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The probable protective Effect of Quercetin Against Doxorubicin-Induced Hepatotoxicity in Adult Albino Rats: A Biochemical and Immunohistopathological Study

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Abstract

Background: Doxorubicin (DOX) is an anthracycline antineoplastic which causes hepatotoxicity by induction of free radicals and inflammatory liver tissues. Quercetin (QCT) is considered as a potent antioxidant that may ameliorates hepatotoxicity. **Aim of the work:** The current study was designed to detect the toxic effects of DOX in liver cells and evaluating the possible protective role of QCT against those toxic alterations in adult male albino rats. **Materials and methods:** forty (40) male albino rates were divided to four groups: Control (group I), DOX-treated (group II): Rats received 18 mg/kg of body weight of DOX. Group III, received 60 ml/kg of QCT combined with DOX. Group IV treated with DOX plus 100 ml of QCT/kg. After scarification of rats, liver enzymes and the bile levels were estimated. The livers were extracted and used in oxidative stress markers, histological and immunological studies. **Results**: Doxorubicin administration induced an increase in liver enzymes, bile level and oxidative stress markers. QCT administration induced an improvement in those levels, especially with the high dose. The microscopic examinations of the DOX-treated sections showed loss of normal architectures of hepatic cells, enlargement and congestion of the central and portal veins and blood sinusoids, vacuolar degeneration, cholelithiasis and necrosis of H&E stained hepatocytes. PAS-stained liver sections in the DOX-treated group showed an increase in the depth of the stain. Quercetin administration improved these changes and hepatocytes which stained with PAS showed moderate to mild staining, especially with the high dose of QCT. Sections of the stained liver caspase-3, in DOX-treated group showed positive results with intensity. QCT treated groups showed little effects of caspase-3, especially with the high dose. **Conclusion:** Doxorubicin had toxic effects on rats' livers, with significant improvement after treatment with QCT as an antioxidant substance, and its high doses were more protective than low doses.

Key words Doxorubicin, Quercetin, hepatotoxicity, albino rats

Introduction

Doxorubicin (DOX) is an anthracycline antineoplastic drug which used in treatment of many malignancies. Its significant dose-dependent chronic toxicity as nephrotoxicity and hepatotoxicity led to its limited clinical usage (Beshay et al., 2011). Although the full mechanisms of DOX-related toxicities are not completely understood yet DOX induces hepatotoxicity by induction of free radicals which generate oxidative damage besides its inflammatory changes in rates' hepatic tissues (Bulucu et al., 2009). Reactive oxygen species (ROS) may cause liver's membrane damage which induces liver enzymes release. DOX-induced toxic effects include increase in superoxide dismutase, catalase and glutathione peroxidase enzymes in liver tissue so, trying to control that oxidative injuries is highly appreciated (Alshabanah et al., 2010). Quercetin (QCT) is the most abundant polyphenolic flavonoid in nature, present in large amounts in vegetables and fruits as onions, broccoli, apples, grapes and green tea. It considered as a potent antioxidant as it has an antioxidant potential four times more than that of vitamin E (Dong et al., 2014). It was reported that QCT has many effects, including anti-allergy and anti-inflammatory effects (Reutrakul et al., 2007). QCT can protect against drug-induced genotoxicity, hepatotoxicity, nephrotoxicity and oxidative stress in vivo (Qader et al., 2014). The antioxidant ability of QCT may be explained due to its high diffusion into cell membrane which permits it to sweep oxy radicals (Moskaug et al., 2004). QCT can ameliorate the hepatotoxic effect of diethylnitrosamine in rats by reducing aminotransferase (ALT) and aspartate aminotransferase (AST) serum levels and improves hepatic lipid peroxidation and hepatic glutathione (GSH) (Gupta et al., 2010) so, QCT may have a protective effect against DOX hepatotoxicity (Tiong et al., 2010).

Aim of the Study

The current study tried to detect the toxic effects of DOX on liver cells and evaluating the possible

protective roles of QCT against those toxic alterations that might be induced in adult male albino rats.

Materials and Methods

The present study was conducted on forty healthy adult male albino rats (12–14 weeks) weighing 180–200 g. which maintained under controlled laboratory conditions of a 12-hours light and 12-hours dark cycle, at 25± 2°C. All animals were provided with water and a standard pellet diet ad libitum. Prior to be utilized for experimental purpose, rats were left for two weeks allowing them to acclimatize to the new environment.

Experimental design: After the two weeks acclimatization period, the rats were divided randomly into four equal groups, each of ten rats as follows:

Group I (control group): Where rats were further subdivided into: Group Ia (negative control) where animals did not receive any treatment and Group Ib (positive control) where animals received a daily saline in a dose equivalent to that of DOX orally through gavage (Hiromasa et al., 2005 and Richter et al., 2007).

Group II (DOX-treated group): Where rats received DOX in a dose of 6 mg/kg intra-peritoneal (I.P.) every week of the experiment (at days 1, 8, and 15 of the experiment) (Milic et al., 2009 and Ali et al., 2015).

Group III (DOX-treated plus QCT-treated low dose group): Where rats received an oral dose of QCT in a dose of 60 mg/kg body weight/day) combined with DOX in the same regimen as Group II (Richter et al., 2007 and Verma and Sangai, 2009).

Group IV (DOX-treated plus QCT-treated high dose group): where rats received an oral daily dose of QCT in a dose of 100 mg/kg body weight combined with DOX in the same regimen as Group II (El-Beshbishy et al., 2012).

After 30 minutes of DOX injection, QCT was administered orally through gavage. All rats were weighed daily, and the doses of the drugs were adjusted accordingly as the experimental period lasted for 21 days.

Drugs: Adricin vial containing 2 mg/ml of DOX was purchased from EIMC United Pharmaceuticals (Cairo, Egypt), and diluted with saline solution. DOX dose was equal to the lowest dose that is commonly used and resulting in serum levels close to those observed in human serum (Richter et al., 2007). An examination of data from sub-chronic and chronic toxicological studies support a dose of 18 mg /kg body weight as the used therapeutic dose of DOX is 60–75 mg/m2 IV once every 21 days. This dose is equivalent to 18-25 mg/kg in rats (Poole et al., 2015 and Hajra et al., 2018). QCT powder as pentahydroxy flavone (Q4951 Sigma) was dissolved in saline solution (Verma and Sangai, 2009). QCT dose was chosen on the basis of that it prevented daunorubicin-induced elevation of plasma concentrations of lactate dehydrogenase and creatine kinase (Guzy et al., 2003)

Sample collection: At the time of sacrifice, the rats were anesthetized with ketamine (80 mg/kg intraperitoneal). Body weight was measured before euthanization and livers were measured after euthanizing the animals and subjected for studies.

Biochemical study:

Measurement of liver function and hepatic necrosis markers:

Blood samples were collected from the retro-orbital venous plexus of all rats and centrifuged at 1500 rpm for 10 minutes at room temperature and the serum was used for the estimation of total liver markers after separation. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB) was determined by enzymatic colorimetric kits from Bio-diagnostic (Egypt) and reflotron plus machine by Roche, USA according to Reitman and Frankel (1957).

Estimation of oxidative stress markers and antioxidants:

Specimens of the liver tissue had washed by solution of ice-cold 0.9% NaCl and homogenized in 9% ice-cold phosphate-buffered saline with PH 7.5. The homogenate had centrifuged at 3000 r.p.m for about 15 min. and the supernatant was collected and kept at -80ºC (Fernadez-Botran et al., 2002). Hepatic level of malondialdehyde (MDA) as oxidative stress marker was measured according to Kei (1978) and glutathione (GSH) concentration as antioxidant marker was estimated according to Beutler et al (1963).

Histological study: Specimens of the liver tissues were fixed in 10% formalin, dehydrated and embedded in paraffin blocks then processed to prepare 4-µm-thick for Haematoxylin and Eosin staining (Hx.&E.) for studying the hepatic lobules architecture. Periodic Acid Shiff staining (PAS) was used for studying glycogen contents (Bancroft and Gamble, 2002). Sections were studied using an image analyzer (Olympus Image J, NIH, 1.41b; Olympus, America Inc., Melville, New York, US (Lirdi et al., 2008).

***Immunohistochemical study*:** Paraffin embedded blocks were further immunohistochemically analyzed using caspase-3 (Rat polyclonal Antibody, CPP32, Ab-4, Thermo, UK) that purchased from LIFESPAN (BIOSCIENCE) for detection of caspse-3 cells markers in liver tissues.

Ethical considerations: After approval of Animal Ethics Committee to the experimental design, the experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care. Animals were handled only by the investigator and maintained in manners that provide their physical comfort. There was no interference except after complete anesthesia and at the time of sacrifice. The rats were anesthetized with ketamine not with ether inhalation as it is forbidden now.

Statistical analysis: All values were presented as mean± SD. The differences among the groups with respect to all measured data parameters were statistically analyzed using one-way analysis of variance (ANOVA) and the post-hoc test using SSPS program, version 17 (IBM Corporation, Somers, New York, USA). The calculations were considered significant if *P* value was less than 0.05.

Results

The body and liver weight: Table (1) showed the rats body weights changes throughout the experiment in all groups. It showed non-significant decrease in all groups in comparison to control group.

Table (2) showed the liver weight values in all groups, as DOX-treated group showed a high significant increase in liver weight compared to control group while the QCT treated group with low and high levels showed significant decrease compared to DOX-treated group and a significant increase compared to control group.

Biochemical results: Table (3) showed that serum level of ALT, AST, ALP and bilirubin are significantly increased in group II (DOX-treated) in a comparison with control group ($p<0.05$). The values showed a significant decrease in groups III and IV (QCT-treated) in comparison with group II (DOX-treated) and they remained significantly higher than their counterparts in control group ($p <0.05$).

Table (4) showed that MDA as an oxidative stress marker was significantly increased in group II (DOX-treated) in a comparison to control group ($p<0.05$), and significant decrease in groups III and IV (QCT-treated) in comparison to group II (DOX-treated). It remained significantly higher than their counterparts in control group ($p<0.05$). The antioxidant marker (GSH) level in DOX-treated group showed a high significant decrease in comparison to control group. The QCT treated groups showed significant increase in comparison to both DOX-treated and control groups.

Liver Histo-Pathological changes: H&E stained sections of control group illustrated, the structural unit of the liver (hepatic lobule) formed of radiating cords of hepatic cells forming a network around the central vein and radiating to the lobular periphery (Figures 1and 2). The hepatic cells appear cuboidal to polyhedral in shape with a centrally located nucleus. The hepatic cords are alternating with blood sinusoids, which lined with a discontinuous layer of flattened and fenestrated endothelial cells. The so-called portal triad consists of a portal venule, a hepatic arteriole and a bile ductule is illustrated in (Figures 3 and 4). Control group PAS stained sections showed faint positive PAS stained hepatocytes with scattered areas of glycogen precipitation (Figure 19). Examination of liver sections of H&E stained hepatocytes in DOX-treated group II, revealed loss of normal architecture of hepatic lobules. Vascular changes such as dilatation and congestion of central and portal venules, hepatic arterioles and blood sinusoids, vacuolar degeneration of hepatocytes, cholestasis, and focal areas of necrosis, inflammatory infiltrations and hepatocytes nuclei showed pyknosis are illustrated in (Figures 5-11). DOX-treated group II showed high positive PAS stained hepatocytes with decreased glycogen contents (Figure 20). Examination of DOX-treated plus QCT-treated (low dose) group III sections which stained with H&E, showed slight restoration of normal architecture of hepatic lobules and normal arrangement of hepatocytes in the centrilobular areas that were decreased and lost towards the periphery (Figures 12-14). DOX-treated plus QCT-treated (low dose) group III showed moderate positive PAS stained hepatocytes with moderate glycogen contents (Figure 21). In DOX-treated plus QCT-treated (high dose) group IV, H&E stained hepatocytes, revealed intact and well defined cellular boundaries, few areas of vacuolar degeneration of hepatocytes. Slight congestion of central, portal and hepatic vessels and dilated congested blood sinusoids with slight inflammatory infiltrations were illustrated in (Figures 15-18). DOX-treated plus QCT-treated (high dose) group IV showed mild positive PAS stained hepatocytes with mild glycogen contents (Figure 22).

