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Original Article

Scanning and transmission electron microscopy of the cells forming the hepatic sinusoidal wall of rat in acetaminophen- and *Escherichia coli* endotoxin-induced hepatotoxicity

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ABSTRACT

Drugs and xenobiotics as well as bacterial endotoxins may reach the liver either systematically or after intestinal absorption. Therefore, cells lining the sinusoidal wall form the last barrier before blood constituents get in contact with the parenchymal cells. In this work, the ultrastructure of the cells forming the sinusoidal wall was studied after acetaminophen and *Escherichia coli* endotoxin treatments. Rats received acetaminophen at a dose of 1000 mg/kg body weight by intraperitoneal injection once in acute and four times with a 1-week interval in chronic treatments, and *E. coli* endotoxin at a dose of 5 mg/kg of body weight by intraperitoneal injection once in acute and four times with a 1-week interval in chronic treatments. Tissue samples were collected for scanning and transmission electron microscopy. Swelling of sinusoidal endothelial cells was noticed in both acute intoxicated groups with narrowing of the fenestrae, whereas large gaps were formed in chronic toxicity. Activation of Kupffer cells was a prominent common feature between the four toxicity groups. Interestingly, hepatic stellate cell activation was evident in both chronic acetaminophen and chronic endotoxin groups. Large amounts of collagen fibers were seen surrounding the hepatic stellate cells and in Disse space.

1. Introduction

Liver is the main organ of metabolism in the body. It has a unique blood supply as well as a unique microvasculature. Blood, which frequently carries toxins and infectious agents with gastrointestinal or systemic origins, passes in the liver through the sinusoids allowing the cells forming the wall of the sinusoids to get in direct contact with all irritants circulating in the blood especially in case of toxemia. Accordingly, sinusoidal cells represent the safeguard against inciting agents such as toxins, infectious agents, and particles delivered to the liver via blood. These cells react under these conditions by some defensive mechanisms to minimize the effect of the irritant on the hepatic cells and on other vital organs. In experiments and diseases, they display different and cell-specific reactions to accomplish their mission [1].

Normally, the fenestrae of the sinusoidal endothelial cells can control the passage of fluids and particles from

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sinusoids to the space of Disse in a selective manner depending on the size of these particles and the width of the openings [2]. Moreover, they exhibit high endocytotic activity aside from their role in cirrhosis and cancer [3]. Endothelial cells were suggested to have an important role in immunological response to some infections [4,5]. Kupffer cells are intrasinusoidal resident macrophages of the liver. Their main function is phagocytosis of the foreign bodies including dead and foreign cells, infectious agents, and foreign particles [1]. In addition, Kupffer cells are able to present antigens to lymphocytes, and they are important to the tissue repair process by clearing damaged tissues [6]. Upon activation, Kupffer cells secrete a wide range of inflammatory mediators and reactive oxygen species to control the tissue reaction against inciting agents as well as to organize the repair process [7]. Hepatic stellate cells (HSCs; also known as Ito cells and fat storing cells) are located in the space of Disse between the endothelial cells and the hepatocytes. They are easily distinguished during ultrastructural examination by numerous and variable-sized fat droplets in their cytoplasm, where vitamin A is stored. When activated, they morphologically transform to a myofibroblast-like cell with loss of fat droplets and extensive production of type I collagen [8,9]. Moreover, they are thought to be involved in the mechanism of metastasis process during malignant tumors [10] and controlling blood flow in the liver [6,11].

Acetaminophen (APAP) is a commonly used analgesic in humans and animals. Although it is considered safe at therapeutic doses, overdose of APAP produces toxic effects in humans and in experimental animals [12]. Accidental or intentional APAP overdose is the most common cause of drug-induced liver injury [13]. Along with the centrilobular necrosis of hepatocytes, toxicity of APAP is accompanied by Kupffer cell activation [14] and endothelial cell swelling [2]. Lipopolysaccharide represents one of the main components of the outer membrane of Gram-negative bacteria. They are able to induce inflammatory reaction upon administration to experimental animals. The inflammatory reaction to lipopolysaccharide was manifested by neutrophil infiltration to the liver tissue along with hepatocellular necrosis and activation to the Kupffer cells and the sinusoidal endothelial cells [4,15].

