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Article

Minimizing the side effects of Doxorubicin Induced Hepatotoxicity by using alcoholic extract of Date Palm in adult rats

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ABSTRACT

Doxorubicin (DOX) is a highly effective drug for chemotherapy. However, hepatotoxicity reduces its clinical utility in humans. Thus, this study was designed to examine Date Palm extract on serum anti-inflammatory markers (interleukin(IL) IL-1B, IL-6 and IL-10). Forty adult rats were divided into 4 groups (G1 control, G2 receiving 2mg\kg of DPE orally, G3 treated with 2mg\kg of DOX IP, and G4 received 2mg\kg of DOX via IP and 2mg\kg of DPE by oral gavage daily for 30 days). At the end of the study, animals were sacrificed, and livers were analyzed histologically. The Dox group showed significantly higher levels of serum IL-1B, IL-6, and IL-10 than the control group, with inflammation and necrosis in hepatic histopathology. In the DPE+ DOX group, it was detected that DOX treatment caused a significant decrease in serum IL-1B, IL-6, and IL-10 levels. Collectively, pre-coadministration of DPE partially mitigated DOX-induced hepatic injuries via its antioxidant, anti-inflammatory, anti-fibrotic, and antiapoptotic protein.

INTRODUCTION

Cancer is characterized by loss of control of cellular growth and development, leading to excessive proliferation and spread of cells. Doxorubicin (DOX) is an anthracycline glycoside antibiotic, a product of Streptomyces peucetius var. caesius, commonly prescribed against several tumors human solid tumors like ovarian breast, lung, uterine, and cervical cancers, Hodgkin's disease, soft tissue, and primary bone sarcomas, as well against several other cancer types and hematological malignancies[1-3]. Rendering non-tissue specific characteristics, Doxorubicin (DOX) has wide indications [4]. Despite the wide range of anticancer efficiency, DOX leads to varied side effects, including cardiomyopathy and renal, hepatic, pulmonary, testicular, and hematological toxicities [5]. Phoenix dactylifera (Date Palm) is widely grown worldwide in semi-arid and arid regions. Date Palm (Phoenix dactylifera) belong to the family Arecaceae, called Nakhla and the Tree of Life by the Arabs. Date extracts were shown to possess a myriad of pharmacological properties such as antioxidant, anticancer, nephroprotective, antimicrobial, antiallergic, immunostimulatory, neuroprotective, antifungal anti-inflammatory,

antiatherogenic, hepatoprotective, antimutagenic and gastrointestinal transition stimulatory activity [6]. The liver is one of the body's essential organs, and it performs an amazing array of vital functions in the body's maintenance, performance, regulation of homeostasis, metabolism, and exogenous (drug) and endogenous substances detoxification. This can explain why the liver is the primary body organ affected by chemotherapy[7,8] Approximately 40% of patients on doxorubicin suffer from liver injury by cell cycle arrest, thus inhibiting the self-regeneration potential of the liver [9]. Cytokines are soluble cell signal transducing proteins or polypeptides with low molecular weight (≈6–70 kDa), manufactured by a variety of cells: macrophages, lymphocytes, monocytes, dendritic cells, neutrophils, endothelial cells, natural killer (NK) cells, mast cells, and stromal cells). They participate in the immune response and act as important mediators associated with the communication network of the immune system. Cytokines are responsible for the dynamic regulation of immune cells' maturation, growth, and responsiveness and are important determinants of health. Different cell types may secrete a single cytokine and can act on several cell types, producing multiple biological activities. Variation in cytokines levels in various biological fluids, such as serum, blood, stool, saliva, and sweat, provides valuable information regarding the diagnosis, stage, and prognosis of various diseases [11-13]. Interleukin-1 (IL1) is one of the major pro-inflammatory cytokines. It is comprised of two. The IL-1 superfamily of cytokines encompasses both pro- and anti-inflammatory members and includes IL-1α, IL-1β, IL-18, IL-33, IL-37, and IL-38. IL-1α and IL-1β are pro-inflammatory, pyrogenic cytokines released by numerous cells, particularly innate immune cells such as monocytes, macrophages, and dendritic cells. IL-1 is primarily produced by innate immune cells, such as macrophages and monocytes. IL-1 exerts pro-inflammatory actions to recruit immune cells and induce secondary cytokine production, resulting in acute phase reactions [20]. The precursor of IL-1β is inactive and does not bind to the receptor. It requires a cleavage to become the active form. The release of IL-1β from blood monocytes is highly controlled and takes several hours in healthy subjects [21]. Interleukin-6 (IL-6) is now recognized as a hormone-like cytokine due to its diverse physiological effects and is a rapid-response inflammatory protein with a short plasma half-life. Synthesis of IL-6 takes place in almost all cell types [14,15] [Rose-John 2020], including cells of the immune system, delineating the pleiotropic nature of a cytokine that also plays a role in regulating metabolism, including insulin sensitivity, energy expenditure, and lipid homeostasis, as well as endothelial function, neuronal activity. and more [16,17]. Interleukin-10 (IL10) was originally named cytokine synthesis inhibitory factor; it is a key pleiotropic immunomodulatory cytokine created by type 2 helper cells (Th2), B cells, monocytes, thymocytes, and macrophages typically delivered locally from insusceptible cells to help settle irritation, and is best described for its capacity to repress macrophage initiation. It can restore equilibrium by inhibiting the development of pro-inflammatory cytokines and stimulating the production of defensive antibodies [18,19]. So, this study aimed to investigate the possible protective effect of pretreatment with DP extract on DOX-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