Immunological results: Caspase-3 immunostaining showed a negative cytoplasmic Caspase-3 immuno-expression in control group I, a dense cytoplasmic Caspase-3 immuno-expression in DOX-treated group II. A few traces of brownish cytoplasmic Caspase-3 immuno-expression in DOX-treated plus QCT-treated (low dose) group III and a very few cytoplasmic Caspase-3 immuno-expression in DOX-treated plus QCT-treated (high dose) group IV illustrated in (Figures 23-26).

Table (1): Effect of Doxorubicin (DOX) and its combination with Quercetin (QCT) on body weight of rats using one-way ANOVA test

Groups	Body weight (gm) Mean ± SD				
	In the first day	After one week	After two weeks	Day of scarification	P
Group I	196 ± 5.5	200 ± 7.7	201 ± 5.6	202 ± 5.6	
Group II	197 ± 5.6	196 ± 4.3	196 ± 1	195 ± 3.4	0.107
Group III	195 ±16	195 ± 4.4	195 ± 1.1	195 ± 1	0.105
Group IV	197 ± 6	197 ± 3.3	197 ± 5.7	196 ± 4.9	0.112

Each group consists of 10 rats, Group I: Control, Group II: DOX-treated group, Group III: DOX-treated plus QCT-treated 60 mg/kg body weight group, Group IV: DOX-treated plus QCT-treated 100 mg/kg body weight group, SD: Standard Deviation

Table (2): Effect of Doxorubicin (DOX) and its combination with Quercetin (QCT) on liver weight of rats using one-way ANOVA test

Groups	Liver weight (gm) Mean± SD	P
Group I	7.5 ± 0.14	< 0.05
Group II	13 ± 0.56*[a]	< 0.05
Group III	11.9 ± 2.5*[a,b]	< 0.05
Group IV	10.7 ± 2.6*[a,b]	< 0.05

*Each group consists of 10 rats, Group I: Control, Group II: DOX-treated group, Group III: DOX-treated plus QCT-treated 60 mg/kg body weight group, Group IV: DOX-treated plus QCT-treated 100 mg/kg body weight group, SD: Standard Deviation, * P < 0.05 significant, a compared to control group, b compared to DOX-treated group.*

Table (3): Effect of Doxorubicin (DOX) and its combination with Quercetin (QCT) on liver functions parameters of rats using one-way ANOVA test

Parameters Mean± SD	Group I	Group II	Group III	Group IV	P
ALT (U/L)	27.34 ± 5.6	133.6 ± 6.9*[a]	75.8 ± 4.7*[a,b]	44.5 ± 4.3*[a,b,c]	< 0.05
AST (U/L)	32.6 ± 5.7	127± 5.7*[a]	67.8 ± 4.3*[a,b]	40.22 ± 6.3*[a,b,c]	< 0.05
ALP (U/L)	148.9 ± 5.2	194.7 ±10.45*[a]	166.76 ± 12*[a,b]	158.96 ± 10*[a,b]	< 0.05
Bilirubin (mg/dl)	0.29 ± 0.2	0.80 ± 0.06*[a]	0.39 ±0.05*[a,b]	0.33 ± 0.03*[a,b]	< 0.05

*Each group consists of 10 rats, Group I: Control, Group II: DOX-treated group, Group III: DOX-treated plus QCT-treated 60 mg/kg body weight group, Group IV: DOX-treated plus QCT-treated 100 mg/kg body weight group, SD: Standard Deviation, * P≤0.05 significant, a compared to control group, b compared to DOX-treated group, c compared to DOX+ QCT-treated group (60mg/kg body weight group).*

Table (4): Effect of Doxorubicin (DOX) and its combination with Quercetin (QCT) on liver oxidative markers of rats using one-way ANOVA test

Parameters Mean± SD	Group I	Group II	Group III	Group IV	P
MDA (mmol/g tissue)	47.17± 4.1	83.16 ± 4.38*[a]	58.66 ± 4.96*[a,b]	50.23 ± 3.2*a,b	< 0.05
GSH (mmol/g tissue)	6.29 ± 0.2	4.26 ± 0.3*[a]	5.62 ± 0.32*[a,b]	6.36 ± 0.37*a,b	< 0.05

*Each group consists of 10 rats, MDA: Malondialdehyde, GSH: Glutathione, Group I: Control, Group II: DOX-treated group, Group III: DOX-treated plus QCT-treated 60 mg/kg body weight group, Group IV: DOX-treated plus QCT-treated 100 mg/kg body weight group, SD: Standard Deviation, * P ≤ 0.05 significant, a compared to control group, b compared to DOX-treated group.*

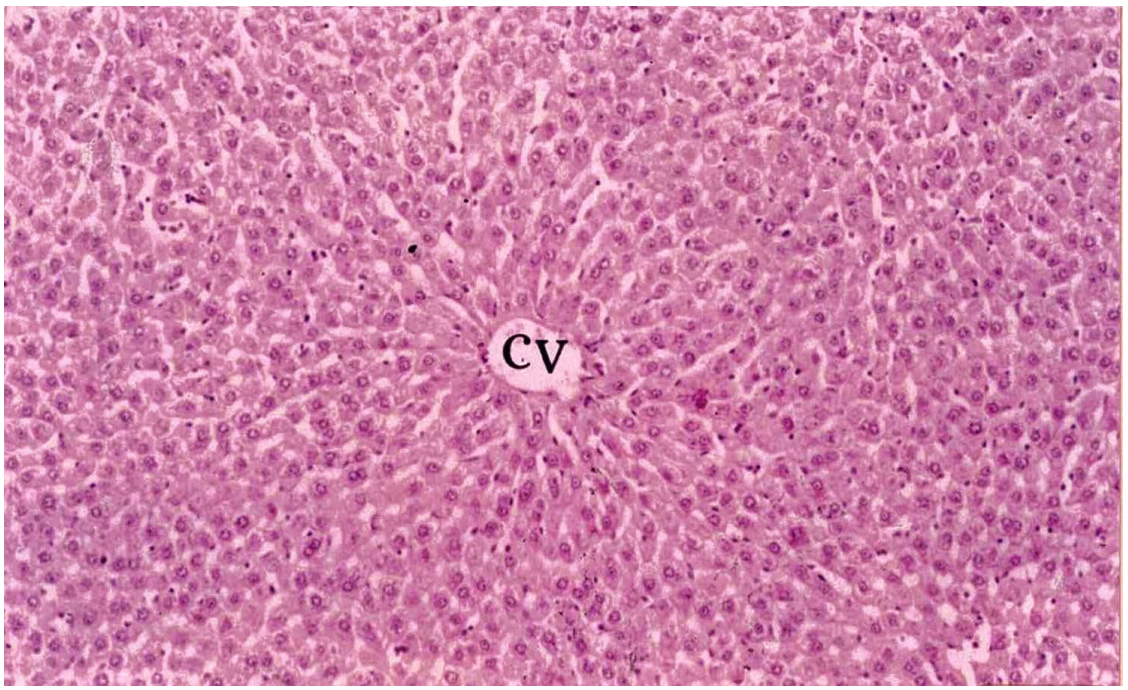

Figure (1): A photomicrograph of liver section in (control group I) showing normal architectural appearance of the hepatic lobules as they arranged in cords radiating from the central vein (CV) (H&E X 100)

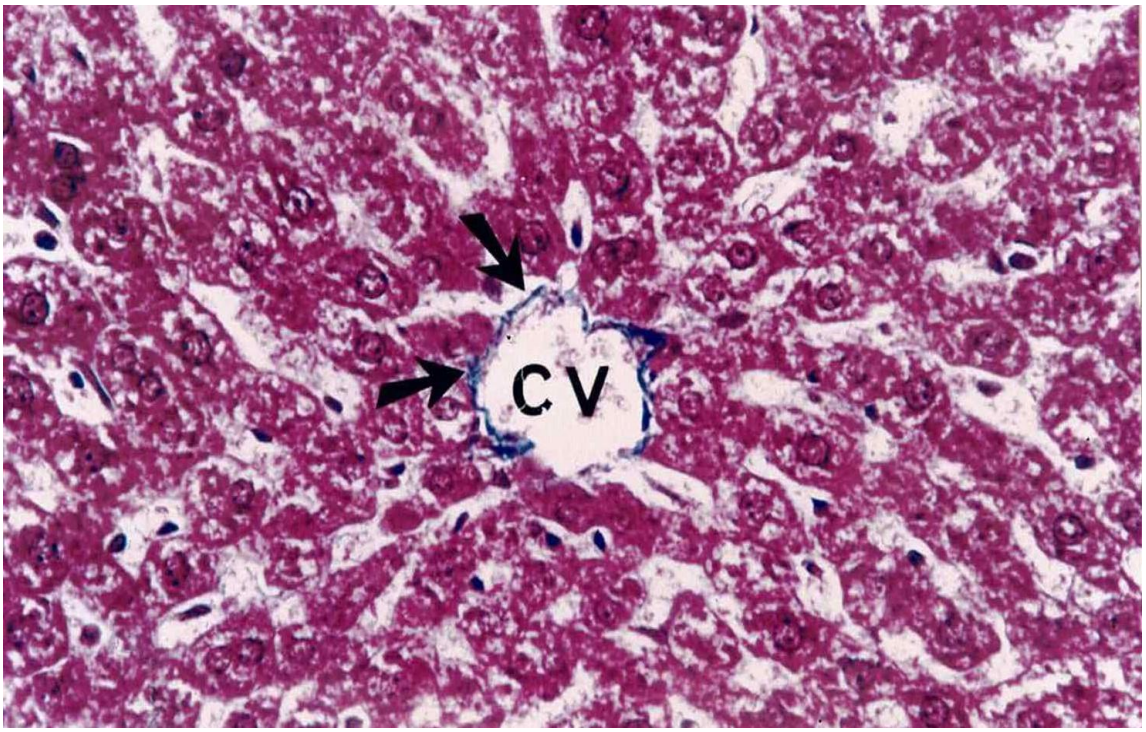

Figure (2): A photomicrograph of liver section in (control group I) showing normal connective tissue (arrows) surrounding the central vein (CV) which observed as a thin layer of collagenous fibers (H&E X 200)

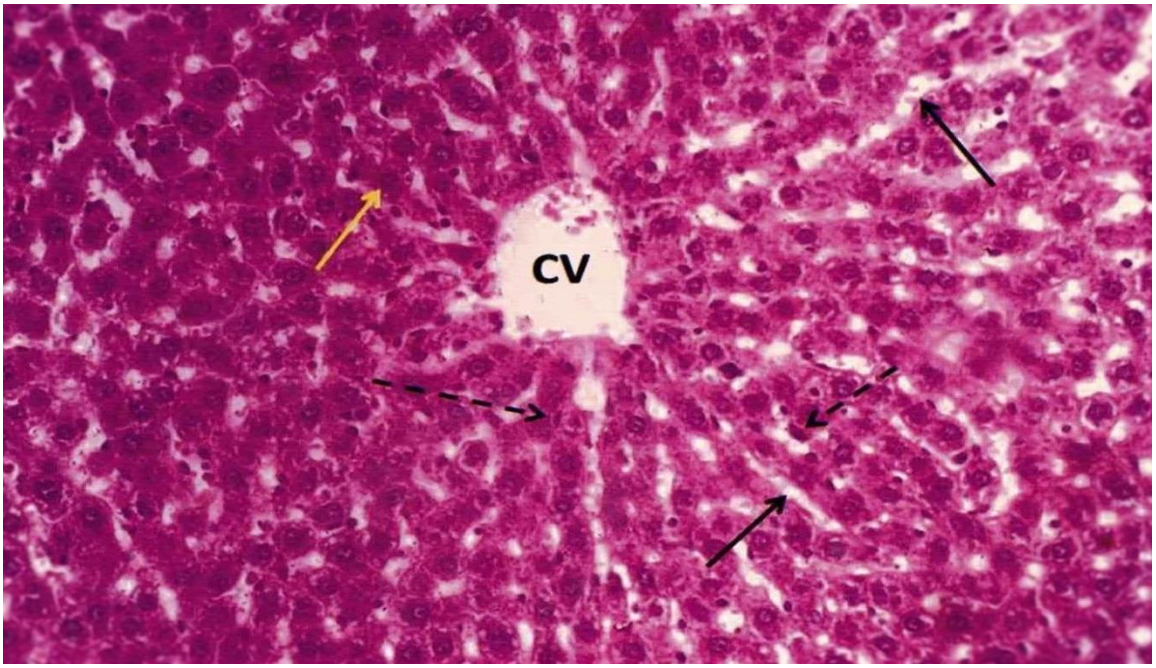

Figure (3): A photomicrograph of liver section in (control group I) showing normal architecture of hepatic lobule formed of hepatocytes (yellow arrow) which arranged in single-cell thick plates radiating around the central vein (CV). These plates are separated by vascular sinusoids (black arrow) that lined by flat kuppfer cells (dashed arrow) (H&E X 200)