In this work, we used two models of toxicity (APAP- and endotoxin-induced hepatotoxicity) to study the morphology of the cells lining the hepatic sinusoidal lumen and their reaction to these two types of toxin hoping to shed light on the mechanisms of reaction of these cells and their role in different liver diseases.

2. Materials and methods

Twenty-five male albino rats were kept on commercial rat diet with free access to tap water at room temperature with a 12-hour light/dark cycle. Every possible effort was made to minimize animal suffering, and the experiments followed the ethical guidelines of Assiut University, Assiut, Egypt. Animals were randomly divided into five groups, each consisting of five rats. Group I served as the control group and received only the vehicle. Group II received a single intraperitoneal injection of APAP (Sigma-Aldrich) at a dose of 1000 mg/kg body weight after 16 hours of fasting [16]. Group III received a single intraperitoneal injection of *Escherichia coli* endotoxin (*E. coli* O139 k82) at a dose of 5 mg/kg of body weight [17]. The endotoxin was prepared according to Ellis et al. [18] and Davis and Goldberg [19]. Briefly, the overnight culture of the bacteria was used to isolate the toxin using a modification of the hot phenol–water method. Group IV received four successive weekly intraperitoneal injections of APAP at a dose of 1000 mg/kg body weight after 16 hours of fasting, whereas group V received four successive weekly intraperitoneal injections of *E. coli* endotoxin at a dose of 5 mg/kg of body weight. The first three groups were subjected to perfusion fixation 24 hours after the single toxin injection, whereas the chronic toxicity groups were sacrificed 1 week after the last injection.

Prior to sampling, rats were anesthetized using intraperitoneal injections of ketamine and xylazine at a dose of 60 mg/kg and 6 mg/kg of body weight, respectively. Perfusion fixation was performed at the time of sampling using warm normal saline solution followed by cold gluteraldehyde 4% fixative. After perfusion, livers were removed and carefully cut with a razor blade under the same fixative into 1-mm^3 blocks. These blocks were immediately transferred to labeled bottles with 4% cold gluteraldehyde and kept for transmission electron microscopy (TEM). Samples

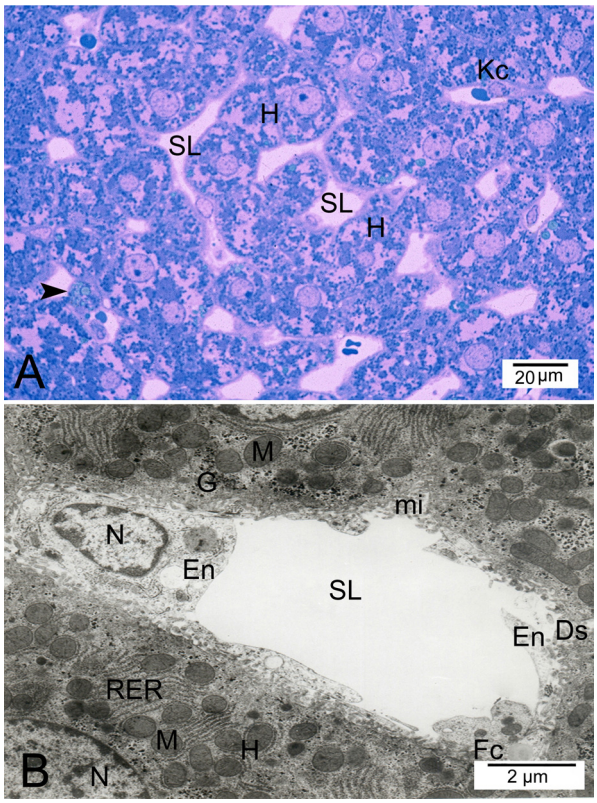

Fig. 1. (A) Light and (B) transmission electron micrographs of normal hepatic sinusoidal wall of rat. Note the presence of hepatic cells (H), sinusoidal lumen (SL), endothelial cells (En), Kupffer cells (Kc), fat storing cells (arrowhead and Fc), Disse space (Ds), microvilli (mi), nucleus (N), rough endoplasm reticulum (RER), mitochondria (M), and glycogen granules (G).