A total of 40 adult male rats of 10 weeks of age and weighing 239 ± 19.97 g were provided from the animal house of the College of Veterinary Medicine, Tikrit University, and were included in this study. The rats underwent an adaptation period for 1 week and were then distributed randomly into 4 groups, as explained in the experimental design. The experimental housing conditions were fixed daylight/night cycles (12 h each) and automated, regulated temperature and humidity of 23 ± 2 ℃ and 60–65%, respectively. All protocols included in this study were

approved by the Research Ethics Committee at Trikit University (Ethical Reference No: (4644/18/7 on 14/3/2019).

Rats were randomly divided into four equal groups, each containing 10 rats, and treated as follows:

- Control group (G1): This group contains 10 adult rats. Each rat received normal saline daily for thirty days.
- Group DPE (G4): This group contains 10 adult rats treated with Date Palm extract 2mg\kg\B.W oral gavage daily for thirty days.
- Group dox (G2): This group contains 10 adult rats treated with DOX 2mg\kg\B.W IP daily for thirty days.
- Group DOX+DPE (G3): This group contains 10 adult rats treated with DOX 2mg\kg\B.W IP and Date Palm extract 2mg\kg\B.W oral gavage daily for thirty days.

At the end of the treatment period, chloroform sacrificed rats, and samples from gastrointestinal cut-out and liver were isolated, cleaned off from the involved connective tissue and fat, and then preserved in 10% formalin for fixation. Next to fixation, samples were dehydrated through ascending series of ethyl alcohol 70%, 80%, 90%, and 100%, two changes each for 2 hours, then cleared with xylene for 1/2 hour. Samples impeded with paraffin wax (58-60 ºc) were then embedded with new paraffin wax to obtain paraffin blocks. Sections of 5-6 μm thickness were obtained using a rotary microtome deparaffinized, stained with hematoxylin and eosin stain, and then tested beneath a light microscope [22,23].

Extraction of Date Palm:

Zahedi dates were purchased from the market in Salah Addin governorate and washed with tap water, followed by air drying in the shade for one week at room temperature. Seeds were manually separated from the fruits and crushed into fine particles, and the fruits were also ground; the final ground fruits and crushed seeds were collected together and then extracted by Soxhlet apparatus using 99.9% ethanol. The extracted solution was collected and exposed to ambient temperature for one week till it became a semi-solid texture and was kept in the fridge until use. Eventual extract diluted with distilled water to be suitable for oral administration [24].