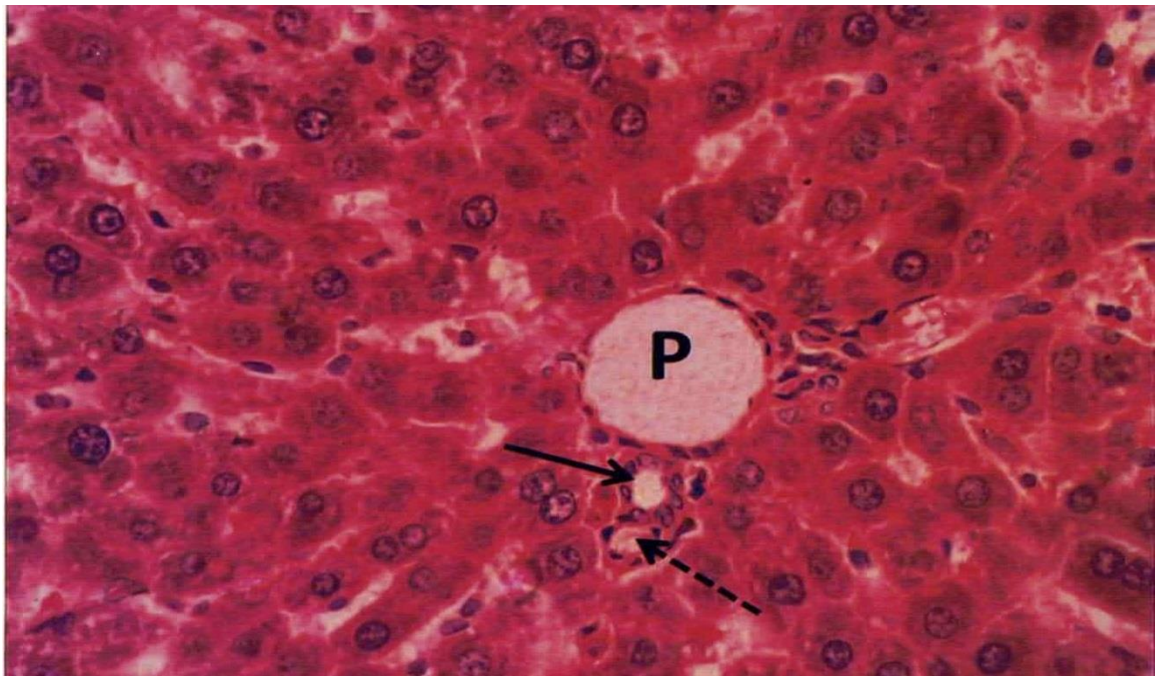

Figure (4): A photomicrograph of liver section in control group showing normal portal triad formed of portal venule (P), bile ductule (arrow) and hepatic arteriole (dashed arrow) (H&E X400)

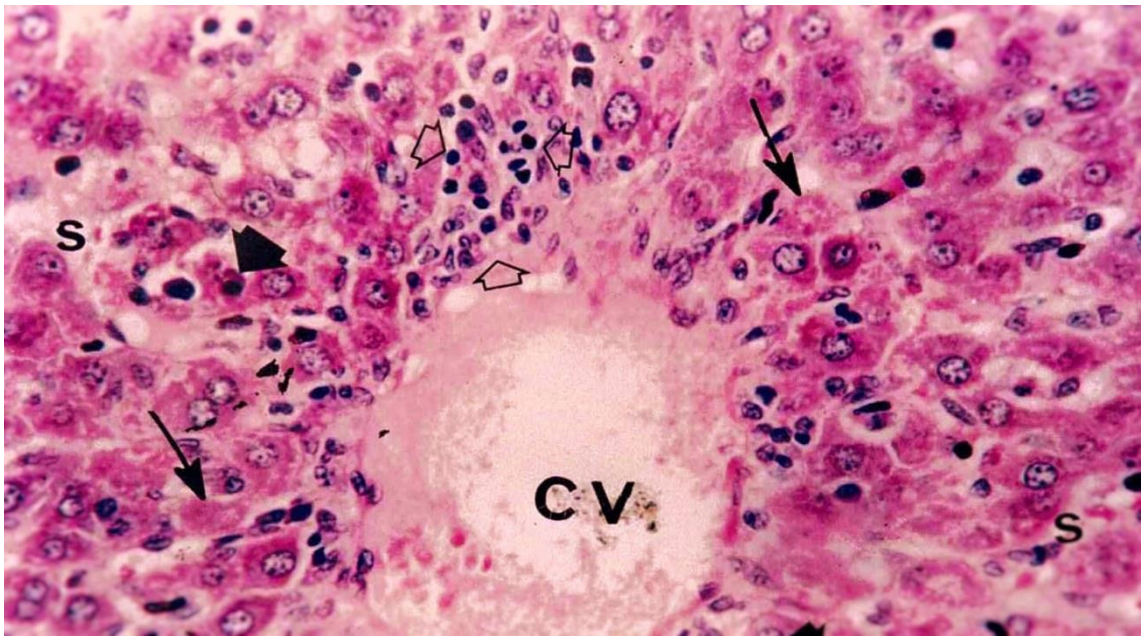

Figure (5): A photomicrograph of liver section in (DOX-treated group II) showing dilatation and congestion of the central vein (CV) resulting in distortion of the normal hepatic lobular architecture with dilatation and congestion of the sinusoids (S) and some centrilobular hepatocytes showed pyknotic or absent nuclei (thin arrows). (H&E X 200)

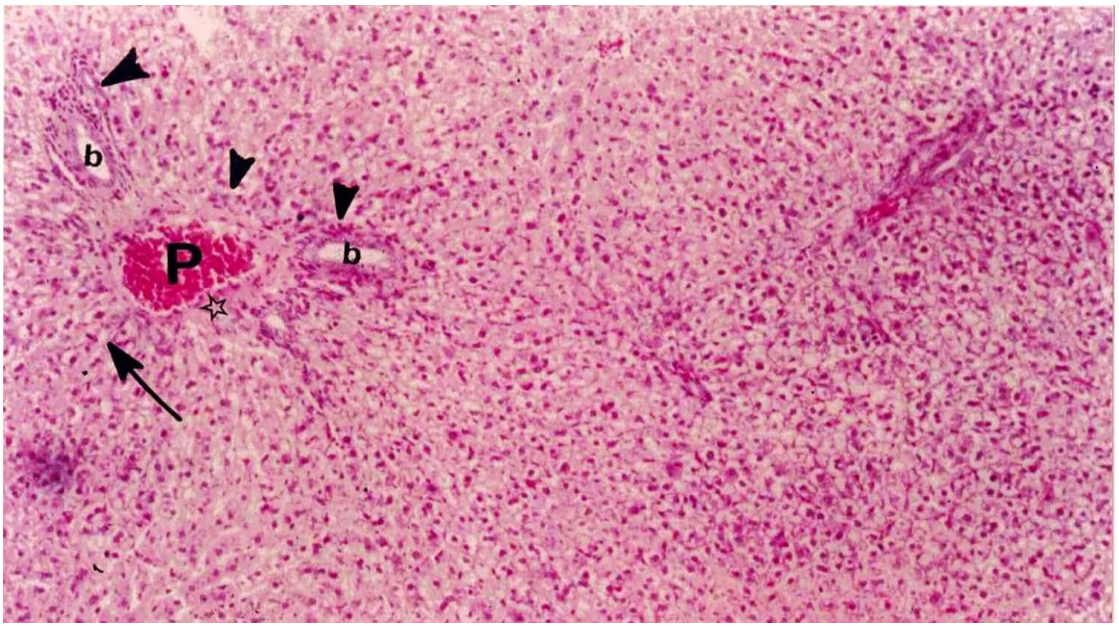

Figure (6): A photomicrograph of liver section in (DOX-treated group II) showing branches of the portal vein (arrow) with dilated bile ducts (b) (head arrows) which frequently encountered in the portal tract (P) (H&E X200)

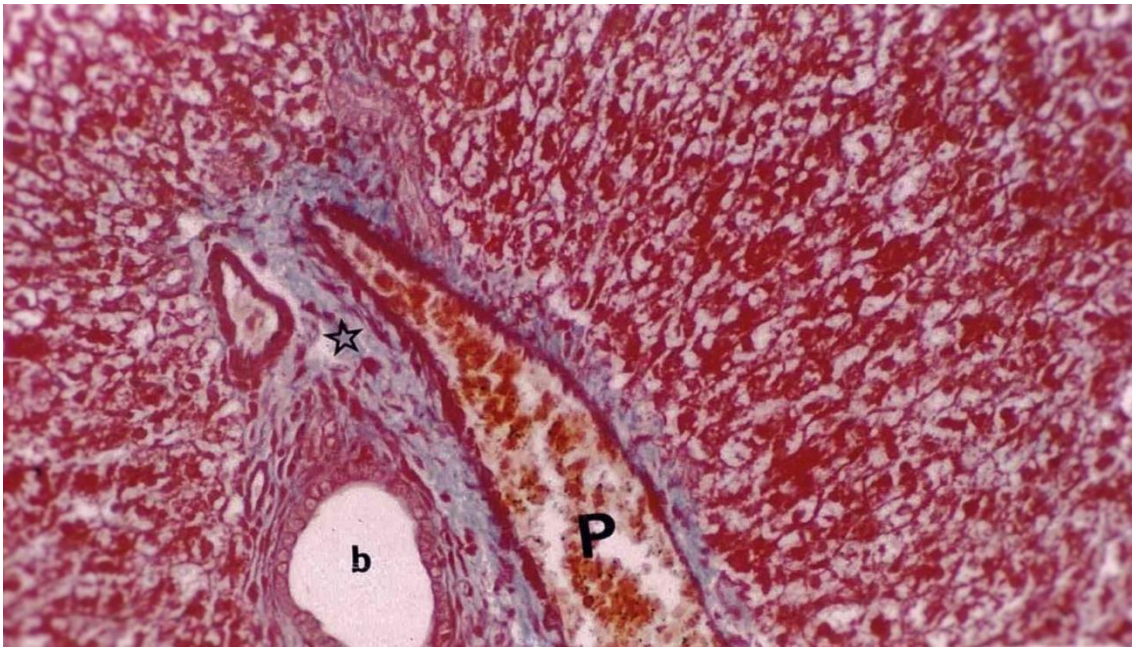

Figure (7): A photomicrograph of liver section in (DOX-treated group II) showing widespread vacuolation of the cytoplasm of the hepatocytes which being more marked peri-portally with bile duct dilatation (b) (H&E X400)