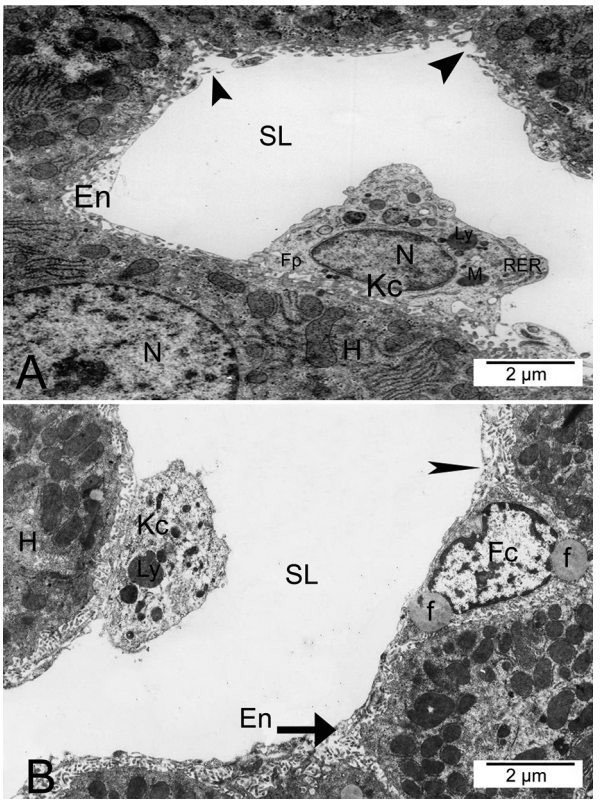

Fig. 2. Transmission electron micrographs of normal hepatic sinusoidal wall of rat. Note the presence of hepatic cells (H), sinusoidal lumen (SL), endothelial cells (En), Kupffer cells (Kc), fat storing cells (Fc), nucleus (N), rough endoplasm reticulum (RER), mitochondria (M), lysosome (Ly), filopodia (Fb), fat globule (f), and endothelial fenestrae (arrowheads).

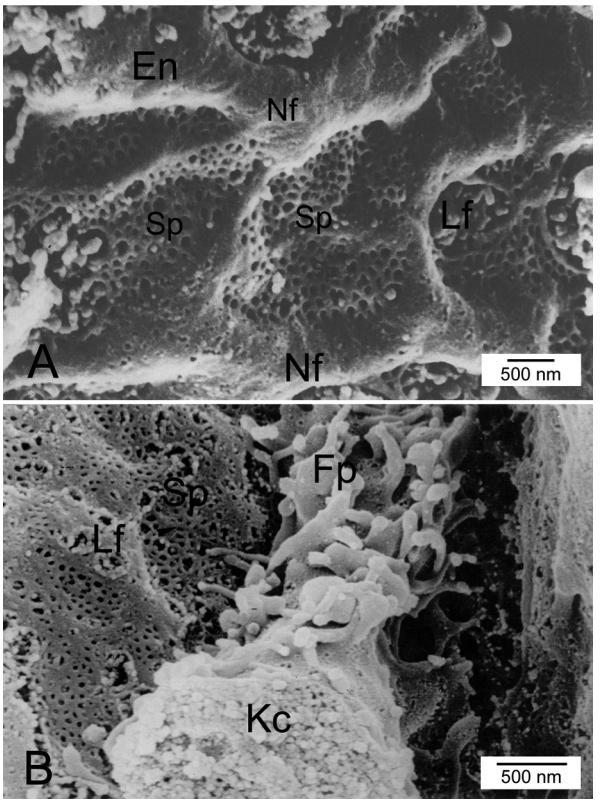

Fig. 3. Scanning electron micrographs of normal hepatic sinusoidal wall of rat. Note the presence of endothelial cells (En), Kupffer cells (Kc), non-fenestrated (nf) and large endothelial fenestrae (Lf) in the sieve plate (Sp), and filopodia (Fp).

were then processed and embedded in Epon. Semithin sections were stained with Toluidine blue and photographed. Ultrathin sections were cut on an LKB Ultrotome III, and photographs were taken with JEOL JEM-100CX II Electron Microscope [20]. For scanning electron microscopy (SEM), larger blocks were taken after perfusion fixation, kept in 4% cold gluteraldehyde, processed for SEM, and then examined using JEOL (JEOL Ltd, Tokyo, Japan) JSM 5400 LV Scanning Microscope.

