رقم البحث (43)

ELECTRON MICROSCOPIC STUDY ON THE THERAPEUTIC EFFECT OF CURCUMIN AGAINST CCL4 INDUCED HEPATIC FIBROSIS

BY

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ABSTRACT

Curcumin used as food additives and traditionally used in Chinese medicine for treatment various chronic diseases as it possess antioxidant and anti-inflammatory properties, so we try to study its effect on hepatic fibrosis as serious prevalent problem among animals and humans, we found that curcumin has potent antifibrotic properties not only stop progression of liver fibrosis but also ameliorated the fibrous tissue in rat liver with preformed fibrosis by CCl4 and improved ultrastructure of the hepatocytes by using TEM examination. So curcumin can treat liver fibrosis through its antifibrotic properties.

Keywords: Curcumin, antifibrotic , Hepatic fibrosis

INTRODUCTION

Curcuma longa or turmeric is a tropical plant native to southern and southeastern tropical Asia. It belongs to the ginger family (Lampe et al., 1910). Turmeric has been used for centuries for the treatment of inflammatory diseases (Chopra et al. 1958 and Nadkarni 1976). Curcumin (CMN), the main yellow pigment of a popular spice, is a polyphenolic compound and is widely used as a food colorant. CMN plays essential pharmacological roles, such as antioxidant, anti-inflammatory agent, anti-fibrotic agent and hepatoprotector (Yang et al. 2005).

Hepatic fibrosis is a progressive pathological process, caused by a variety of agents including viral hepatitis, alcohol abuse, drugs, and metabolic diseases involving an overload of iron or copper, autoimmune diseases, or congenital abnormalities (Friedman 2000 and

Pinzani et al. 2001). In respond to liver injury, myofibroblasts promote replacement of normal hepatic tissue with a scar-like matrix composed of cross linked collagen (Elsharkawy et al. 2005).

The CMN protects against fibrogenesis induced by CCl4 by attenuating oxidative stress through improving the antioxidant activity (Ruby et al. 1995), inhibiting of lipid peroxidation (Rajakumar and Rao, 1994 and Sreejayan and Rao et al. 1994), suppressing inflammation through significantly reducing the levels of proinflammatory cytokines, including the TNF-α and IL-6 (Fu et al. 2008) and inhibiting activation of hepatic stellate cell (HSC) through interruption of the signaling pathways for transforming growth factor-beta (TGF-β), platelets derived growth factor (PDGF), and epidermal growth factor (EGF) (Fu et al. 2008 and Xu et al. 2003). Objectives of this research study were to elucidate the therapeutic effects of curcumin to alleviate the hepatic fibrosis by using the electron microscope.

MATERIALS AND METHODS

Experimental Animals

Sixty female Sprague-Dawley rats (200-250 gm B.wt) were purchased from the Animal Research House located in Holding Company for Biological Products & Vaccines (Agoza, Giza). They were housed in plastic cages and kept under strict hygienic condition. The animals were fed on a standard laboratory diet, besides fresh water ad libitum, when the rats attained a weight of 200–250 g, 50 rats were equally divided randomly into sex equal groups.

Treatment regimen was as follow:

Group one: Negative control (10 rats)

Group two:(experimental control for olive oil): Ten rats were orally given olive oil orally (3-4 ml) daily for 6 weeks.

Group three:(experimental control for curcumin): Curcumin (purchased from Sigma Chemical, Co. Ltd. St. Louis, MO, USA) was orally given at a dose of 200 mg/kg B wt, dissolved in olive oil (He et al. 2006 and Shu et al. 2007) daily for about 6 weeks.

Group four : (model group for hepatic fibrosis): Ten rats were IP injected with CCl4 (purchased from ADWIA Co, Egypt) (50% v/v) in olive oil (1 ml/kg) every 3 days for a period of 6 weeks, for development of hepatic fibrosis. (kucharska et al. 2004)

Group five: (therapeutic group): Ten rats were IP injected with CCl4 (50% v/v) in olive oil (1 ml/kg B wt) every 3 days for a period of 6 weeks. Then CMN was daily administerated for about one week via stomach gavage, at dose of 200mg/kg B wt (Maiti et al. 2007 and Park et al. 2000) dissolved in olive oil.