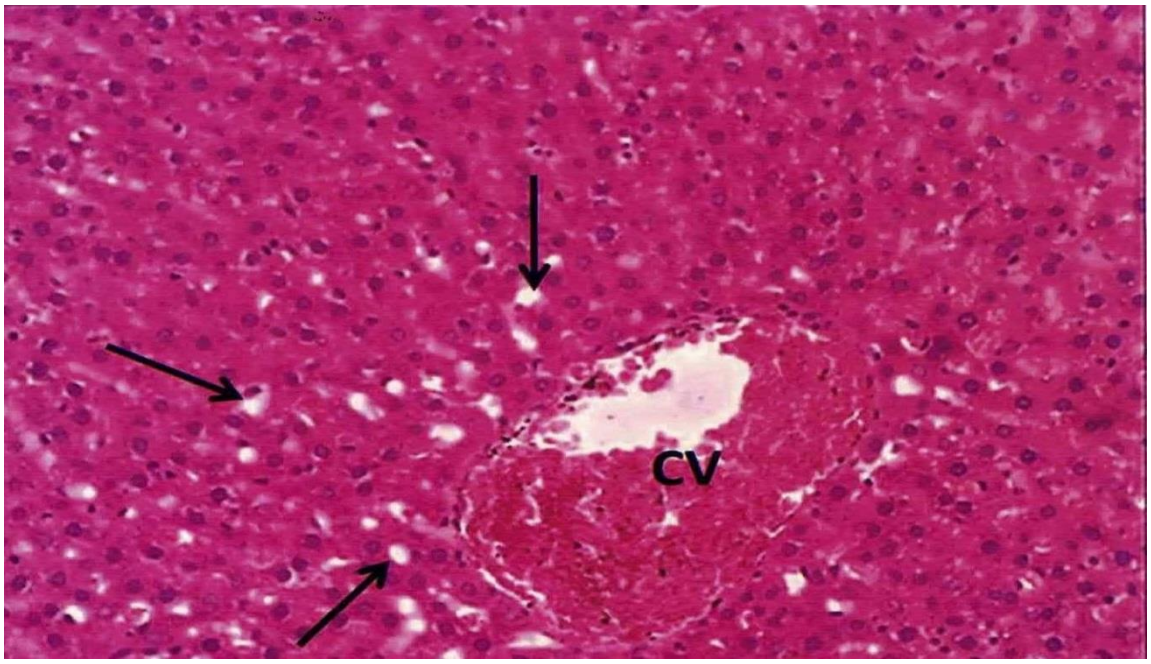

Figure (8): A photomicrograph of liver section in (DOX-treated group II) showing vacuolar degeneration (arrows) and dilated congested central vein (CV) (H&E X200)

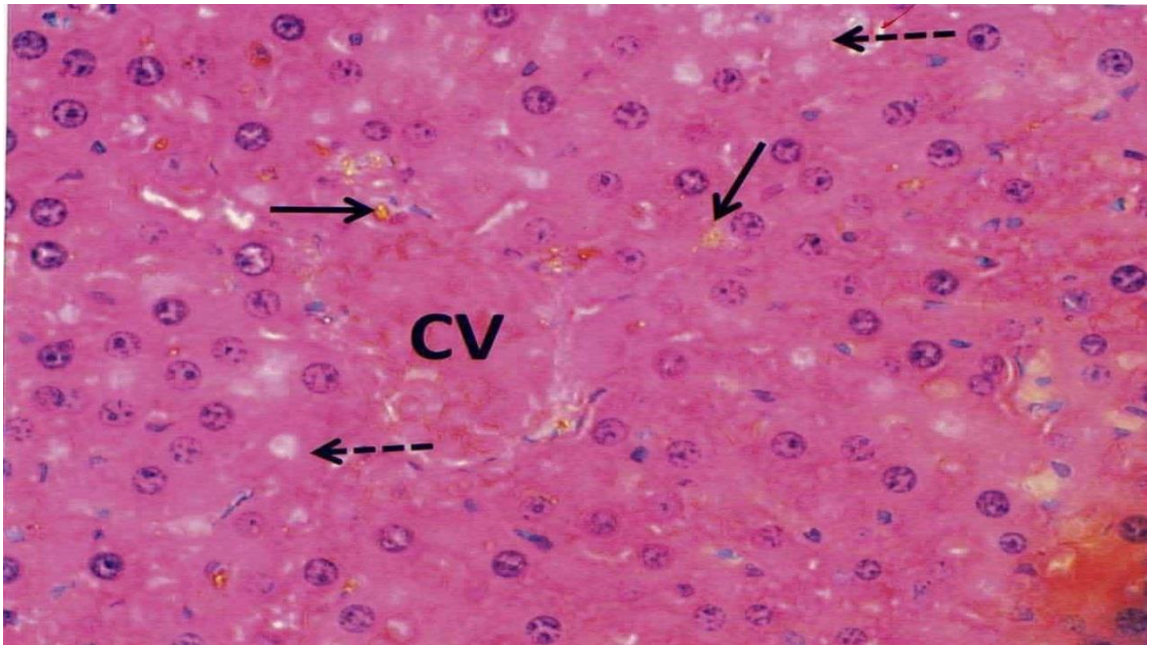

Figure (9): A photomicrograph of liver section in (DOX-treated group II) showing congested central vein (CV), loss of normal arrangement and cellular boundaries of hepatocytes, cholestasis (arrows) and areas of necrosis (dashed arrow) (H&E X400)

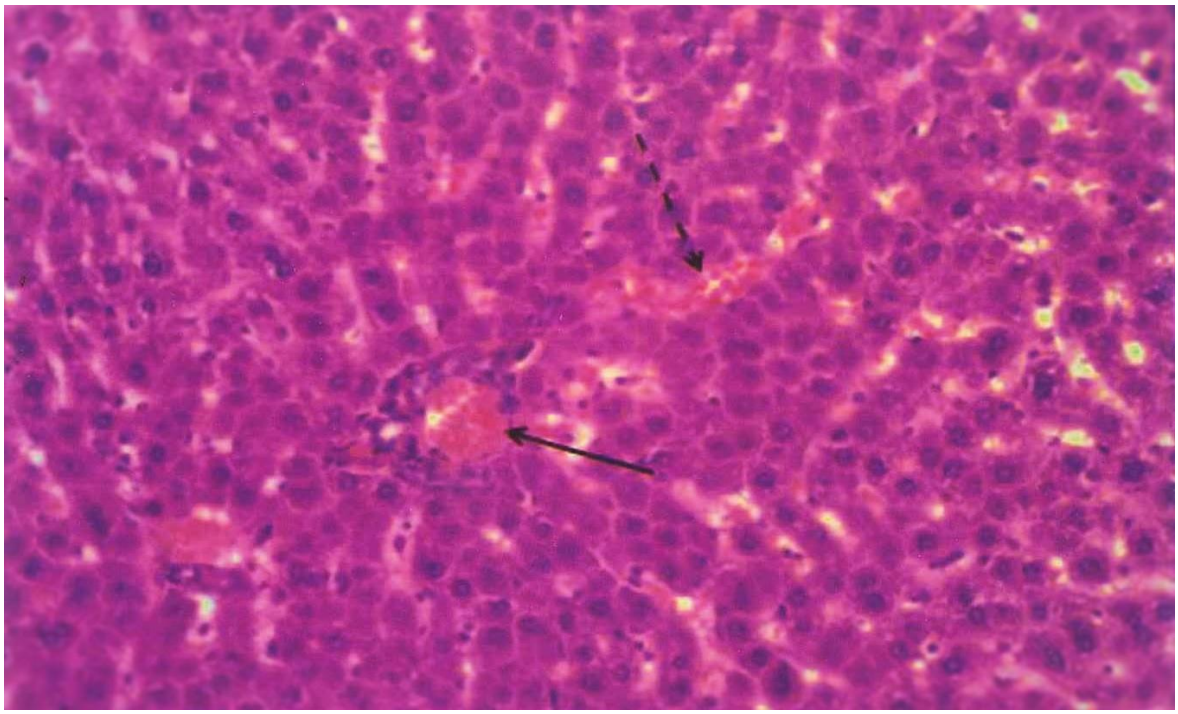

Figure (10): A photomicrograph of liver section in (DOX-treated group II) showing congested portal venule with inflammatory cell infiltration (black arrow) and dilated congested blood sinusoids (dashed arrow) (H&E X200)

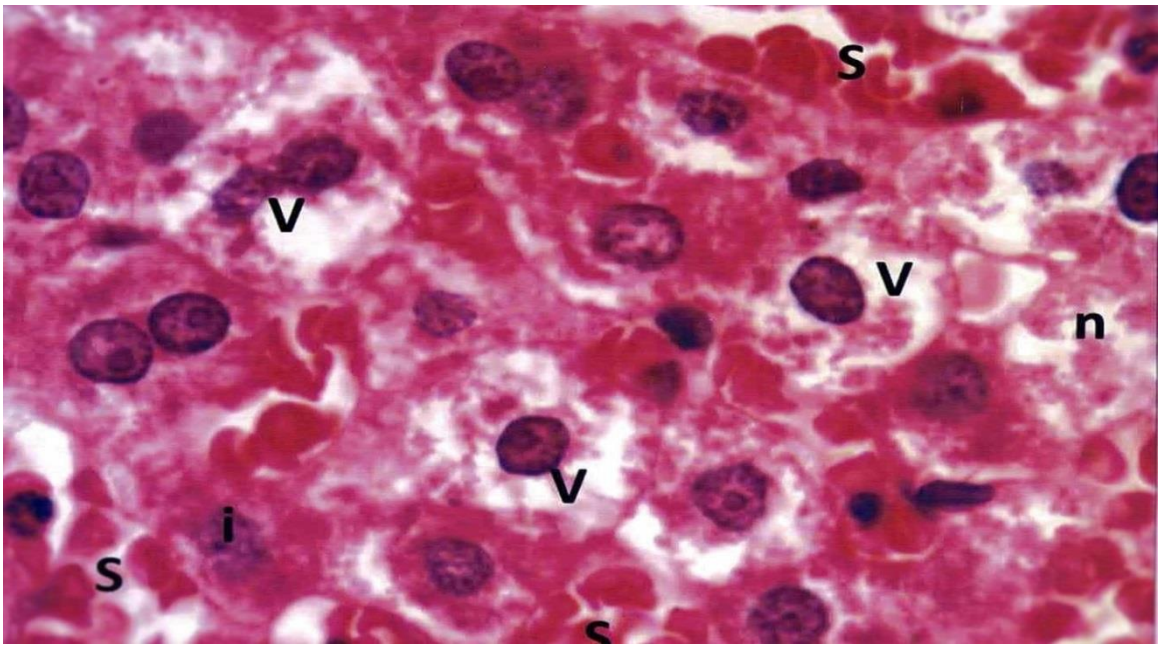

Figure (11): A photomicrograph of liver section (DOX-treated group II) showing hepatocytes with vacuolar degeneration (V), necrosis (n), karyolitic nuclei (i), separated by dilated congested blood sinusoids (S) (H&E X 1000)

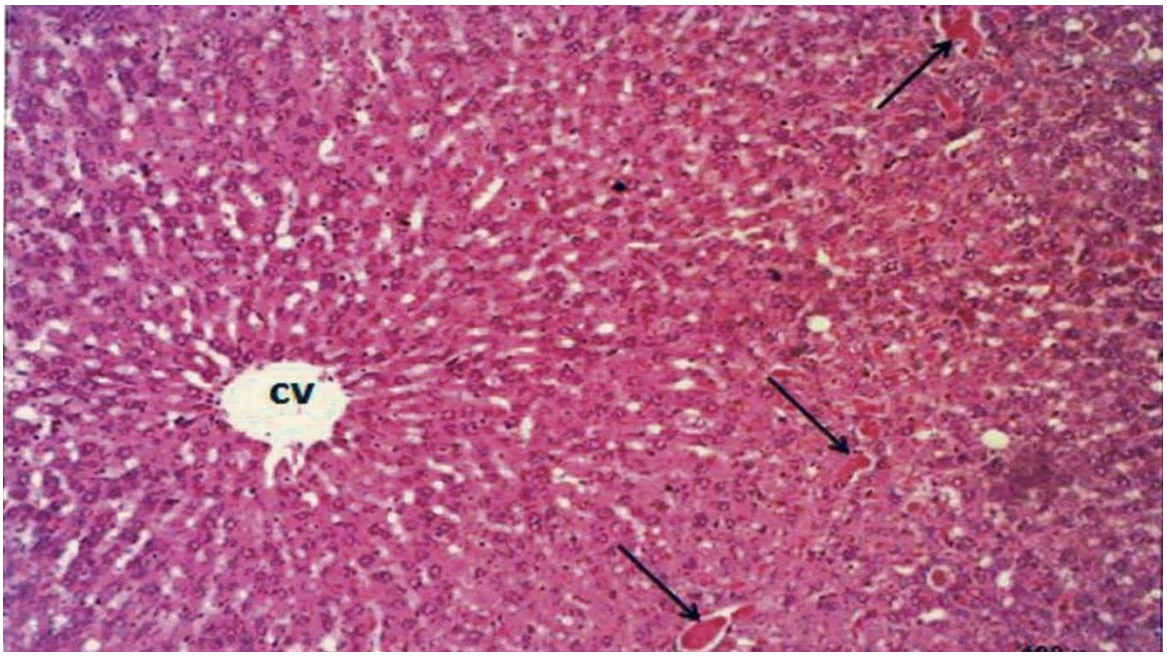

Figure (12): A photomicrograph of liver section in (DOX-treated plus QCT low dose group III) showing normal architecture of hepatic lobule and normal arrangement of hepatocytes around the central vein (CV) with dilated congested blood sinusoids (arrows) (H&E X100)