3. Results

In normal liver, three types of cells were found to constitute the wall of the hepatic sinusoids (Figure 1). The flat and fenestrated endothelial cells appeared forming the wall of the sinusoids where they group to form the sieve plates. Beside the small fenestrae in the sieve plate, large fenestrae could frequently be seen, alongside with the nonfenestrated areas. Endothelial cells had scanty cytoplasm and few cellular organelles (Figures 1–3). Kupffer cells were found protruding from the luminal surface of the endothelium and extending microvilli and filopodia through the fenestrae of the endothelial cells (Figures 1A, 2, and 3B). The cytoplasm of Kupffer cells contained variable-sized vacuoles and microvesicles along with lysosomes, mitochondria, rough endoplasm reticulum (RER), ribosomes, and nucleus (Figures 1A, 2, and 3B). The fat storing cell contained rounded nucleus that sometimes appeared compressed by the characteristic, variable density fat droplets present in the cell cytoplasm (Figures 1 and 2B).

Examination of acute intoxicated animals with APAP showed various degrees of degenerative changes in the liver parenchymal cells such as hydropic and fatty degeneration. Hydropic degeneration in the centilobular areas of the hepatic lobules could be seen around the central vein (star) accompanied by fatty degeneration in the cytoplasm of the hepatocytes (arrowheads, Figure 4A). Sinusoidal endothelial cells appeared swollen with ratified cytoplasm, which apparently led them to overlap each other and eventually led to narrowing of their fenestrations (Figure 4B, arrow). Activation and swelling of Kupffer cells could be also seen with the presence of some leukocytes in the sinusoids (Figure 4C). Similar changes in Kupffer cells were seen in chronic APAP-intoxicated animals (Figures 5 and 6B). Activation of Kupffer cells was made evident by proliferation, swelling, and the large number of vesicles. As expected, fat storing cells did not react in acute stages of the toxicity, but they showed extraordinary production of large amounts of collagen fibers that could be easily encountered around themselves, in Disse space and in between the hepatic cells in chronic APAP-induced hepatotoxicity (Figures 5B, 6A, and 6B). Some degree of

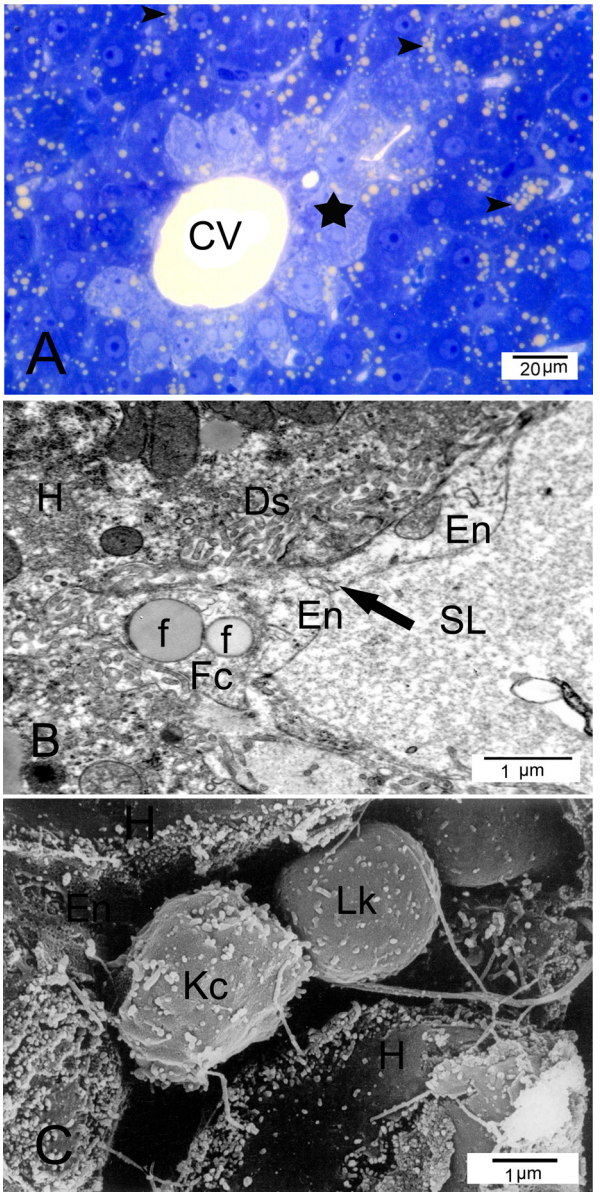

Fig. 4. (A) Light, (B) transmission electron, and (C) scanning electron micrographs of the cells forming sinusoidal wall in acute acetaminophen toxicity. Hydropic degeneration of the hepatocytes around the central vein (star) was seen with fatty degeneration all over the hepatic lobule (arrowheads, panelA). Swelling of the endothelial cells (En) led to narrowing of the fenestrae (arrow) was observed (panel B). Activation of Kupffer cells (Kc) was also seen (panel C). Note also hepatocytes (H), fat globules (f), sinusoidal lumen (SL), Disse space (Ds), and leukocytes (Lk).