Rats were sacrificed under ether anesthesia and liver was fixed in 2.5% glutaraldehyde for electron microscopic examination.

Transmission electron microscopy

Two freshly cut liver sections (size, about 1 mm3 each) from the left lobe of the livers were taken. The liver samples were immersed in buffered 5 % glutaraldehyde for 2-24 hours. then washed in cacodylate buffer (0.1 M, pH 7.2) 3-4 times for 20 minutes at every time and then post fixed in 1% osmium tetroxide for 2 hours. After repeated washing in cacodylate buffer (4 X 20 minutes), by using ascending grades of ethyl alcohol up to 100% (30, 50, 70, 80, 90 and 100% /2 hours) dehydration was done and using gelatine capsule they were embedded in Epon 812. For polymerization, the embedded samples were kept in incubator at 35 C° for one day, at 45 C° for another day and three days at 60 C°. From prepared blocks, using LKB ultra microtome, semithin sections in thickness of 0.5-1µ were prepared. The sections were stained by toluidine blue, examined by light microscope, photographed and regions for preparation of ultrathin sections were oriented and by Leica ultramictotome the ultrathin sections at a thickness of 500-800 Å were made and fixed on copper grids (200µ meshes). The ultrathin sections were then contrasted in uranyl acetate for 15 minutes and lead citrate for 5 minutes and examined by a transmission electron microscope (Jeol, CX11) in the electron Microscope Unit, of Assiut University.

RESULTS

Control groups (1, 2 and 3), the TEM revealed hepatocytes with healthy mitochondria and nucleus. Moreover, the fat storing cell (Ito cell) was observed among healthy hepatocytes. The later contained normal mitochondria and RER. No collagen fibers were seen in the Disse-space (fig. 1).

Meanwhile, the toluidine-blue-stained-sections in the group four revealed cellular necrosis, dysplasia, microvesicular hepatic steatosis, intralobular fibrosis and little regenerative capacity (Fig. 2). Moreover, the TEM revealed damaged and swollen mitochondria, besides apoptotic Kupffer cells, and deposition of fibrillar collagen in space of Disse. Collagen fibers could be seen in Disse-space with inter-communicating spaces in an adjacent hepatocyte (figs. 3& 4). Kupffer cell activation could be noticed along with lipofuscin pigments in an adjacent hepatocyte. Activated hepatic stellate cell (fat storage cell) and activated Kupffer cells were seen.

In contrary the group five semi-thin sections revealed highly regenerative capacity of hepatocytes, microvesicular fatty change, and intralobular fibrosis (fig. 5). The TEM, of the ultra-thin section showed medium density fat globules in the cytoplasm of hepatocyte with somewhat intact mitochondria and RER, besides activated Kupffer cell protruding in the lumen of the hepatic sinusoid with minimum collagen fibers were seen in Disse-space (fig. 6). The mitochondria appeared as O or C shape, indicating an increased energy-production.

DISCUSSION

The present study showed that the oral administration of curcumin, a major component of the food additive turmeric, treated rats against CCl4-toxicosis. Curcumin ameliorated the hepatic-degenerative, necrotic and fibrotic effects of CCl4. The TEM revealed swollen mitochondria and apoptosis of the Von kupffer cells, besides deposition of fibrillar collagen in the spaces of Disse. The presence of activated Kupffer cells along with lipofuscin pigments in an adjacent hepatocyte is due to formation of autophagosomes and deposition of fat soluble pigment which indicates sublethal cellular injury. Activated hepatic stellate cells (fat storage cell) and Kupffer cells could be attributed to reactive free radical metabolites, such as trichloromethyl (CCl3) and trichloromethyl peroxyl radical (OOCCl3) which impaired the

cellular functions, depending on membrane integrity (loss of calcium homeostasis) and ultimately apoptosis and cell death (Boll et al. 2001 and Weber et al. 2003).

