

The effect of high fructose diet on the structure of liver of albino rat and the possible protective role of cinnamon. Light and electron microscopic study.

Faika H. El Ebiary and Gehan Khalaf

Histology Department, Faculty of Medicine, Ain Shams University

Abstract:

Background: Fructose consumption has largely increased over the past few decades. Recent studies suggested that overconsumption of fructose might increase the risk of obesity, metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). **Aim of the work** was to evaluate the effect of high fructose diet (HFD) on the liver structure and the possible protective role of cinnamon. **Material & methods:** Twenty adult male albino rats were used and equally divided into four groups. Group I; control rats were fed control diet, group II; rats were fed control diet in concomitant with oral administration of cinnamon extract (80mg/kg/day), group III; rats were fed HFD and group IV; rats were fed HFD in concomitant with oral administration of cinnamon extract (80mg/kg/day). All rats were sacrificed after 60 days and the liver was processed for light and electron microscopic examination.

Results: The liver of rats of group III (HFD group) showed cytoplasmic vacuolation of the hepatocytes around the central vein with a significant increase in the area percentage of the collagen fibers in between the hepatocytes as compared to that of the control group. Electron microscopy examination of the liver of rats of group III (HFD group) revealed numerous swollen mitochondria with loss of their cristae. Collagen fibrils in close proximity to hepatic stellate cell (HSC) and macrophage were frequently seen in between the hepatocytes. The liver of rats of group IV (HFD and cinnamon) revealed structural profile comparable to that of the control group.

Conclusion: the present work revealed that HFD produced a remarkable injurious effect on the liver structure which was markedly ameliorated by concomitant cinnamon extract administration. Reduction of the daily consumption of HFD and increase of cinnamon intake is highly recommended

Key words: High fructose diet- liver- cinnamon- histopathology- fibrosis

I. Introduction

Table sugar (sucrose) and high fructose corn syrup (HFCS), which is present in soft-drinks, juice beverages and many convenient pre-packaged foods such as breakfast cereals, are the two major dietary sources of fructose. Fructose consumption has largely increased over the past few decades. The use of HFCS, which contains between 55–90% fructose, has been markedly increased between 1970 and 1990[1]. High fructose consumption might not be as benign as it was previously thought as it was linked to weight gain, the rise in obesity and metabolic syndrome particularly in children and adolescents and increases the risk of non-alcoholic fatty liver disease NAFLD [2].

Unlike glucose, fructose ingestion could rapidly cause fatty liver in animals in association with the development of leptin resistance [3]. It was proved that increased fructose consumption increased fat mass, de novo lipogenesis and induced insulin resistance and postprandial hypertriglyceridemia, particularly in overweight individuals [4]. Furthermore, it was indicated that the development of NAFLD might be associated with excessive dietary fructose consumption [5]. It was evidenced that childhood obesity and pediatric NAFLD were becoming epidemic, particularly in young boys who tend to consume soft drinks [6].

Cinnamon extract, derived from cinnamon bark, has been shown to improve symptoms associated with the metabolic syndrome in rats. In a model of fructose-induced insulin resistance, the concomitant treatment of rats with cinnamon extract enhanced muscular insulin signaling [7]. It was reported that cinnamon and components of cinnamon have beneficial effects on all of the factors associated with metabolic syndrome including insulin sensitivity, glucose, lipids, antioxidants, inflammation, blood pressure and body weight [8].

High fructose consumption might be a potential risk factor for the liver impairment. So this study aimed to evaluate the effect of high fructose consumption on the structure of the liver of albino rat and the possible protective role of cinnamon.

II. Material and methods

Animals

Twenty adult male albino rats weighing 180- 200 gram were purchased and raised in **Medical Research Center Ain Shams University**. They were housed at 22±2C in an air-conditioned room in plastic cages with mesh wire covers and were given a prepared diet and water ad libitum. The rats were sacrificed according to the Ethics Committee recommendations of Ain Shams University.

Diet:

Two diets were prepared in the Tests and Consulting Unit (Food analysis and design for special groups) at the National Research Centre. One was a control diet (60 gm starch/100gm diet) and the other was a high fructose diet HFD (60 gm fructose/100gm diet) [9] as presented in table 1.

Table (1): showing the composition of the control diet and HFD.