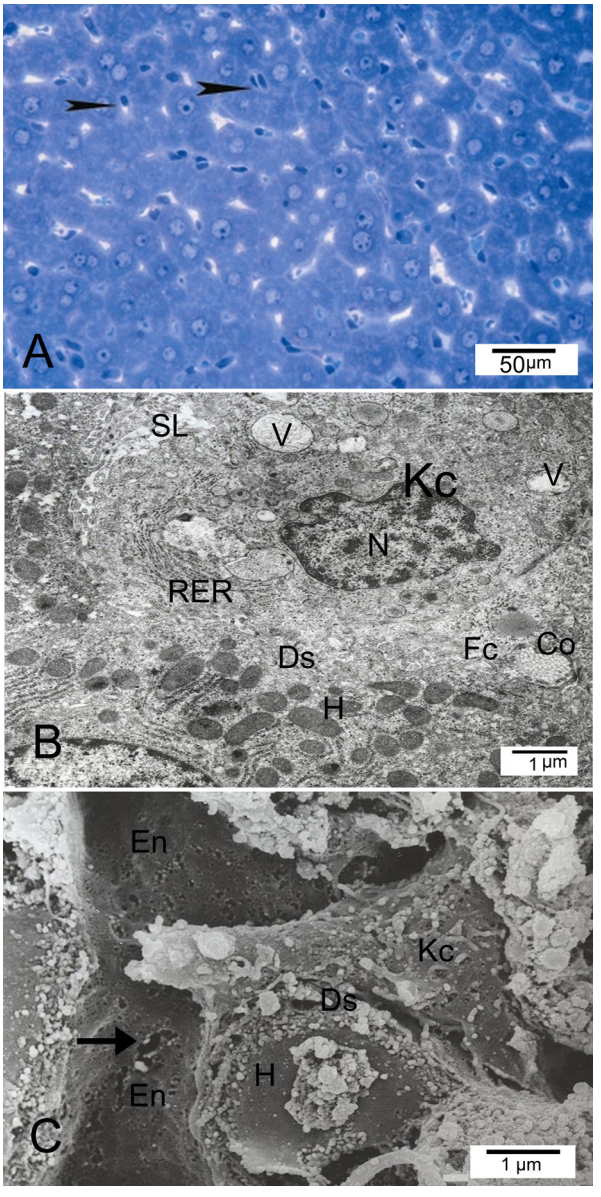

Fig. 5. (A) Light, (B) transmission, and (C) scanning electron micrographs of the cells forming sinusoidal wall in chronic acetaminophen toxicity. Increase in number of Kupffer cells (Kc, panel A) and signs of their activation could be observed with increase in number of vesicles (V) in their cytoplasm (panel B). Collagen deposition (Co) could be seen adjacent to the fat storing cell (Fc) and the Disse space (Ds, panel B). Large gap formation (arrow) was seen in the sieve plate (panel C). Note also Hepatoctes (H), sinusoidal lumen (SL), endothelial cells (En), and rough endoplasmic reticulum (RER).

fatty degeneration was also seen in the cytoplasm of the hepatocytes (Figure 6B).

Similar changes were observed in endotoxin-induced hepatotoxicity. Activation of the Kupffer cells in acute endotoxin-treated animals was a prominent feature. They showed an increase in size and number (Figure 7A) with a large number of phagosomes, some of them containing red blood cells (Figure 7B). Kupffer cells appeared hypertrophied to the point that they sometimes appeared occluding the sinusoidal lumen (Figure 7B). Endothelial cell swelling was also observed in Figures 7A and 7B (arrows). Hydropic degeneration as well as fat droplets indicating fatty degeneration could be seen in both acute (Figure 7B) and chronic (Figures 8B and 8C) endotoxin-induced hepatotoxicity. Again, fat storing cells reacted clearly after chronic endotoxin treatment. They could be easily recognized by their anatomical location and the characteristic fat globules in their cytoplasm (Figure 8).