The hepatic stellate cells (HSC) account for 5–8% of the total cell-count in the normal liver. They are located in the perisinusoidal spaces of Disse, in between the sinusoidal endothelium and hepatocytes, with a higher frequency in the periportal areas than centrilobularly (Giampieri et al. 1991 and Wake, 1995). The HSC are the primary cells, in the liver, that are responsible for excess collagen-synthesis during hepatic fibrosis (Hanauske-Abel, 2003), Meanwhile, our results revealed less fibrillar collagen in the spaces of Disse of treated groups more than model group, besides restoration of the cellular organelles as mitochondria and a marked improvement of the cellular ultra-structure, with nearly absence of fibrillar collagen from the spaces of Disse. Our results are in accordance with Abu-Rizq et al. (2008), who trearted the CCl4-induced cellular hepatic damage with CMN. They found a significant hepatic- recovery, at the level of TEM.

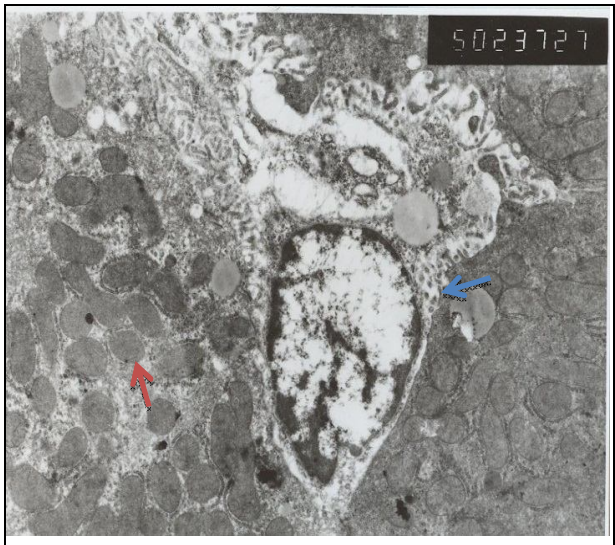

Fig. (1): liver showing fat-storing cell (blue- arrow) present between two healthy hepatocytes. The later, contain normal mitochondria (red-arrow). (TEM, UA, 100000x).

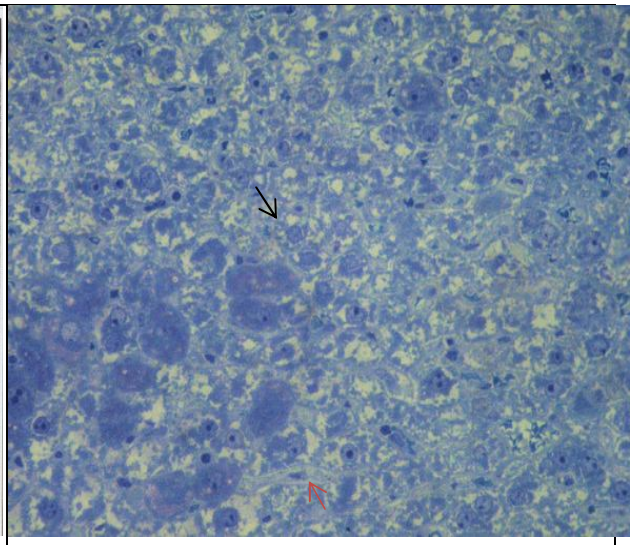

Fig. (2): liver shoeing marked cellular degeneration and necrosis (lower L), small cell dysplasia (arrow) with lost normal lobular architecture. Early intralobular fibrosis in the form of mild fibroblastic proliferation. (Toluidin blue, 300x)

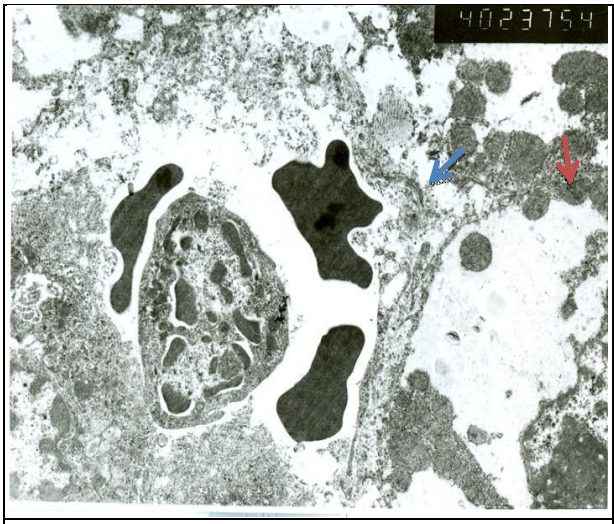

Fig. (3): Liver showing hepatic sinusoids containing RBCs and neutrophils. Collagen be seen in Disse-space (blue- arrow) with intercommunicating spaces in an adjacent hepatocytes (red-arrow). (TEM, UA, 80000x).