RESULTS AND DISCUSSION

The molecular mechanisms of DOX-induced hepatotoxicity are thought the DOX is metabolized in the liver by cytochrome P450 and carbonyl reductases, a semiquinone radical that generates reactive oxygen species (ROS), and increases its toxicity, inducing inflammation, calcium overload mitochondrial dysfunction [25]. This eventually causes hepatocyte death and leakage of hepatic enzymes into the circulation [26]. The present study revealed a significant increase in the serum IL-1B, IL-6, and IL-10 in Dox- (2 mg/kg.BW/IP)- treated rat comparing to (G1,&G4) (213.08±5.89 vs 13.55 ±0.885, & 13.55 ± 1.232pg/ml), (396.14±4.93 vs 79.80±2.114, &79.80 ± 3.51 pg/ml :P<0.00001), and (192.88±8.34 vs115.15 ± 5.57, and 115.97 ± 6.11 pg/mL:P<0.00001). Treatment with DPE at doses of (2mg/kg.BW/orally) significantly reduced IL-1B, IL-6, and IL-10 activities relative to the DOX-alone treated group (47.09±3.92 vs. 213.08±5.89 pg/ml: ($p < 0.0001$), (227.13±5.42 vs 396.14 ± 4.93 pg/ml: P<0.00001), and (143.80±4.63 vs 192.88±8.34 pg/ml: P<0.00001) respectively. (fig.1). Results of the present study supported a key role for inflammation in the pathogenesis of DOX-induced hepatotoxicity, demonstrating significant elevations in hepatic IL-1β and IL-6 in the DOX group compared to control. The principal underlying mechanism promoting this increase in inflammatory markers is not fully understood; however, it is possible that impaired tissue antioxidant capacity, heightened levels of ROS, and

subsequent lipid peroxidation are triggering factors for these changes. However, they were reduced following pre-cotreatment with DPE, suggesting its competency to maintain the normal integrity of hepatic muscle and to inhibit DOX-induced hepatic damage.