Composition	control diet 100 gm	HFD 100 gm
Protein	20	20
Fat	10	10
Starch	60	---
Fructose	---	60
Cellulose	5.5	5.5
Salt mix	3.5	3.5
Vitamin mix.(water soluble)*	1	1

* Fat soluble vitamins were given separately in a dose of 0.1 ml/rat/week orally

Preparation of cinnamon extract:

The cinnamon bark was purchased from the local market, Cairo, Egypt. The bark was finely powdered in a mechanical mixer. Eight gram powder was dissolved in 100 ml water in a water bath for 2hrs at 60C and then filtered. The filtrate was diluted with water (1:10), so each 1ml contained 8 mg cinnamon. The cinnamon extract was given to rats orally at dose of 80mg/kg/day [7].

Experimental procedure:

The experiment was conducted on 20 adult male albino rats weighing 180-200 gram. They were equally divided into 4 main groups

Group I (Control group): Rats were fed on a control diet for 60 days.

Group II (Cinnamon group): Rats were fed on a control diet for 60 days in concomitant with administration of cinnamon bark extract orally in a dose of 80mg/kg/day.

Group III (HFD group): Rats were fed on a HFD for 60 days

Group IV (HFD and Cinnamon group): Rats were fed on a HFD for 60 days in concomitant with administration of cinnamon bark extract orally in a dose of 80mg/kg/day.

At the end of experiment the rats were sacrificed and liver specimens were removed and processed for light and electron microscopic examination.

Methods

For light microscopic examination, specimens were fixed in 10% formol-saline, dehydrated, cleared and embedded in paraffin. Thin serial sections (5 µm) were cut and stained with H&E and Masson's trichrome stain for collagen.

For Transmission electron microscopic (TEM) examination, Small liver specimens (1mm³) were fixed in 2.5% gluteraldehyde solution. They were then post-fixed in 1% osmium tetroxide, dehydrated and embedded in Epon. Semithin sections were stained with 1% toluidine blue. Ultrathin sections were cut, stained with uranyl acetate and lead citrate and then examined using TEM1010- EXII (Joel, Tokyo, Japan) at the **Regional Mycology and Biotechnology Unit, AL Azhar University, Cairo, Egypt**.

Morphometric study

Using the image analysis system Leica Q500 MC (Germany) in the **Histology Department, Faculty of Medicine, Ain Shams University**, The area percentage of the collagen content in between hepatocytes was measured using Masson's trichrome-stained sections. The measurements were carried out in five non overlapping peri-venular fields from five different sections of five different rats in each group at 400 magnification.

Statistical analysis

The data were collected, revised and then subjected to statistical analysis using Student's *t*-test. The significance of the data was determined by the *P*-value, where $P > 0.05$ is non significant and $P < 0.05$ is significant.

III. Results

Light microscopic results:

By examination of the liver sections of the rats of the group I (control group), The hepatocytes appeared with granular acidophilic cytoplasm and were arranged in branching and anastomosing cords radiating from the central veins (figure 1A,B). In toluidine blue stained semithin sections, hepatocytes appeared with basophilic cytoplasmic granules and vesicular nucleus with prominent nucleolus. They were separated by blood sinusoids that were lined by endothelial cells and kupffer cells (figure 2). The liver sections of rats of the group II (cinnamon group) were comparable to that of the control group. The liver sections of rats of group III (HFD group) showed highly vacuolated hepatocytes mainly around the central veins (figure 3A,B). Hepatocytes contained many vacuoles and clear areas in the cytoplasm. Some hepatocytes contained darkly stained condensed nuclei (figure 4). Sections of the liver of rats of group IV (HFD and cinnamon group) revealed that most of the hepatocytes showed histological profile comparable to that of the control group (figure 5A,B). However, few scattered hepatocytes around the central vein appeared vacuolated (figure 6).

As regard the Masson's trichrome stained sections, the sections of the liver of rats of group I (control group) revealed few scattered collagen fibers around central vein and in between hepatocytes (figure 7). The collagen content of group II (cinnamon group) was comparable to that of the control group. An apparent increase in the collagen fibers in between hepatocytes were detected in group III (HFD group) as compared to that of the control group (figure 8). Liver sections of rats of group IV (HFD and cinnamon group) revealed collagen content comparable to that of the control group (figure 9).

Electron microscopic results

Electron microscopic examination of hepatocytes of group I (control group) showed many mitochondria, rough endoplasmic reticulum and glycogen rosettes in the hepatocyte's cytoplasm (figure 10A). The blood sinusoids in between hepatocytes were lined by endothelial cells and kupffer cells. The wall of blood sinusoids were separated from the surface of hepatocytes by space of Disse that contained microvilli of hepatocytes (figure 10B). The hepatocytes of rats of group III (HFD group) showed structural changes in the form of vacuolated cytoplasm (figure 11A), swollen mitochondria with disrupted cristae (figure 11B,C). Hepatic stellate cell appeared as elongated cell with large euchromatic nucleus in between hepatocytes and associated with collagen fibrils (figure 11D,E). Numerous collagen fibrils were seen in between hepatocytes in close proximity to HSCs. Macrophages were also observed in vicinity of collagen fibrils contained many lysosomes (figure 11F,G).