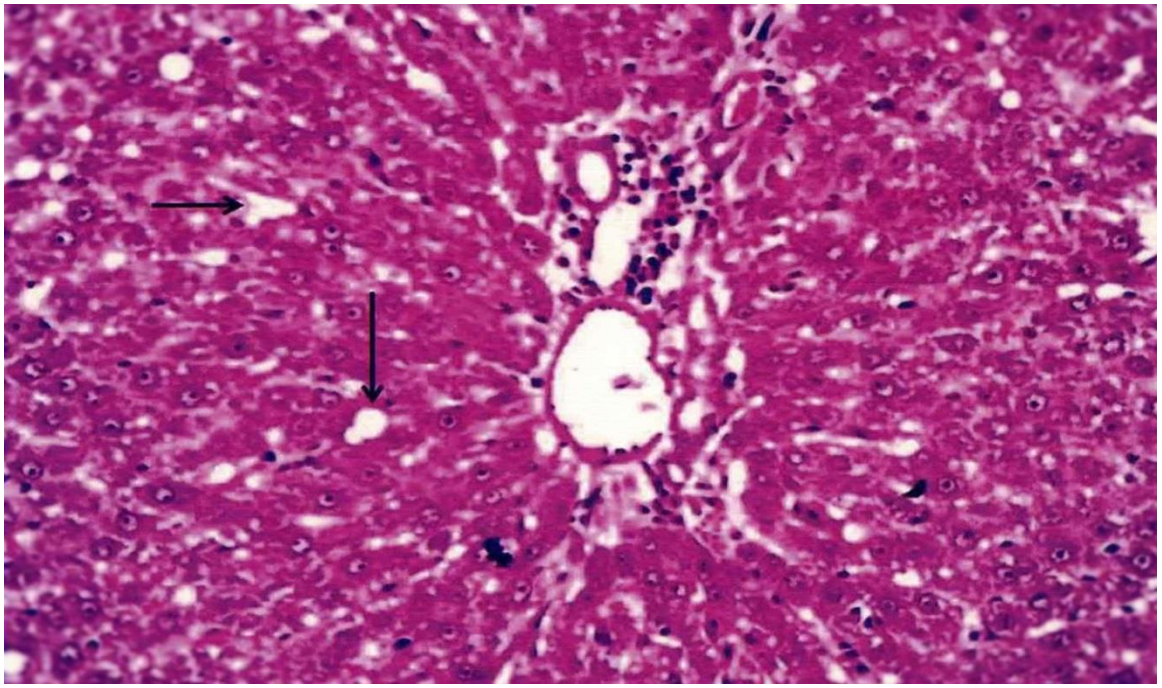

Figure (13): A photomicrograph of liver section in (DOX-treated plus QCT low dose group III) showing intact portal triad, normal sinusoids and normal arrangement of hepatic cords with few areas of vacuolar degeneration of hepatocytes in the periphery (arrows) (H&E X200)

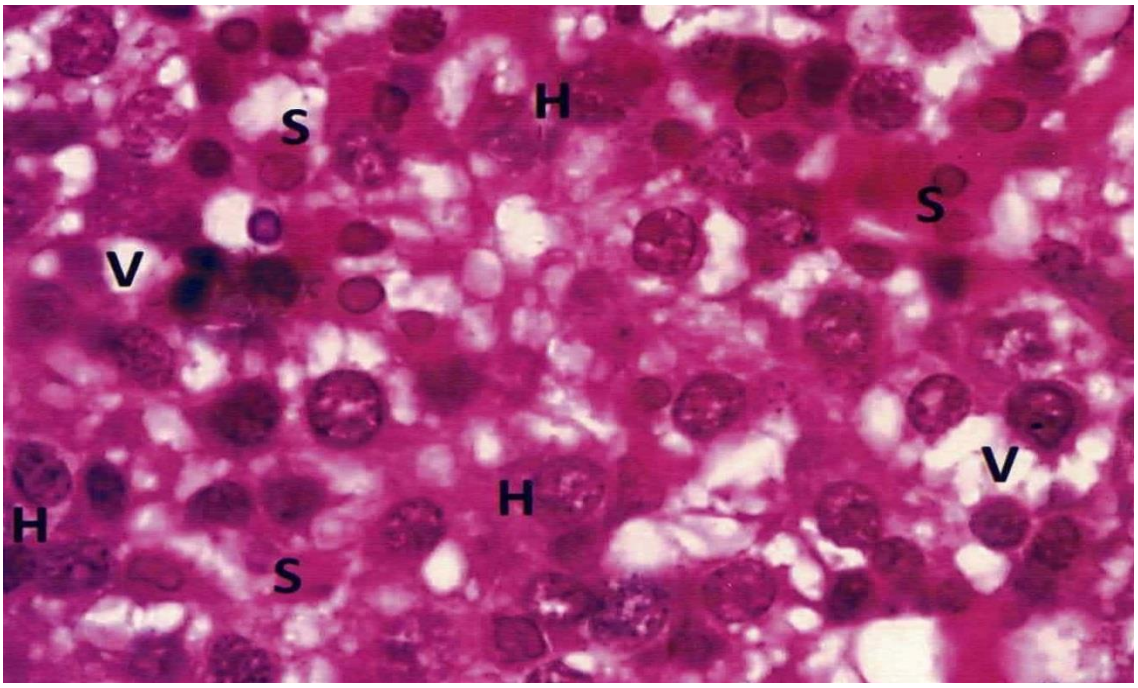

Figure (14): A photomicrograph of liver section in (DOX-treated plus QCT low dose group III) showing numerous intact hepatocytes (H) separated by narrow sinusoids (S) filled with hematopoietic cells with few areas of vacuolar degeneration (V) (H&E X1000)

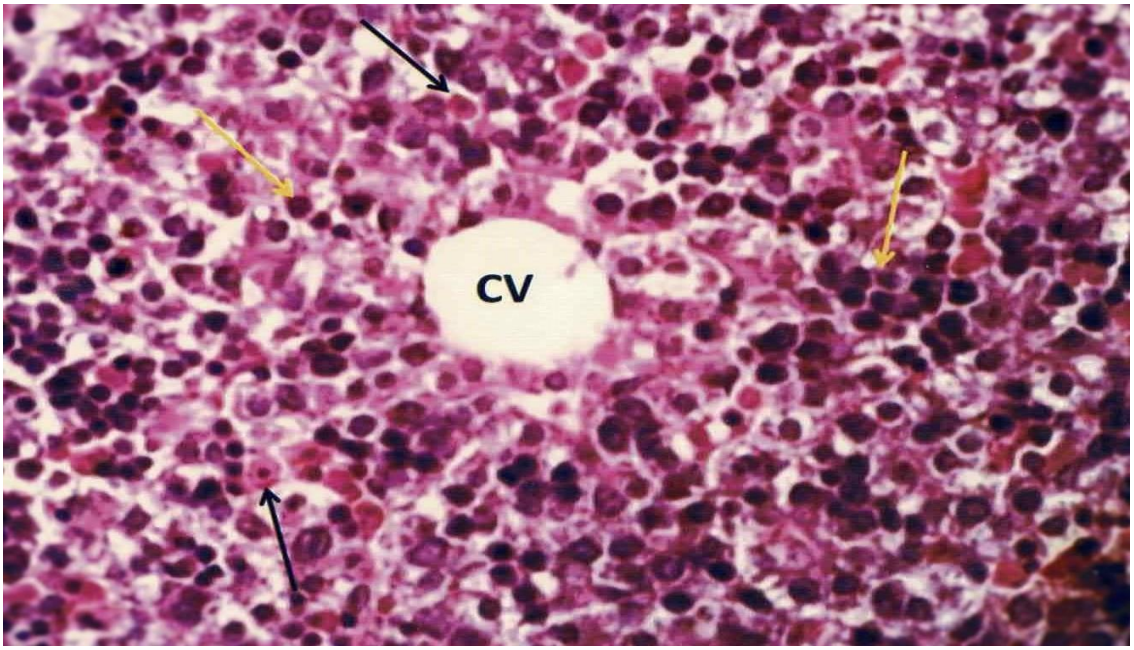

Figure (15): A photomicrograph of liver section in (DOX-treated plus QCT high dose group IV) showing polyhedral shaped hepatocytes arranged in cords around the central vein (CV) and dispersed in the periphery of hepatic lobules. Hepatocytes are separated from each other by blood sinusoids filled with hematopiotic cells mostly lymphocytes (yellow arrows) and nucleated immature red blood cells (black arrows) (H&E X400)

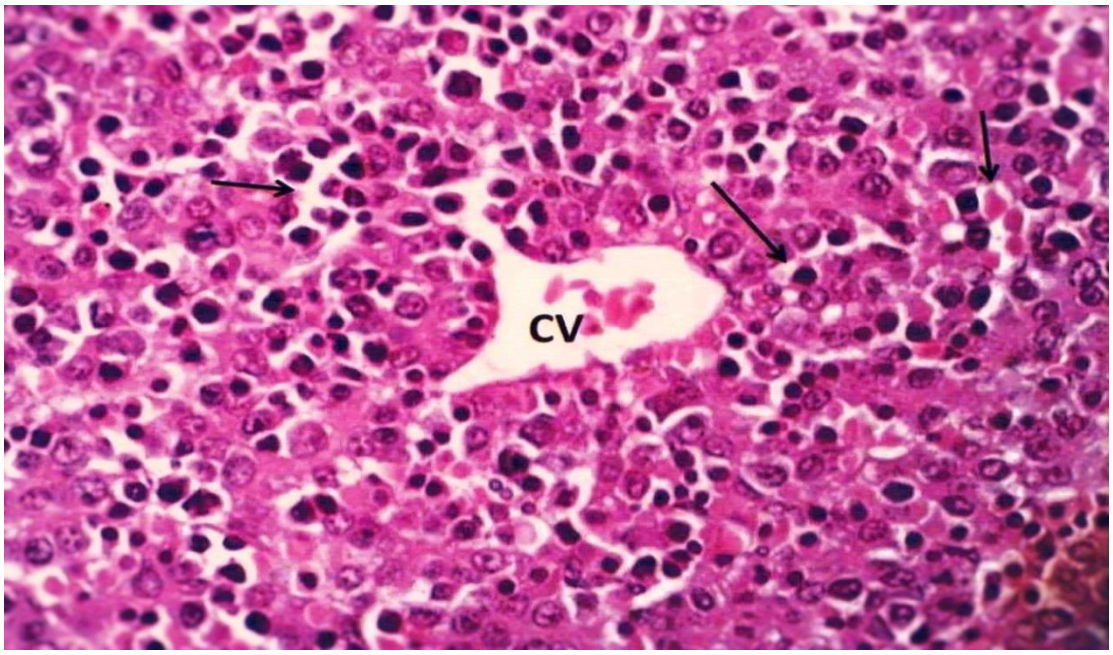

Figure (16): A photomicrograph of liver section in (DOX-treated plus QCT high dose group IV) showing central vein (CV) surrounded by intact hepatocytes separated by sinusoids filled with hematopiotic cells (arrows) (H&E X400)