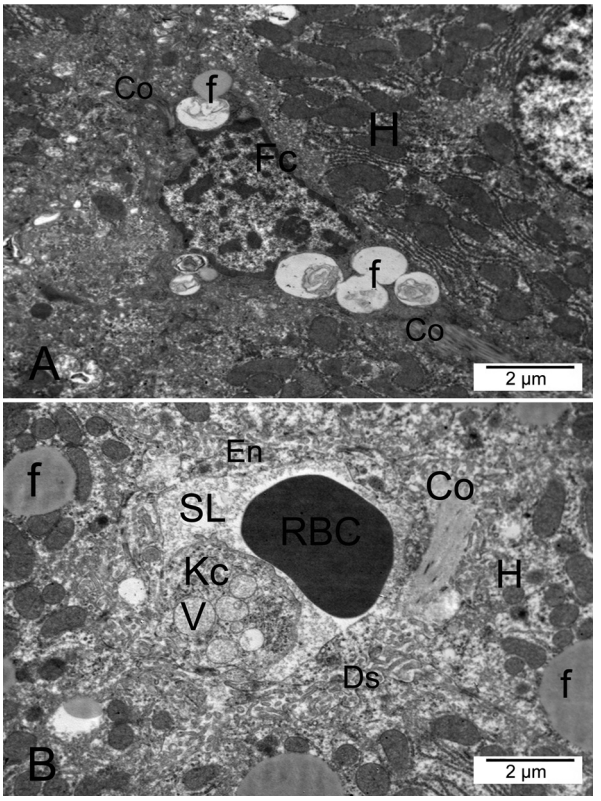

Fig. 6. Transmission electron micrographs of fat storing cells in chronic acetaminophen-induced toxicity. The collagen fibers (Co) could be seen deposited adjacent to the space of Disse (Ds) and around the fat storing cells (Fc). Fatty degeneration (f) was noticed in the hepatocytes (H) as in panel B. Note also the presence of Kupffer cells (Kc) with multiple vacuoles (V) in their cytoplasm.

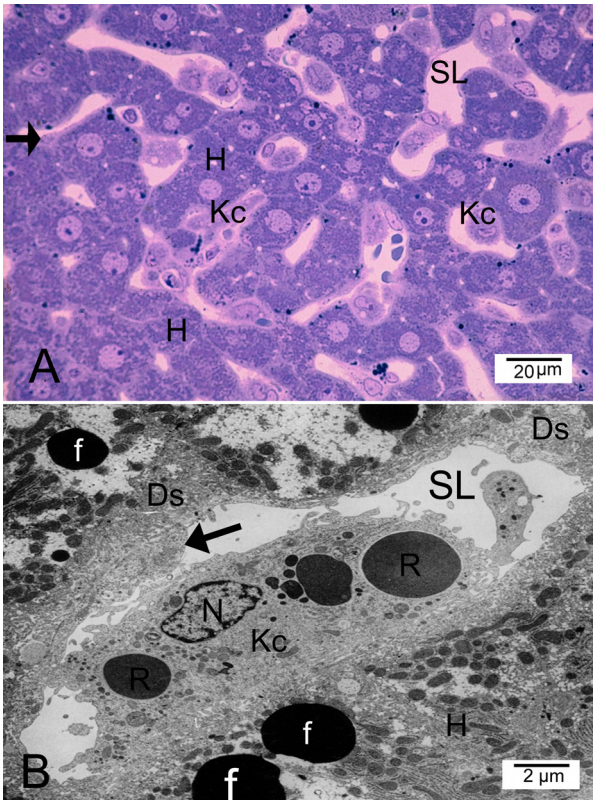

Fig. 7. (A) Light and (B) transmission electron micrographs of the cells forming sinusoidal wall in acute *Escherichia coli* endotoxin toxicity. An increase in both size and number of Kupffer cells (Kc) was observed sometimes filling the sinusoidal lumen (SL) and sometimes containing red blood cells (R) in phagosomes. Endothelial cell swelling was also evident (panels A and B, arrows). Fat globules (f) in hepatocytes (H) were also observed (panel B).