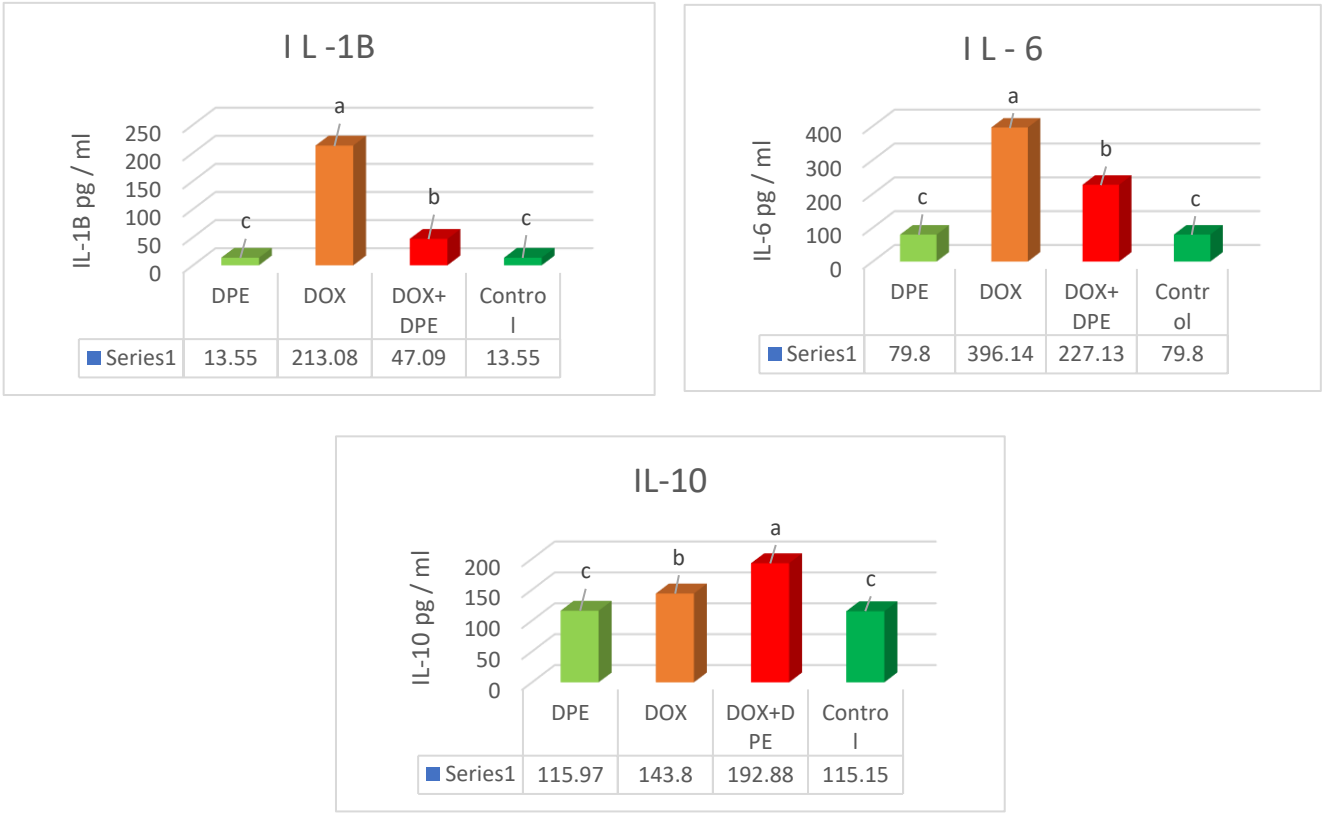

Figure 1. The protective effects of date palm extracts on IL-1β, IL-6, and IL-10 in rats treated with doxorubicin group.

Histopathological Examination of Liver Tissue:

Control Group

The liver tissue had the central vein in the lobule, which had RBCs and continuous from its periphery with blood sinusoids, which were present in network channels containing kupffer cells. Columns of liver cells surrounded those channels. Each cell had a spherical nucleus and eosinophilic cytoplasm; specific cells had two nuclei (fig.2).

Date Palm extract group (G2)

The portal area of liver tissue has the portal vein, a branch of the hepatic artery and bile ductules, which are surrounded by several WBCs; the liver cells are arranged in a radial pattern, surrounded by blood sinusoids with the presence of kupffer cells inside these channels (fig.3).

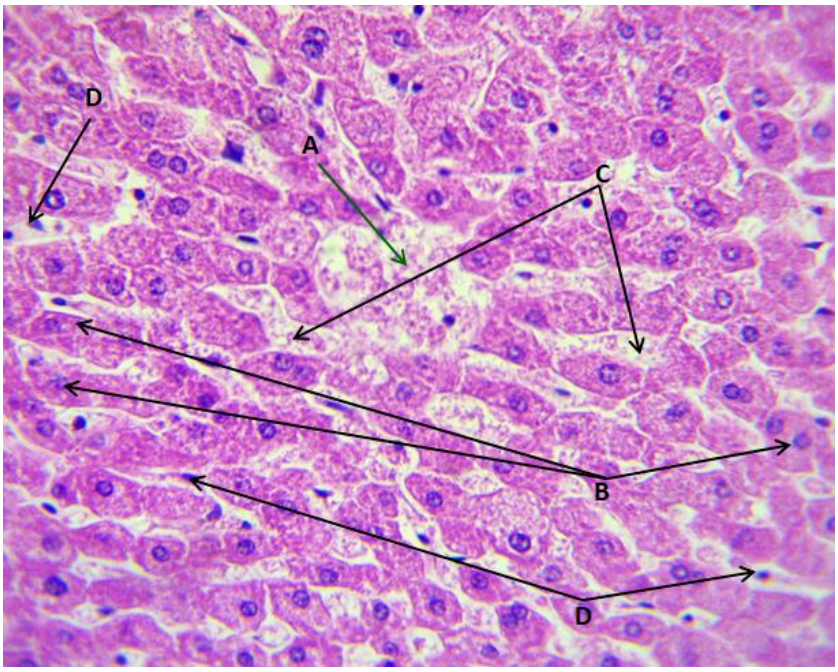

Figure 2. Liver lobules, central vein (A) liver cells columns (B) blood sinusoids (C) kupffer cells (D) (H & E x40).