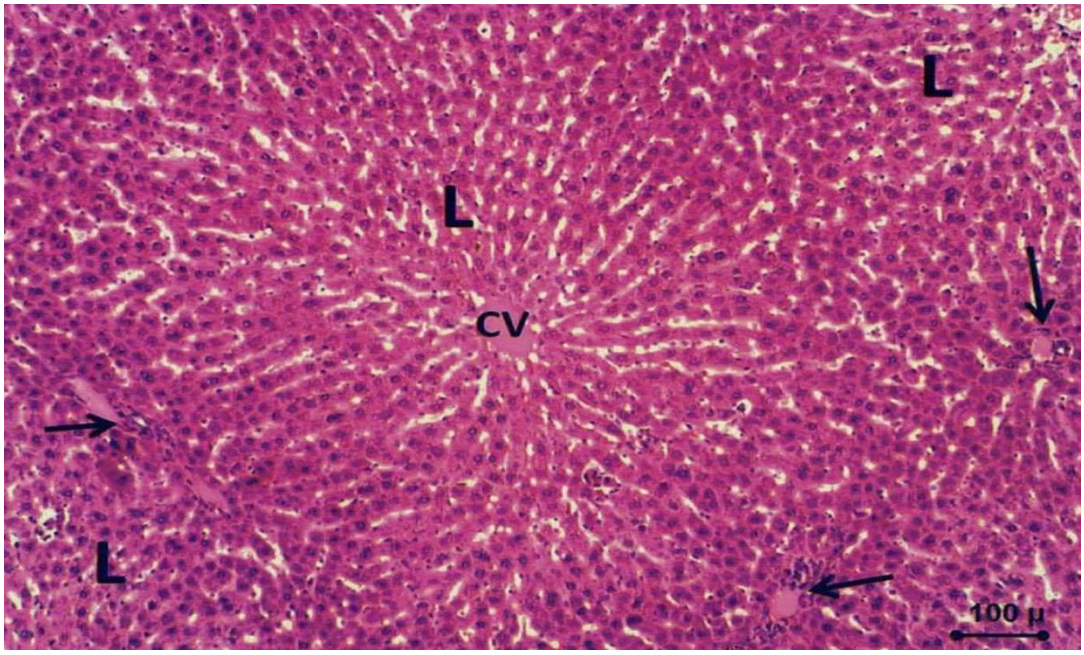

Figure (17): A photomicrograph of liver section in (DOX-treated plus QCT high dose group IV) showing hepatic lobules (L) formed from cords of hepatocytes radiating around the central vein (CV) and portal triads (arrows) in the angles of each lobule (H&E X100)

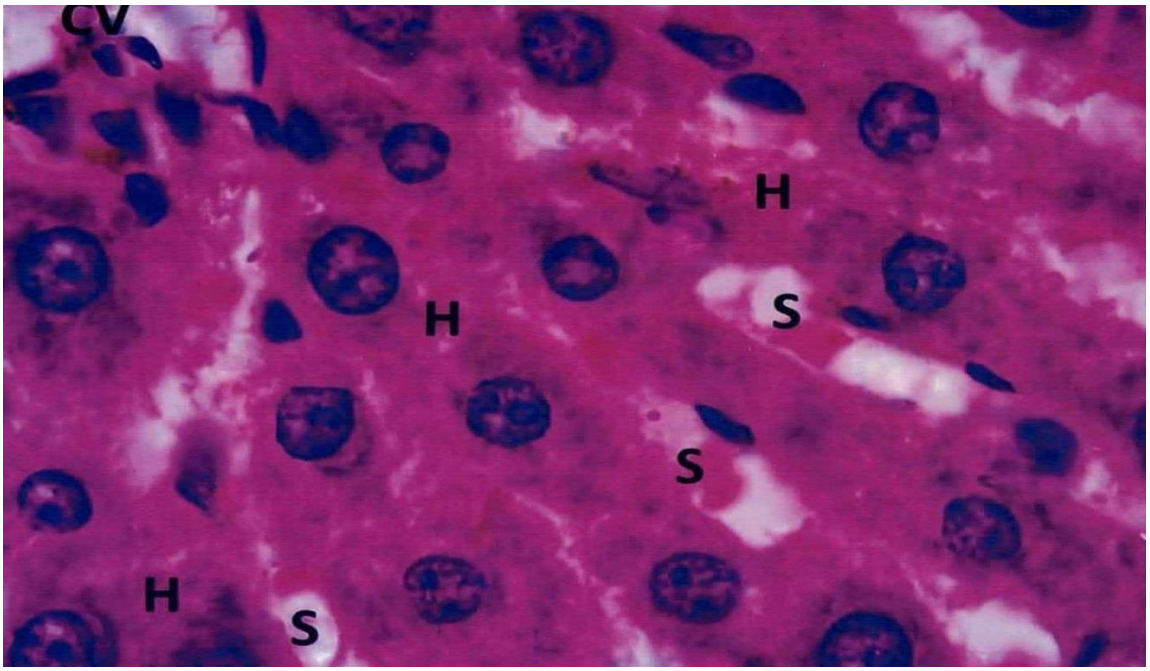

Figure (18): A photomicrograph of liver section in (DOX-treated plus QCT high dose group IV) showing arrangement of intact hepatocytes (H) in cords radiating around the central vein (CV) separated by blood sinusoids (S) (H&E X1000)

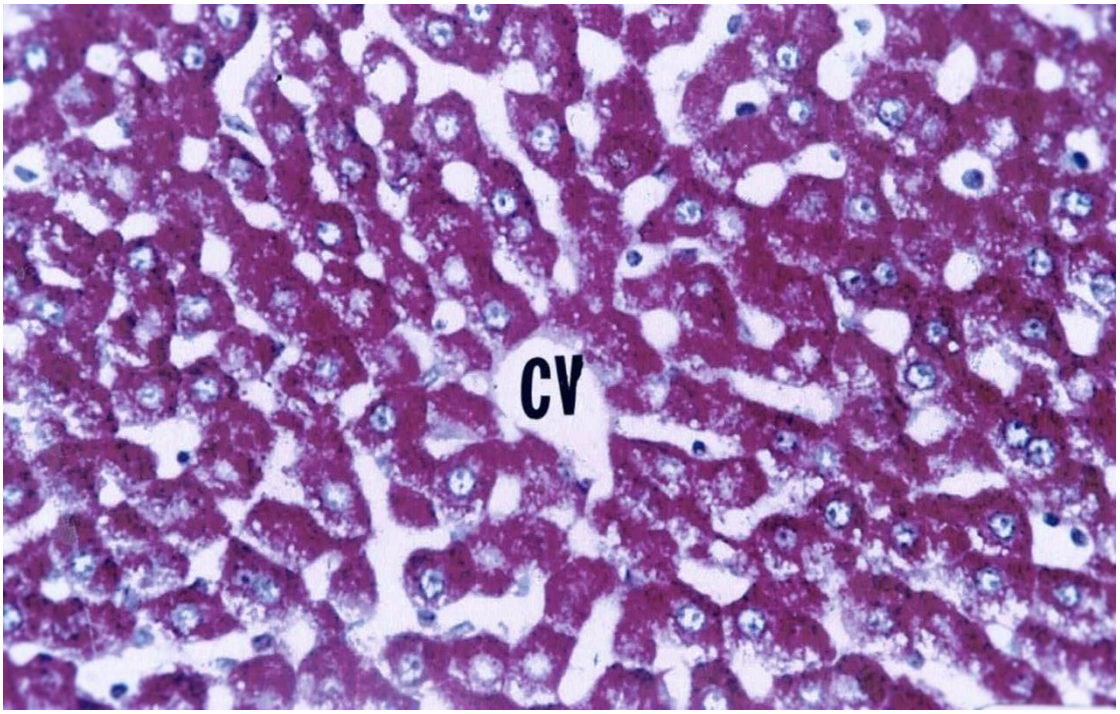

Figure (19): A photomicrograph of liver section in (control group I) showing faint positive PAS stained hepatic cells with increased glycogen deposits in the different zones of hepatic lobules especially around the central vein (CV) (PAS X 400)

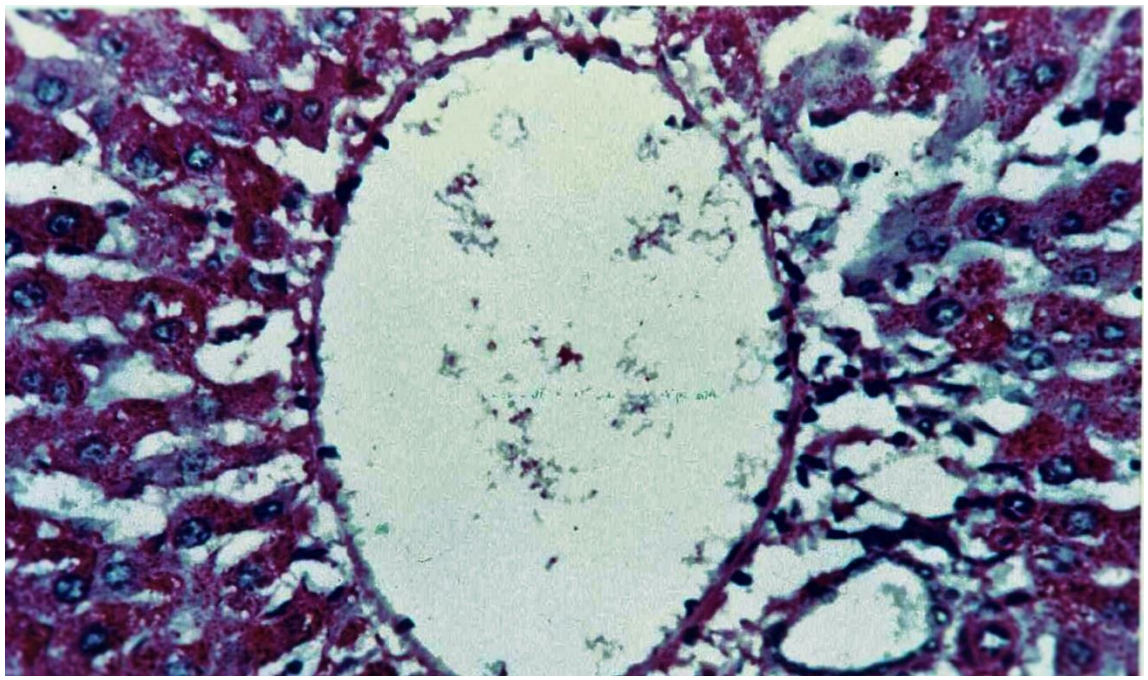

Figure (20): A photomicrograph of liver section in (DOX-treated group II) showing dense positive PAS stained hepatic cells with severe decrease of glycogen content especially around the central vein (PAS X1000)

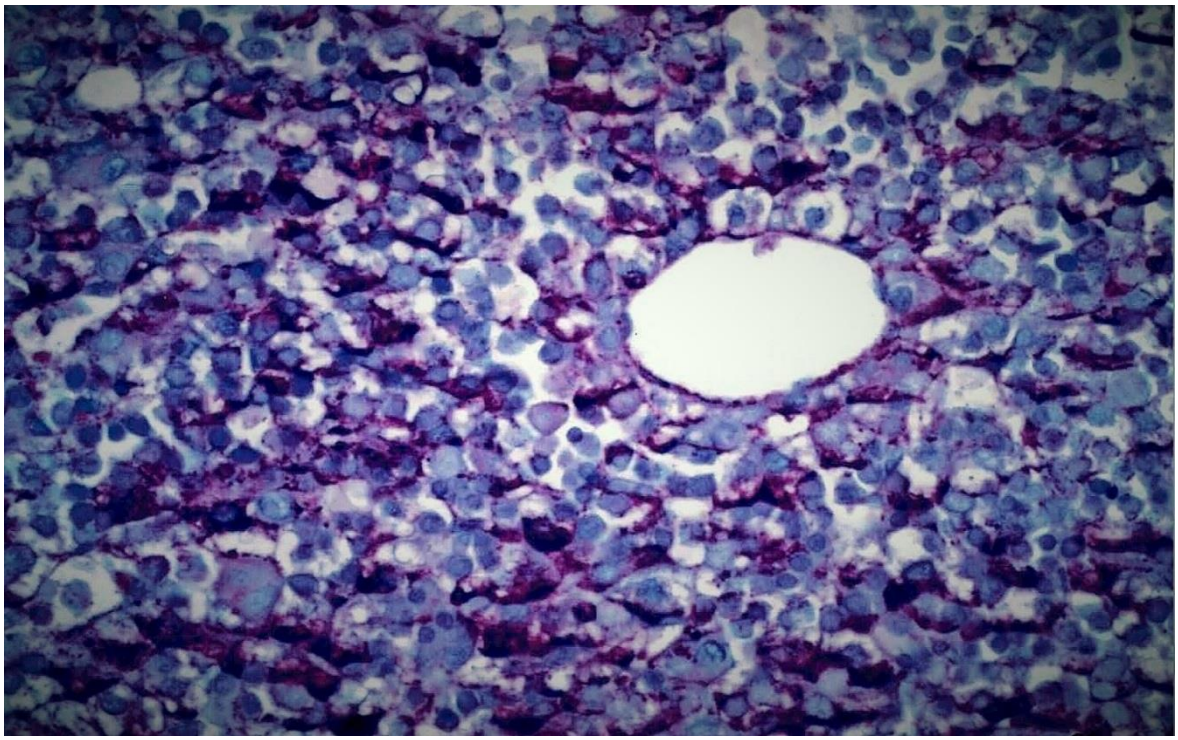

Figure (21): A photomicrograph of liver section in (DOX-treated plus QCT low dose group III) showing faint positive PAS stained hepatic cells with moderate decrease of glycogen content especially in the portal zone (PAS X400)