The liver of rats of group IV (HFD and cinnamon group) appeared with ultra-structural profile comparable to that of the control group (figure 12A). Few collagen fibrils and HSC were occasionally seen in between hepatocytes (figure 12B).

Morphometric and statistics results

The area percentage of collagen fibers in between the hepatocytes of rats of the group I (control group) was 5.6%. Group II (cinnamon group) revealed non significant change in the area percentage of collagen fibers ($6.7\% \pm 0.52, p > 0.05$) as compared to that of the control group. The area percentage of the collagen in between the hepatocytes of rats of group III (HFD group) was significantly increased ($10.23 \pm 1.69, p < 0.05$) as compared to that of the control group. Non significant change ($7.11 \pm 1.87, p > 0.05$) was noticed in group IV (HFD and Cinnamon group) as compared to that of the control group (table 2).

Table 2. The mean area percentage of collagen fibers in between hepatocytes in the different groups:

	Mean (%) \pm standard deviation	P value	Significance
Group I (Control group)	5.6 % \pm 0.91		
Group II (cinnamon group)	6.7% \pm 0.52	> 0.05	Non- significant
Group III (HFD group)	10.23 \pm 1.69	< 0.05	Significant
Group IV (HFD and Cinnamon group)	7.11 \pm 1.87	> 0.05	Non- significant

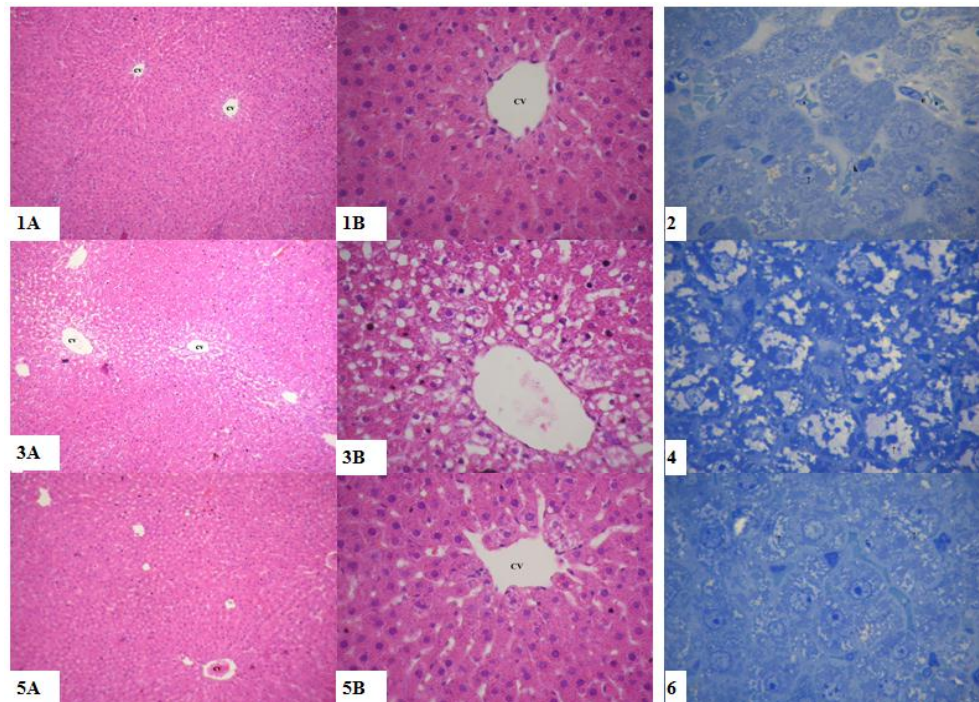


Figure 1: Shows branching and anastomosing cords of hepatocytes radiating from the central vein (cv).

Group I (Control group). H&E. Ax100, B x640.

Figure 2: Shows hepatocytes that contain basophilic cytoplasmic granules and vesicular nucleus with prominent nucleolus (↑) and are separated by blood sinusoids (s) which is lined by endothelial cells (e) and kupffer cells(k).

Group I (Control group). Toluidine blue x1000.

Figure 3: Shows highly vacuolated hepatocytes mainly around the central veins. **Group III (HFD group).** H&E. Ax100, B x640.