TEM examination revealed extensive collagen production radiating from them toward the liver parenchyma (Figures 8B and 8C). Some necrobiotic changes such as fatty and hydropic degeneration were also observed in chronic endotoxin-treated rats (Figures 8B and 8C). SEM examination of this group showed wide gaps formation in the sieve plates of the endothelial cells (Figure 9A) as well as swelling of the Kupffer cells (Figure 9B).

4. Discussion

Sinusoidal endothelial cells act as a dynamic barrier controlling the passage of fluids and solute through its fenestrae, which are devoid of basal lamina. Moreover, they can transport macromolecules from blood to hepatocytes and vice versa as well as to neighboring cells by endocytosis [2,3]. In our experiment, the sinusoidal endothelial cells of acute intoxicated animals with both APAP and endotoxin appeared swollen with narrowing of the fenestrations as well as overlapping each other. These results are in accordance with a previous report that described defenestration of the hepatic sinusoidal endothelial cells after endotoxin treatment [21]. Loss of fenestrae represents the main reaction of sinusoidal endothelial cells against toxins [2].This reaction can be regarded as a defense mechanism by the endothelial cells to minimize the entry of toxins to the space of Disse and hence to the hepatocytes. The loss of endothelial fenestrae is thought to be an early step in the pathogenesis of liver cirrhosis [3]. By contrast, chronic treatment with APAP and endotoxin resulted in formation of wide gaps because of the destruction of the membranes. Sinusoidal endothelial cells are very sensitive to oxidative stress [22], which explains their damage after chronic treatments with APAP and endotoxin since both, like many toxicities, usually result in oxidative stress [12].

In both acute and chronic treatments, Kupffer cells appeared hypertrophied and active, and contained numerous vesicles and phagosomes sometimes containing red blood cells. Activation is also morphologically evident by roughness of the cell surface and increase in number of micovilli and filopodia [2]. This reaction was noticed by other authors and appeared to characterize the reaction of Kupffer cells against various toxins [23]. Endocytosis by activated Kupffer cells and sinusoidal endothelial cells is regarded as the main clearance route of some toxins [24]. Moreover, activated Kupffer cells secrete various inflammatory mediators including superoxide, nitric oxide, platelets activating factor, leukotrienes, interleukins, interferon α/β, and tumor necrosis factor α. These mediators