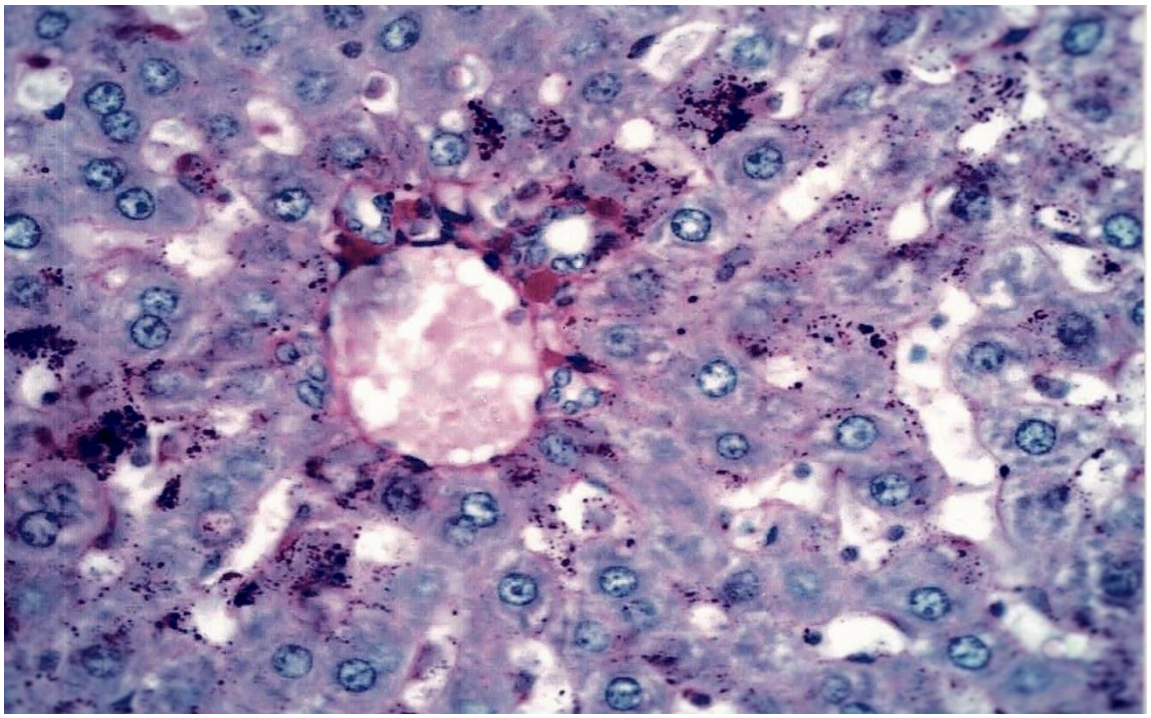

Figure (22): A photomicrograph of liver section in (DOX-treated plus QCT high dose group IV) showing faint positive PAS stained hepatic cells with mild decrease of glycogen content especially in the portal zone (PAS X400)

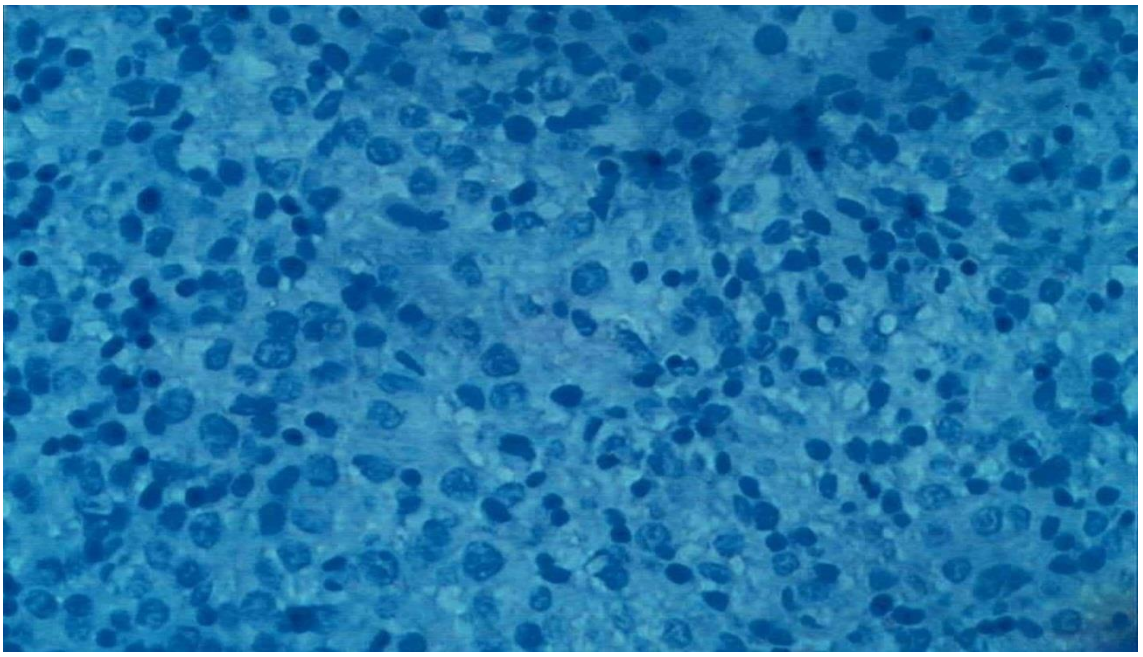

Figure (23): A photomicrograph of liver section in (control group I) showing a negative cytoplasmic Caspase-3 immuno-expression (X400)

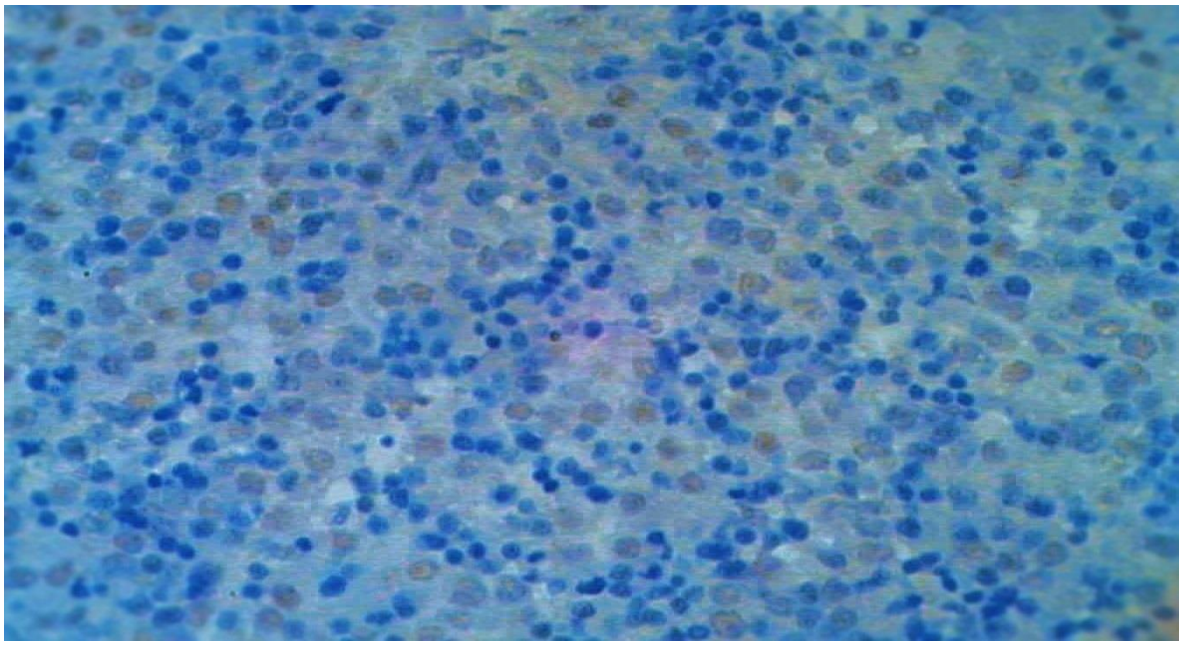

Figure (24): A photomicrograph of liver section in (DOX-treated group II) showing a dense positive cytoplasmic Caspase-3 immuno-expression (X400)

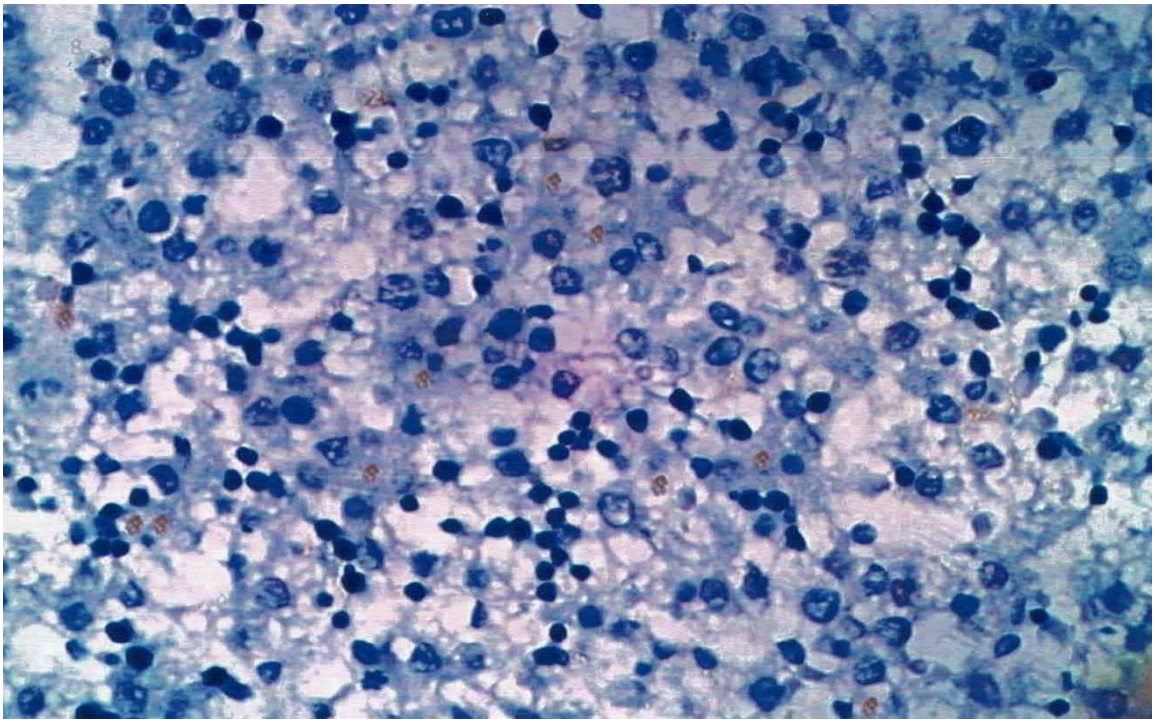

Figure (25): A photomicrograph of liver section in (DOX-treated plus QCT low dose group III) showing few traces of positive cytoplasmic Caspase-3 immuno-expression (X400)