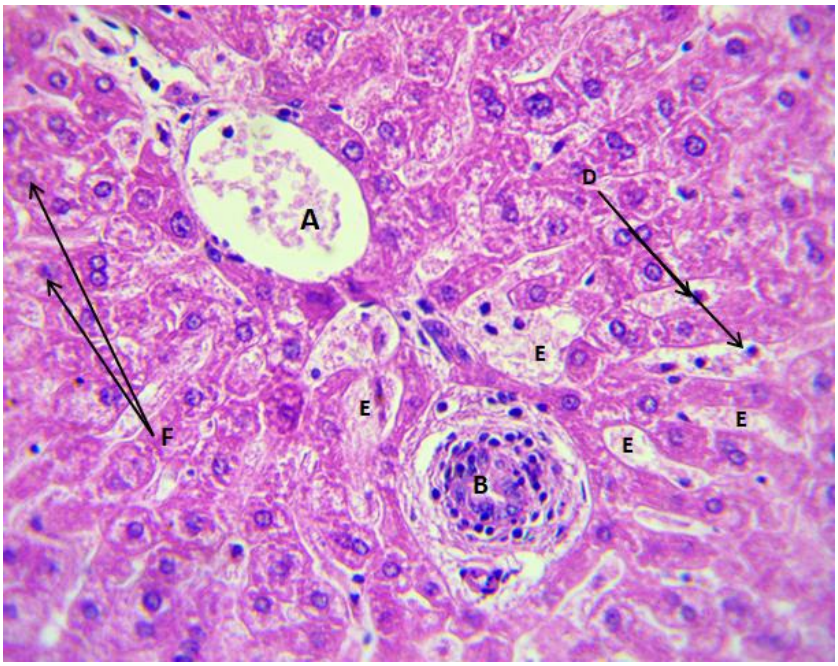

Figure 3. The portal area: portal vein (A), bile ductile (B), WBCs (C), kupffer cells (D), inside blood sinusoids (E), liver cells (F) (H & E x40).

Doxorubicin group (G3)

The liver tissue had degeneration of liver cells and necrosis of others, which lost its nuclei. With extensive vacuolation of its cytoplasm, pyknotic nuclei were seen, and kupffer cells were present in narrow blood sinusoids (fig.4).

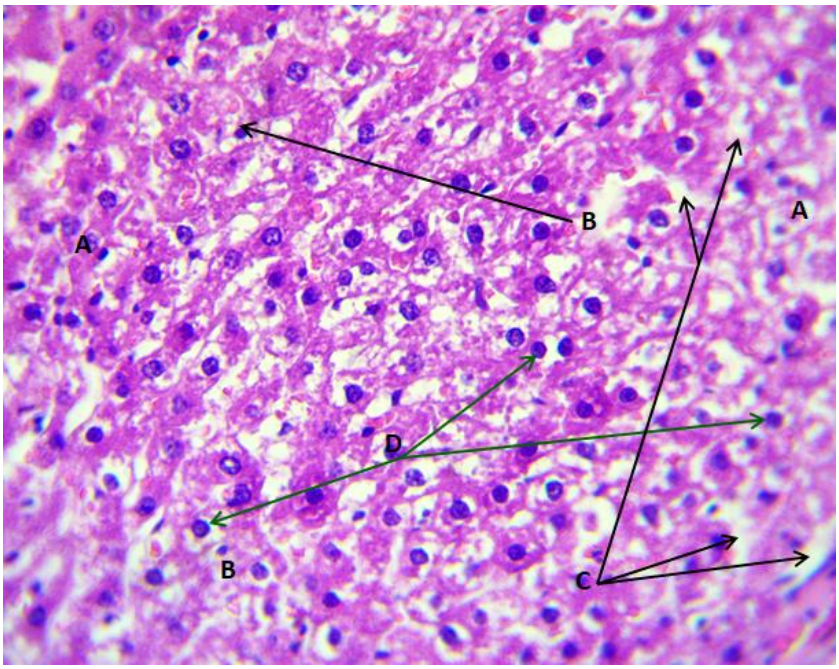

Figure 4. Degeneration, necrosis of liver cells and loss of nuclei (A) kupffer cells (B) in blood sinusoids (C) pyknosis of liver cell nuclei (D) (H & E x40).