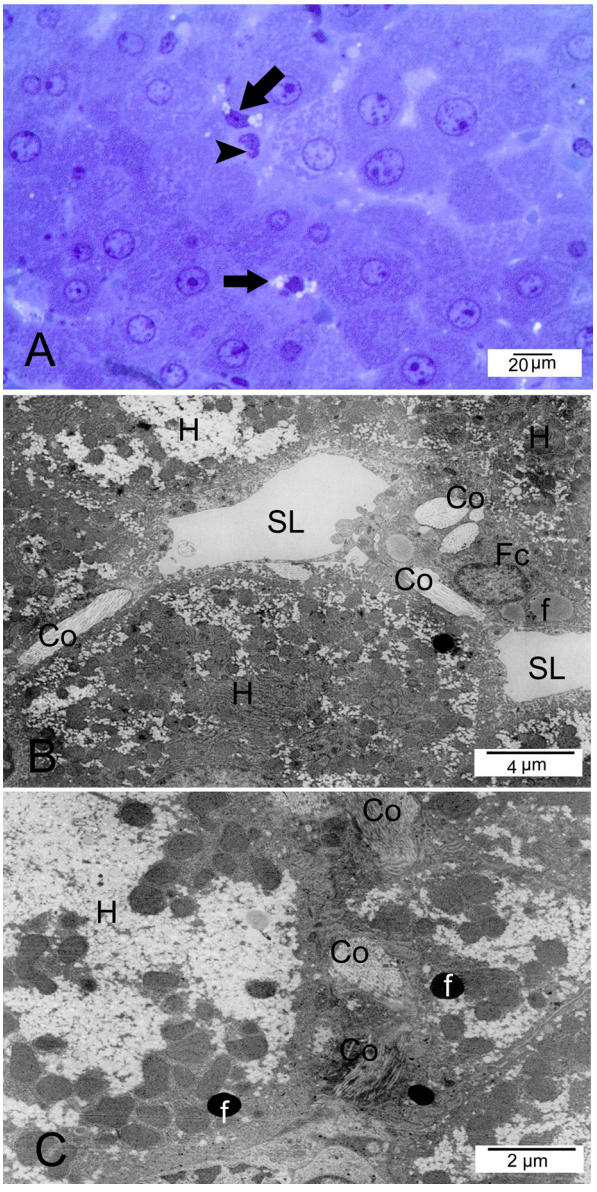

Fig. 8. (A) Light and (B and C) transmission electron micrographs of fat storing (Arrows and Fc) cell in chronic *Escherichia coli* endotoxin-induced toxicity. Fat storing cells (arrows) and Kupffer cells (arrowhead) were observed in panel A. The collagen fibers (Co) could be seen deposited adjacent to the space of Disse (Ds) and around the fat storing cells (Fc, panels B and C). Fatty degeneration (f) could be noticed in the hepatocytes (H) along with hydropic degeneration as in panel C.

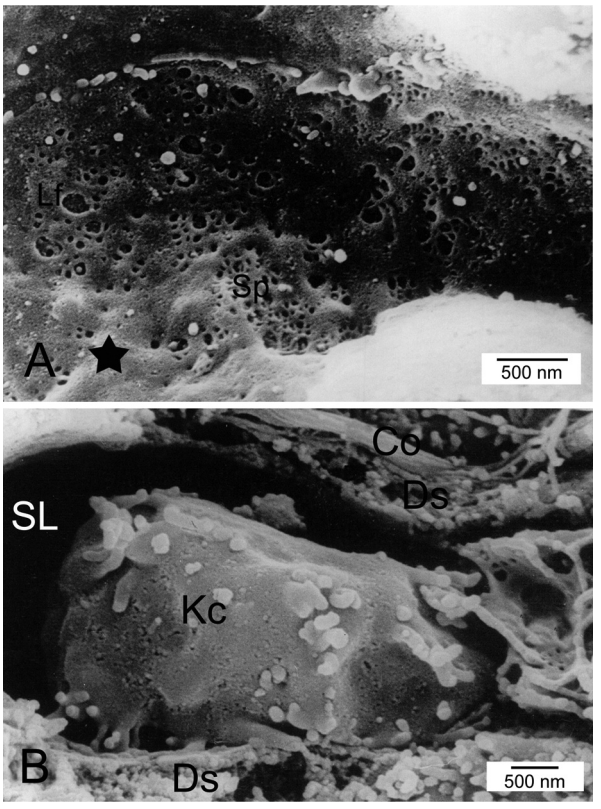

Fig. 9. Scanning electron micrographs of the cells forming sinusoidal wall in chronic *Escherichia coli* endotoxin toxicity. Some areas of the sieve plate (Sp) lost fenestration (star). Note also the presence of large fenesterae (Lf, panel A). An increase in size of Kupffer cells (Kc) was observed sometimes filling the sinusoidal lumen (SL) as can be seen in panel B. Note also the presence of collagen fibers (Co) in the space of Disse (Ds).

start and control many pathological mechanisms in the liver to counteract the effects of the toxins and promote the detoxification process [1,23].

Our results showed activation of fat storing cells evidenced by transforming to fibroblast-like cell producing extraordinary amounts of collagen fibers in chronic stages. These fibers were seen around them, in Disse space, and in between the hepatic cells. Activation of HSCs is important to the detoxification process because it contains several enzymes involved in both intermediary metabolism and detoxification xenobiotics [8,11]. Although HSCs were always thought to react in chronic conditions, they were suggested to have an early role in conducting the inflammatory signal from the sinusoids to the parenchymal cells based on their production of many inflammatory mediators in an early stage after exposure to an irritant [25,26]. Shen et al. [27] reported a more severe hepatic injury induced by APAP after depletion of HSCs, a fact that proves their protective role in the mechanism of APAP-induced hepatotoxicity.

5. Conclusion

In conclusion, our results suggest that these three cells lining the sinusoidal wall synergistically play the most important role in the process of reaction of the liver against different types of toxins. Each cell undergoes morphological transformation to meet the new functional demand according to the nature of the irritant and the time of exposure. Owing to the anatomical location, the unique structure, and physiology, these three cells act as the gatekeepers of the liver against bloodborne irritants. Further investigations are required to fully understand their role in liver diseases and especially in case of toxicity.

6. Conflicts of interest

The Authors declare no conflict of interest.

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