study, in the sections from the liver of animals received therapeutic dose of cisplatin and treated by grape seed extract, group (CIS+GSE) clearly showed that there was

a significant alleviated hepatotoxicity, since histopathological changes were markedly less pronounced compared to animals treated with cisplatin alone group (CIS). The central vein was appeared more or less normal. The hepatocytes regained their normal organization and

architecture. Moderate dilatations of blood sinusoids and mild congested in portal area were also noticed. These findings were in agreement with the previous studies [61,62,63,64].

In the present work, the combination of grape seeds extracts with cisplatin ameliorated most of these obvious previous findings. Kart et al, 2010; Abdelmeguid et al., 2010 who demonstrated the structural changes in the hepatic parenchyma in cisplatin-treated rabbits or rats respectively, and its reversal by different antioxidants such as caffeic acid phenethyl ester or silymarin [47,51].

This could be due to an increase in the flow of blood within the vasculature of the liver by the action of grape seeds extract. This explained the mild congestion that found within the central vein and the portal area manifested in our study. It was determined that grape seed extract co-treatment was markedly efficient in reducing ROS accumulation and strengthening endogenous antioxidant systems leading to a considerable attenuation of the hepatotoxicity as evidenced by histopathological examinations [52]. In addition, the grape seed extract contains polyphenolic compounds that have powerful free-radical-scavenging effect and antioxidant activity, which exert vasodilatory, anticarcinogenic, anti-inflammatory and immune-stimulating activities [65].

Cisplatin which is a chemotherapeutic drug used in the treatment of various cancer types was determined to cause liver toxicity and to weaken the antioxidant defense systems in liver tissue and to increase oxidative stress. Whereas the grape seed extract application can be considered as

a supportive adjuvant therapy able to reduce or prevent the hepatoxic effects of cisplatin [49].

These facts support our findings that the hepoatocytes regained their normal organization and architecture. The cytoprotective effect of grape seed 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 grape seed 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 cisplatin-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. A combination of these antioxidants with cisplatin produced a significant decrease in liver enzymes when compared with the cisplatin-treated alone. The improvement of liver 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].

Nuria et al., 2008 said that, cisplatin toxic side effects seemed to be associated with mitochondrial injury both with in vivo treatment with the drug and in vitro exposure to it. They also showed that cisplatin caused a direct and significant impairment of mitochondrial DNA and RNA synthesis [68]. Moreover, the histopathological findings of cisplatin-treated animals were confirmed at the ultrastructural level, where size of the hepatocyte, dilated rough endoplasmic reticulum, degenerated mitochondria, disturbed nuclear envelope, reduced amount of condensed heterochromatin on the nuclear envelope around the wide nuclear pores with an absence of nucleolus, have been observed. By using of various antioxidants, most of these ultrastructural changes were significantly improved. This finding reflected the decrease of the cytotoxic effects of cisplatin in conjunction with these different antioxidants where, the number of mitochondria was significantly increased with subsequent production of an excessive amount of glutathione that overcame the cytotoxicity of cisplatin. Thus, an increase in number and function of mitochondria in combined different antioxidants and cisplatin-treated animals might reflect the target chemotherapeutic and toxic effects of cisplatin on mitochondria; therefore, mitochondrial dysfunction could be a major mechanism of cisplatin-induced hepatotoxicity [46,47,49,51,52,69]. Considering that, the grape seeds extract is one of the most important antioxidants, thus and based on our findings, it contributes significantly to the improvement of histological alterations in rabbit liver caused by cisplatin.

CONCLUSION

From the findings of our present study, we conclude that cisplatin has toxic and various other adverse effects on rabbits given cisplatin injections in doses equivalent to the human therapeutic dose. General toxicity demonstrated in prominent disorganized architecture and loss of liver lobulation, distortion of the arrangement of parenchyma of the liver, loss of radial arrangement of sinusoids from the central vein of the liver, Dilatation and congestion of central veins, sinusoids and portal traids, degenerative changes in hepatocytes and pyknotic nuclei and marked vacuolar hydropic ballooning degeneration and hyper activation of inflammatory cellular infiltrations.

It was evidenced that, the reactive oxygen resulting from oxidative stress in the tissue causes a significant alteration in the cellular structure resulting in tissue injury and cell damage. Moreover, cisplatin treatment reduced antioxidant enzyme activities, reduced cell viability and caused hepatic DNA fragmentation which ultimately leads to cellular necrosis.

Grape seeds extract is one of the well-known plants with remarkable anti-carcinogenic and antioxidant properties. Using of grape seed extract accompanied with cisplatin clearly showed that there was a significant alleviated hepatotoxicity, since histopathological changes were markedly less pronounced compared to animals treated with cisplatin alone.

RECOMMENDATION:

Our results may provide histological evidence that proved that the cisplatin cause direct hepatic toxicity. Appropriate protective measures must be applied with anticancer treatment for improving liver function, which could be used as the basis for determining the appropriate dose of these drugs to reduce their hepatotoxic effects. Thus, we recommend to use grape seed extract concomitant with this drug based on our findings, it contributes significantly to the improvement of histological alterations in rabbit liver caused by cisplatin.

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