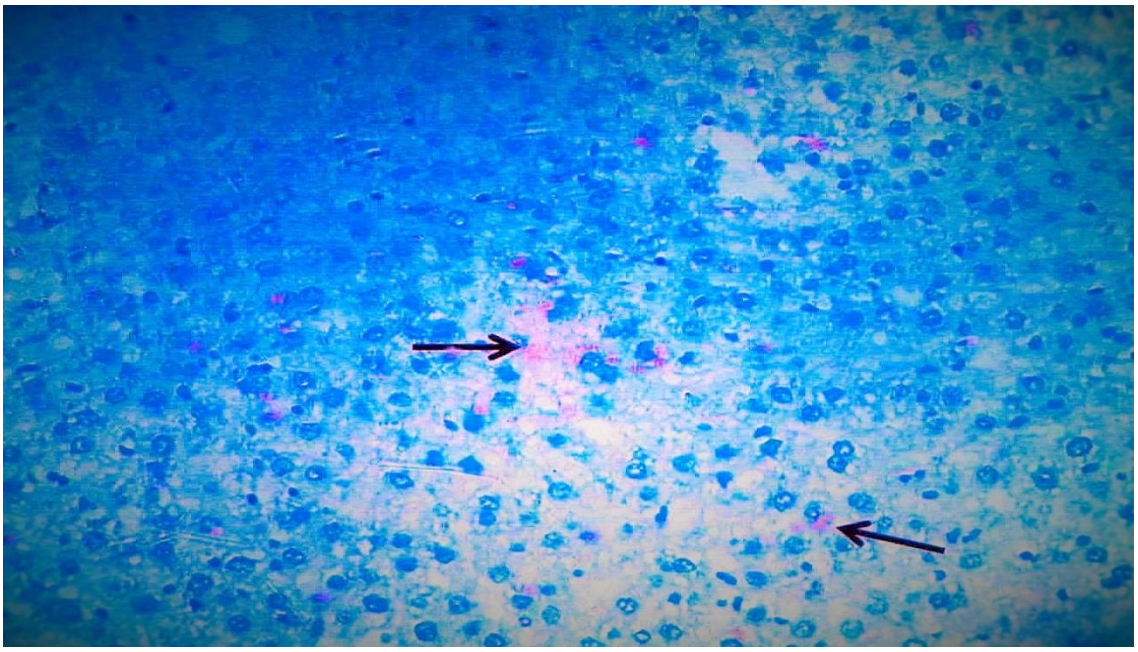

Figure (26): A photomicrograph of liver section in (DOX-treated plus QCT high dose group IV) showing very few traces of positive cytoplasmic Caspase-3 immuno-expression (arrows) (X400)

Discussion

Liver maintains the body's internal environment as it controls the metabolism of carbohydrate, protein and fats and it has a role in both metabolism and detoxification of toxicants, which may result in liver injury. Liver injury is attributed to oxidative stress, which can result in liver diseases that range from transient elevation of liver enzymes to fibrosis and cirrhosis (Pandit et al., 2012). The current study stated that, the liver weight increase in the DOX-treated rats which may be attributed to the oxidative stress while the body weight decrease may be due to decreased food intake. Those results are in accordance with Beshay et al, (2011) study results which stated that DOX-treated rats lose about 5% of body weight when compared with control. Serum ALT and AST are markers for detection of hepatotoxicity so, serum enzyme levels is used to assess liver damage as membrane necrosis lead to diffusion of the intracellular enzymes to the serum and elevation of their levels which indicate liver membranes integrity loss (Etim et al., 2006). ALP and total bilirubin high levels were also associated with hepatic cell dysfunction (Shehab et al., 2015). The current study stated that DOX administration led to significant increase in ALT, AST, ALP, total bilirubin serum level and MDA in comparison to control. That results denote a hepatocellular damage and hepatic dysfunction as stated by Alshabanah et al, (2010) results as liver damage may induced by the free radicals and their oxidative stress. DOX tend to generate superoxide anions and peroxynitrite radicals in drug metabolism in liver which initiate Reactive Oxygen Radicals (ROS)-lipid peroxidation and lead to damage of hepatocytes and elicit ALT and AST to serum (Barakat et al., 2018). Significant improvement in liver enzymes and GSH level were observed in QCT treated groups in comparison to control and DOX-treated groups. Those results are in accordance with Jambhulkar et al, (2014) results which stated that the altered biochemical parameters were appeared near their normal ranges on QCT administration with DOX group. The mechanism may be attributed to that QCT has the ability to increase the mRNA expression of liver enzymes which involved in drug metabolism in an isoenzyme-specific manner (Odbayar et al., 2009). QCT have been reported to interact with membrane lipid components, with a resultant protection of the membranes against oxidative damage (Verstraeten et al., 2015). A recent study stated that DOX-treated group can increase both ALT and AST enzymes activity because of the liver damage and it is confirmed by the histological results (Barakat et al., 2018). The present results showed that DOX-treated group sections which stained with H&E showed focal hepatocyte necrosis and the hepatotoxicity that resulted are in accordance with Jambhulkar et al, (2014) and Mete et al, (2016) results which stated a parenchymal necrosis and proliferation of biliary ducts in DOX-treated group. Vacuolation of hepatocytes was reported as one of cellular defensive mechanism as they can collect the harmful elements and prevent their interfering with the biological functions of these cells and Pyknosis and karyolysis of the nuclei may be attributed to the loss of functional efficiency (AL-Mosaibih, 2013). The present results showed that DOX-treated sections appeared with high positive PAS stained hepatocytes which illustrated increase of glycogen contents and that results are in accordance with results of Ahmed et al (2020) study. QCT treated sections which stained by H&E, illustrated its protective effect as the normal architecture was observed and the histo-pathological changes which found in the DOX-treated group were decreased in the DOX plus QCT treated groups. The present results are in accordance with Jambhulkar et al, (2014) results

which stated that administration of QCT with 100 mg/kg protected hepatic tissues from DOX toxicity. The protective effect of QCT against hepatotoxicity in the current study is agree with a recent study which stated that QCT limit the methotrexate induced hepatotoxicity in rats as it is considered as a potent antioxidant (El-Bana and Kamal 2019). Studies documented that acute DOX toxicity alters cytochrome P450 expression in mice liver while QCT can increase the P-450 reeducates activity in human liver, and suppresses the expression of the pro-apoptotic Bax gene and increases the anti-apoptotic Bcl-2 gene in cells under the oxidative stress (Zordaky et al., 2011 and Suematsu et al., 2011). The QCT treated sections which stained with PAS showed faint staining which indicated low glycogen contents in hepatocytes. It indicated that QCT protecting the liver cells as it may has a benefit in DOX treatment as it can increase its therapeutic efficacy against hepatic cancer. The results are in accordance with Wang et al, (2012) results which documented that QCT can reverse hepatic damage which resulted from DOX administration in mice. The present results documented that DOX treated animals' sections revealed increased apoptosis which attributed to the oxidative stress and increased cytoplasmic Caspase-3 immune-expression activation (El-Sayyad et al, 2014). DOX prompted hepatotoxicity, may be due to hepatocytes' apoptosis that is associated with a significant DNA degradation along with increased Caspase-3 protein expression (Barakat et al., 2018). QCT administration led to improvement of the Caspase-3 immuno-expression activation as it aids in preventing apoptosis which induced by lipid peroxidation as it decreases the hepatic lipid peroxides because it is considered as a potent anti-oxidant (Arzu e al, 2004). ROS and oxidative stress are considered as apoptosis triggers and modulators. ROS-induced apoptosis requires other cell death signaling pathways as c-Jun N-terminal Kinase (JNK) to regulate the expression of various apoptosis proteins engaged in hepatotoxicity (Chao et al., 2009). The mechanism of JNK-dependent apoptosis has been suggested to involve activation of Caspase-3 via phosphorylation of Bcl-2 family proteins. Studies stated that QCT is able to attenuate the toxicant-induced apoptosis by the inhibition of JNK activation and can prevent apoptosis by altering the expression of both Bax, Bcl-2 and Caspase 3 (Shi et al., 2009 and Kim et al., 2009).

Conclusion

This study concluded that DOX had toxic effects on the liver of rats, with significant improvement after treatment with QCT as an antioxidant substance, and that high doses of QCT were more protective than low doses. QCT may be beneficial in DOX administration for increasing its therapeutic efficacy against liver cancer.

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التأثير المحتمل للحماية بالكورستين ضد السمية الكبدية الناجمة عن الدوكسوروبيسين في ذكور الجرذان البيضاء البالغة: دراسة بيوكيميائية وهستوباثولوجية مناعية

سحر محمد مصطفى[١] منى حسن على[٢]

الملخص العربي

المقدمة: يعتبر الدوكسوروبيسين مضاداء للأورام ولكنه يسبب تسمم الكبد من خلال توليد الجذور الحرة بالإضافة إلى الضرر التأكسدي، و سُميته تحفز التغيرات الإلتهابية في أنسجة الكبد في حين يعتبر الكورستين أحد مضادات الأكسدة القوية مما يمكنه من تخفيف السمية الكبدية.

الهدف من البحث: الكشف عن التأثير السمي للدوكسوروبيسين على أنسجة الكبد وتقييم الدور الوقائي المحتمل للكورستين ضد التغيرات السمية التى قد يسببها الدوكسوروبيسين في كبد ذكور الجرذان البيضاء البالغة.

مواد وطرق البحث: تم إستخدام عدد أربعين من ذكور الجرذان البيضاء و تم تقسيمهم بالتساوي الى أربعة مجموعات وتمت معالجتهم على النحو التالي: المجموعة الأولى: المجموعة الضابطة، المجموعة الثانية المعالجة بالدوكسوروبيسين: حيث تلقت الجرذان ١٨ مجم /كجم من وزن الجسم من الدوكسوربيسين. المجموعة الثالثة المعالجة بالدوكسوربيسين بالإضافة إلى الكوريستين حيث تلقت الجرذان ٦٠ مل لكل كجم من الكورويستين جنباء إلى جنب مع جرعة الدوكسوروبيسين .المجموعة الرابعة المعالجة تلقت ١٠٠مل من الكوريستين لكل كجم جنباء إلى جنب مع جرعة الدوكسوروبيسين. بعد التضحية بالجرذان تم تقدير معدل إنزيمات الكبد ونسبة الصفراء و إستخرجت الأكباد وإستخدمت في تقدير علامات الإجهاد التأكسدي و الدراسات النسيجية والمناعية.

النتائج: نتج عن إعطاء الدوكسوروبيسين إلى الجرذان البيضاء زيادة ملحوظة في إنزيمات الكبد و نسبة الصفراء وعلامات الإجهاد التأكسدي و قد أدي إعطاء الكوريسيتين إلى تحسن ملحوظ في هذه النسب خاصة مع الجرعة العالية. وأظهر الفحص المجهري الضوئي لأنسجة الكبد في المجموعة التي عولجت بالدوكسوروبيسين فقد التوزيع الطبيعي لخلايا الكبد و إتساع و إحتقان بالأوردة المركزية و البابية و الجيوب الدموية و إنحطاط فجوي بخلايا الكبد و ركود صفراوي و نخر بالخلايا الكبدية. و قد أظهرت قطاعات الكبد المصبوغة بصبغة PAS في المجموعة المعالجة بالدوكسوربيسين زيادة و عمق الصبغة. أسفر إعطاء الكوريستين عن تحسن ملحوظ في هذه التغيرات و قد ظهرت خلايا الكبد المصبوغة بصبغة PAS بصورة متوسطة إلى خفيفة خاصة مع الجرعة العالية للكوريستين. أما قطاعات الأكباد المصبوغة caspase-3 فقد أظهرت المجموعة المعالجة بالدوكسوروبيسين نتائج إيجابية بكثافة في حين أن المجموعة المعالجة بالكوريستين أظهرت أثاراء قليلة لصبغة ال caspase-3 خاصة مع الجرعة العالية.

الخلاصة: خلصت هذه الدراسة إلى أن الدوكسوروبيسين له آثار سمية على كبد الجرذان البيضاء مع حدوث تحسن ملحوظ بعد معالجتهم بالكوريستين كمادة مضادة للأكسدة وأن الجرعات العالية من الكوريستين تفوقت في تأثيرها الوقائي على الجرعات الصغيرة.

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