The liver lobule had a wide central vein. Its endothelial cells were present resting on the thick basement membrane, liver cell masses were degenerated with vacuolated cytoplasm, and the nuclei of most cells were present in the narrow blood sinusoids (fig.5).

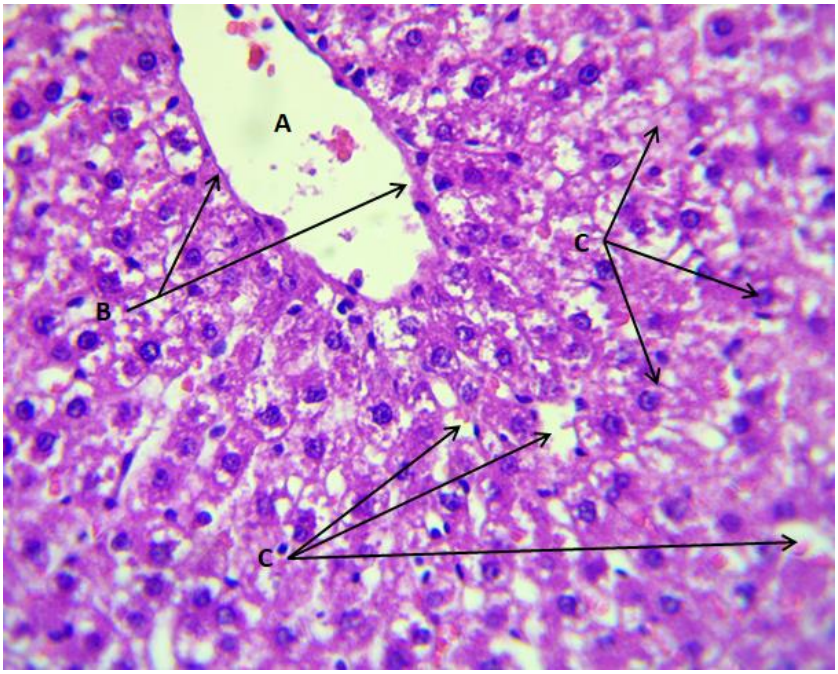

Figure 5. liver lobule, central vein (A), thickness of basement membrane (B) degeneration and necrosis of liver cells (C) with its pyknotic nuclei, sinusoids (D) (H & E x40).

Doxorubicin with Date Palm extract group (G4)

The portal area of the liver parenchyma had congested blood in the portal vein and bile duct, which are surrounded by WBC infiltration around its walls. A few liver cells near the portal area where the blood sinusoids contained many kupffer cells and many liver cells in its columns were atrophied (fig.6).

