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Lemon Crude Extract Modulates Ito Cells Activation in High Cholesterol Diet-Induced Liver Steatosis in Male Mice

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Abstract

Background: Different types of polyphenols exist in many sources such as fruits, vegetables and herbs. For example, lemon has mainly flavonoids (such as Hesperidine, Eryoketrine, Narignine, Hesperidine, routine and chlorogenic acid). Research conducted *in vivo* and *in vitro* showed that lemons have different health benefits, such as anti-cancer effect, antimicrobial, antihyperlidemia and protective effect against liver disease. In addition, lemons are used to treat liver disorders. Therefore, current research was aimed to investigate the protective effect of the prolonged use of Lemon Crude Extract. **Methods**: Sixty male albino mice (4 weeks old, weighing between 20-25g) were used. They were divided into 3 groups (n= 20) for each group during the period from January 2018 to May 2018. Group I was used as control, Group II induction group was fed with (HCD), Group III was fed a (HCD) and treated with 1:1m/v of 100% (LCE) for 12 weeks.: **Results** Histological variations were identified in Group II by disturbed hepatic architecture, congestion in blood sinusoids and portal veins and infiltration of lymphocytes. Also, there was peri portal steatosis that was observed in HCD-fed mice. In addition, there was significant increase in Ito cells that was identified by desmin antibody. Moreover, these variations were less noticeable in Group III. **Conclusion:** Lemon has a protective effect against activation of Ito cells in fatty liver.

***Key words**: Steatosis, LCE, Ito cells, HFD, fatty liver.*

Introduction

Modern lifestyle has had an impact on most food habits in humans leading to eating high-calorie food filled with high carbohydrate and fat, which are known as (fast food). This leads to increased risk of increasing weight, obesity and chronic non-transmissible diseases, such as liver disease [(1)]. Several studies dealt with the importance of active compounds that constitute foods which have maintaining and prevention of various diseases. For example, polyphenols have an effective role in the healing and treatment of many diseases [(2)]. They contain an enzyme capable of modifying the immune function [(3)]. Different types of polyphenols exist in many sources such as fruits, vegetables and herbs. For example, lemon has mainly flavonoids (such as Hesperidine, Eryoketrine, Narignine, Hesperidine, routine, and chlorogenic acid) [(4)]. Research conducted *in vivo* and *in vitro* showed that lemons have different health benefits, such as anti-cancer effect, antimicrobial, antihyperlidemia and protective effect against liver disease [(5)]. In addition, lemons are used to treat liver disorders [(6)].

Ito cells, also called hepatic stellate cells, fat-storing cells, lipocytes, peri-sinusoidal cells,vitamin A-storing cells, are located in the space of Disse between hepatocytes and sinusoidal endothelial cells [(7)]. These cells constitute about 5% of the total number of liver cells, they help in reducing the turnover of parenchymal cells and normalize liver regeneration [(8)]. Ito cells are transformed to myofibroblasts which cause fibrosis then cirrhosis. Myofibroblasts secrete a large amount of extracellular matrix proteins like collagens type I and III, proteoglycan, glycoproteins cytokines and chemokines [(9)]. They stimulate hepatic fibrogenesis, together with fibroblasts and parenchymal cells, into mesenchymal cells [(10)].

Materials and Method

Lemon Crude extract

Fresh mature (lemon citrus) fruits are used. The taxonomic identity of the plant was made by the Biology Department, Faculty of Science/ University of Babylon. Extract preparation was modified from [(11)]. The fruits

with their peel about (500g washed well with deionized water then cut to small pieces, after that their seeds were removed then blended and squeezing using a commercial blender and was filtered to remove the residues by filter with Whitman filter paper (2ǂ).

Preparation of high fatty diet

Fatty diet was prepared by adding 1% cholesterol to the standard diet. Cholesterol was purchased from Sigma Company. The high-fat diet was prepared every 2 days, kept at 4°C until used and left at the room temperature for 1h before use [12].

Histological and Immunohistochemistry

Light microscopic observations

The livers were collected then as soon as fixed with 10% buffered formalin and embedded in paraffin. Sections (5µm) were prepared and then stained with hematoxylin-eosin dye for photo microscopic observations.

Immunohistochemical Technique

The following markers were used in this study (Dako Cytomation Denmark):

Monoclonal Mouse Anti-Human Desmin, Clone: D33, Code Number: M 0760. After DE waxing, dehydration, washing (with distilled water) and then pre-treatment of tissues with heat-induced epitope retrieval in Micro Wave Oven was done prior to staining (using Labeled Streptavidin Biotin LSAB™+/HRP kit, code number K0697 detection system). The staining procedure followed dakocytomation technique [13].

Results

Histological findings

The liver sections obtained from Group I showed hepatocytes with one cell thickness arranged in cords and can identify the central vein at its center and Ito cells lined the space of Disse (Figure 1). The liver sections obtained from Group II showed many variations in the form of narrowing and congestion in sinusoids, most hepatocytes showed cytoplasmic vacuolation appeared with ballooned hepatocytes and cellular infiltration in addition to multiple small microvesicular steatosis (Figure 2). . The liver sections obtained from Group III showed that most of the hepatocytes showed renewal of their cytoplasm (Figure 3).