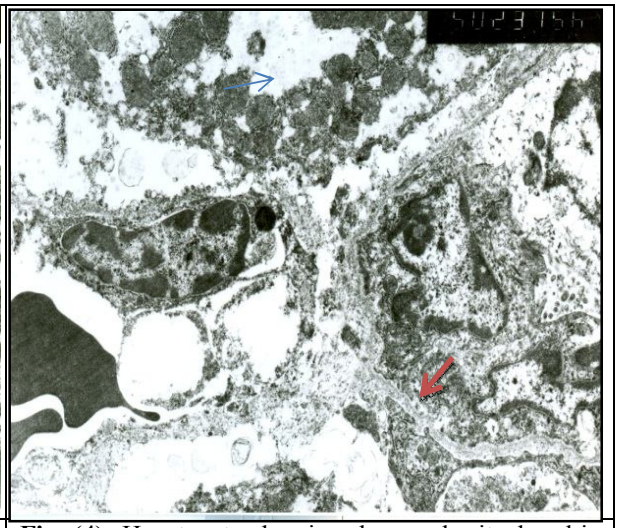

Fig. (4): Hepatocyte showing damaged mitochondria (blue-arrow). Also collagen be seen in Disse-space(blue- arrow) with intercommunicating spaces in an adjacent hepatocytes (red-arrow). (TEM, UA, 100000x).

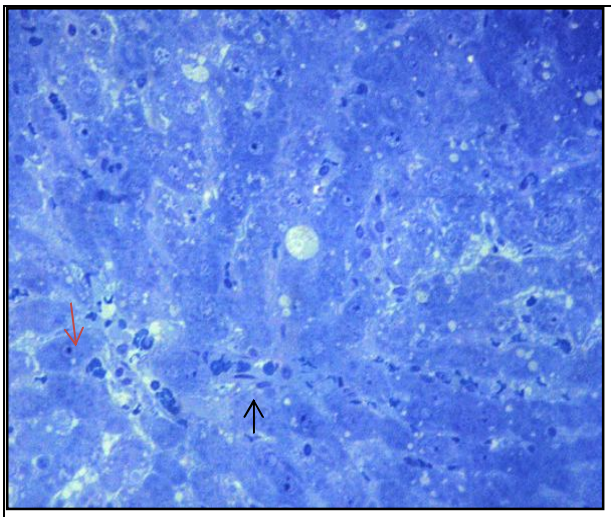

Fig. (5): liver showing intralobular fibrosis (arrow), besides focal mild regeneration (red-arrow). Mild steatosis is also seen. (Toluidin blue, 400x)

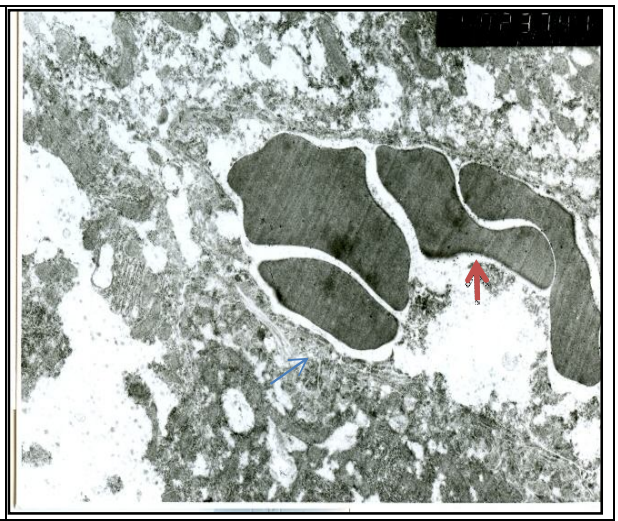

Fig. (6): liver showing hepatic sinusoids containing RBCs (red arrow) with minute amount of collagen fibers in Disse-space (blue arrow). (TEM, UA, 100000x).

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