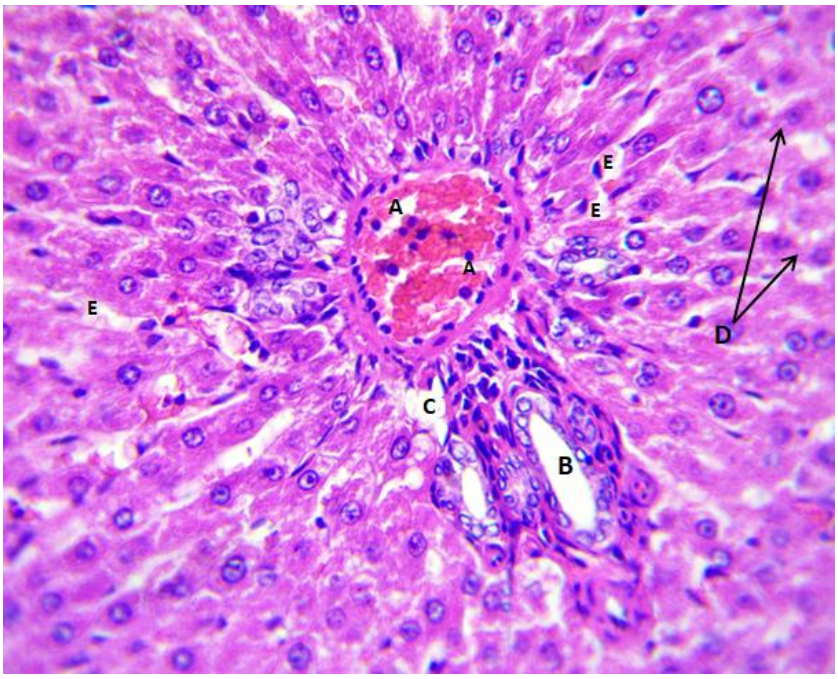

Figure 6. Portal area, congested blood of portal vein (A) bile ductule (B), WBCs infiltration (C) atrophy of liver cells (D) blood sinusoid with kupffer cells (E) (H & E x40).

The central vein was engorged with blood, which was continuous with blood sinusoids, and the sinusoids had kupffer cells with RBCs, liver cells were hypertrophied, and others were atrophied (fig.7).

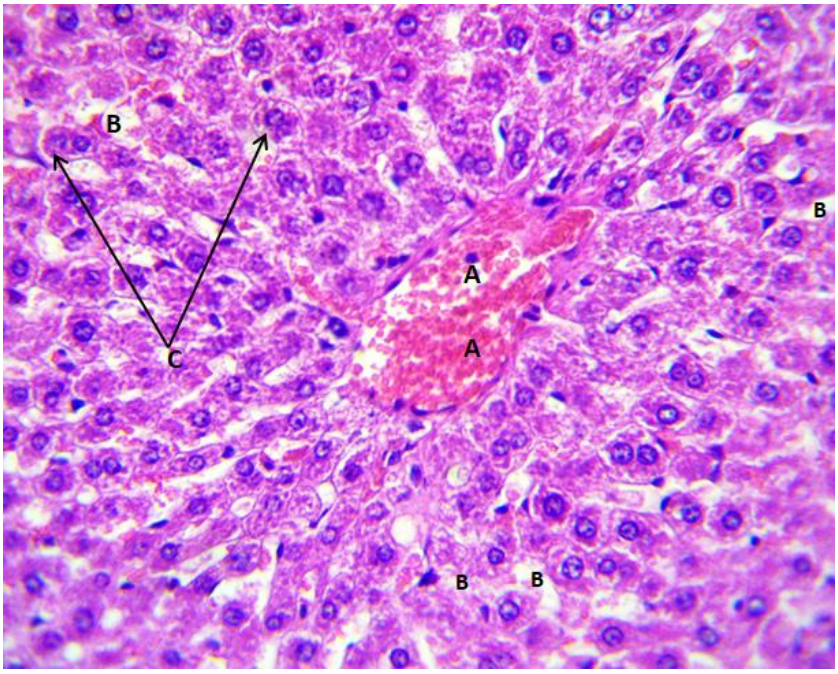

Figure 7. congested central vein with blood (A) blood sinusoid with kupffer cells (B) RBCs, hypertrophy of liver cells (C) atrophy of liver cells (D) (H & E x40).

DISCUSSION

An earlier study showed that antioxidant compounds could decrease hepatic function biomarkers in DOX-intoxicated rats. This results in agreement with [27,28].

CONCLUSIONS

The treatment with Date Palm alcoholic extract reduced oxidative damage in the hepatic tissue of experimental rats. DPE's antihepatotoxic, antioxidant, and membrane-stabilizing effects are mediated through biochemical marker regulation. DPE's active and beneficial components, such as flavonoids, vitamins, amino acids, polyphenols, carotenoids, minerals, fatty acids, and organic acids, have been connected to various pharmacological effects. Furthermore, the data imply that DPE could be useful in identifying potential therapies for DOX-induced hepatic damage.

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