Immunohistochemical findings

Immunohistochemistry was used to demonstrate the presence of Ito cells. Examination of anti- desmin immunohistochemical-stained sections of both Group I and group III revealed anti-desmin positive cells in between hepatocytes (Figures 1 and 3). Examination of anti-desmin immunohistochemical-stained sections obtained from group II revealed an apparent increase in number of anti-desmin-positive cells (Figure 2).

Discussion

Lemon is one of the main citrus, it is extensively cultivated in the middle area. It has antimicrobial, anti-parasitic, antiviral and anticancer effects. It was proved that it lowers blood sugar, inhibits low-density lipoprotein oxidation [14,15]. The current study dealt with the protective role of Lemon Crude Extract (LCE) against fatty liver induced in male albino mice. In this study, Livers obtained from group II showed disturbed hepatic architecture which was explained as increased oxidative damage in hepatocellular proteins or necrotic changes in hepatocytes that lead to abnormality in the orientation of the hepatocyte plates and disturbing hepatic architecture, dilatation of central veins, blood sinusoids and portal veins were attributed to inflammatory changes or ischemia and hypoxia following high-fat diet [15]. In addition, cellular infiltration was observed, this result is considered diagnostic of steatohepatitis. The adipocytes in steatosis secrete many immune modulator elements in the form of pro-inflammatory cytokines (IL-6, TNF-α and ROS). All these factors contribute to chronic inflammatory condition and to hepatocytes injury [16,17].

Microvesicular steatosis is an abnormality in metabolism, synthesis and export of lipids which is associated with defective beta-oxidation of fatty acids [18]. Furthermore, cytoplasmic vacuolation was attributed to lipid peroxidation because of oxidative stress that damages cell membrane as well as membranes of cell organelles leading to an increase in their permeability and disturbance of the ions concentrations in the cytoplasm and cellular organelles [19].

The present study showed an increase in the numbers of Ito cells. These findings may be attributed to fibrosis and altered phenotype called capillarization that lose the ability to prevent Ito cells activation and inactivate activated hepatic stellate cells [20]. In the present work, we observed that daily administration of LCE with HCD diet ameliorates previous changes. A previous

study revealed that daily administration of lemon juice has hepato-protective role against steatosis in alcohol-induced liver injury in Mice [21]. Lemon contains many compositions including phenolic compounds, vitamins, carotenoids, essential oils, minerals and dietary fiber [22]. The hepato-protective effect of lemon may be attributable to preventing oxidative damage including lipid peroxidation [23]. Some studies concluded that vitamin C alone could reduce oxidative stress induced by ethanol and the hepato-protective effect of vitamin C treatment was more effective than silymarin, quercetin and thiamine [24, 25]. Flavonoids interact with hydroxyl radicals, and then inhibit oxidases [26]. In a previous study [27], lemon Flavonoids were shown to possess hepato-protective effects on liver damage induced by carbon tetrachloride, the mechanism of the protective effect was related to the antioxidant activity [27].

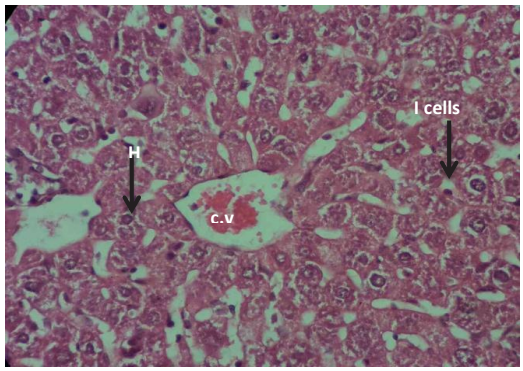

Figure 1 Liver sections for control group showed central vein (C.V) , hepatocytes (H) and Ito cells (I) (H&E A 200X).

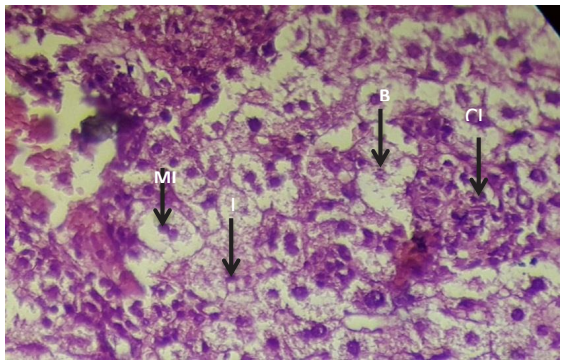

Figure 2 Liver sections for GII showing cellular infiltration (CI), Ito cells (I) ballooning degeneration (B) with microvesicular steatosis (MI) (H&E 200X).

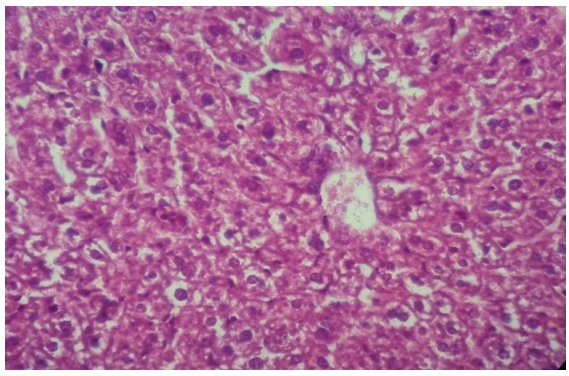

Figure 3 Liver sections for GIII showing normal structure of liver (H&E 200X).

Conclusion

Lemon has a protective effect against activation of Ito cells in fatty liver.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of interest: The authors declare that they have no conflict of interest.

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