Figure 4: Hepatocytes contain many vacuoles and clear areas in the cytoplasm. Some hepatocytes contain darkly stained condensed nuclei (↑). **Group III (HFD group).** Toluidine blue x1000.

Figure 5: Shows branching and anastomosing cords of hepatocytes radiating from the central vein (cv). **Group IV (HFD and cinnamon group).** H&E. Ax100, B x640.

Figure 6: Few hepatocytes appear vacuolated (↑). **Group IV (HFD and cinnamon group).** Toluidine blue x1000.

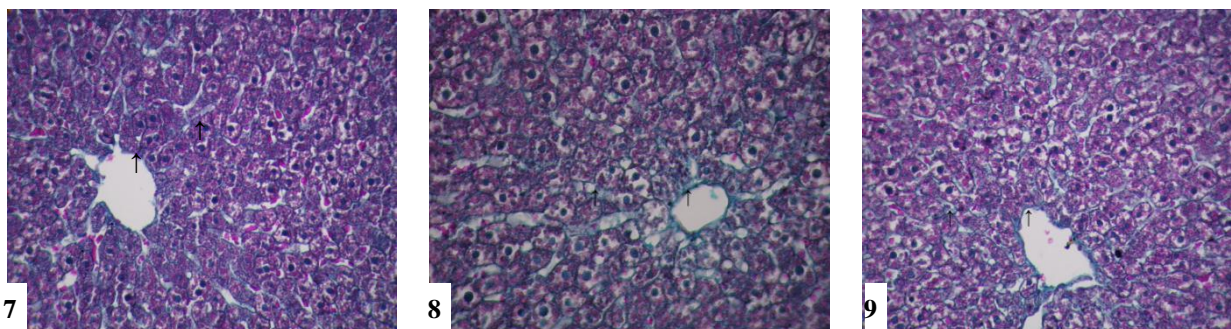


Figure 7: Shows a minimal amount of collagen fibers (↑) around central vein and in between hepatocytes. **Group I (Control group).** Masson's trichrome stain x400

Figure 8: Shows an apparent increase of collagen fibers (↑) in between hepatocytes. **Group III (HFD group).** Masson's trichrome stain x400

Figure 9: Shows a minimal amount of collagen fibers (↑) in between hepatocytes. **Group IV (HFD and cinnamon group).** Masson's trichrome stain x400

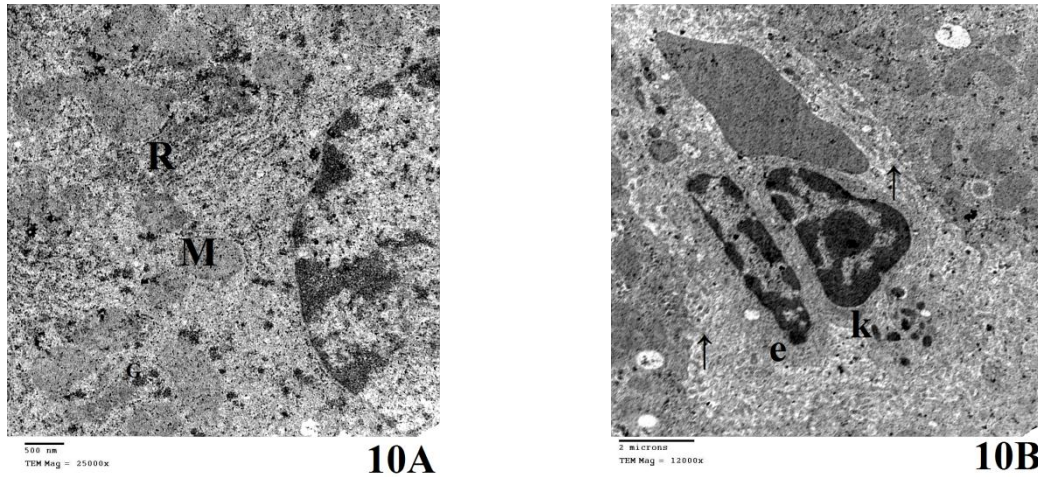


Figure 10: Shows liver sections of rats of group I (**control group**). **A:** Shows many mitochondria (M), rough endoplasmic reticulum (R) and glycogen rosettes (G) in the hepatocyte's cytoplasm. TEMx25000

B: Shows endothelial cells (e) and kupffer (k) cells line the blood sinusoids. Notice presence of microvilli (↑) of hepatocytes in space of Disse. TEM Ax 25000- Bx12000

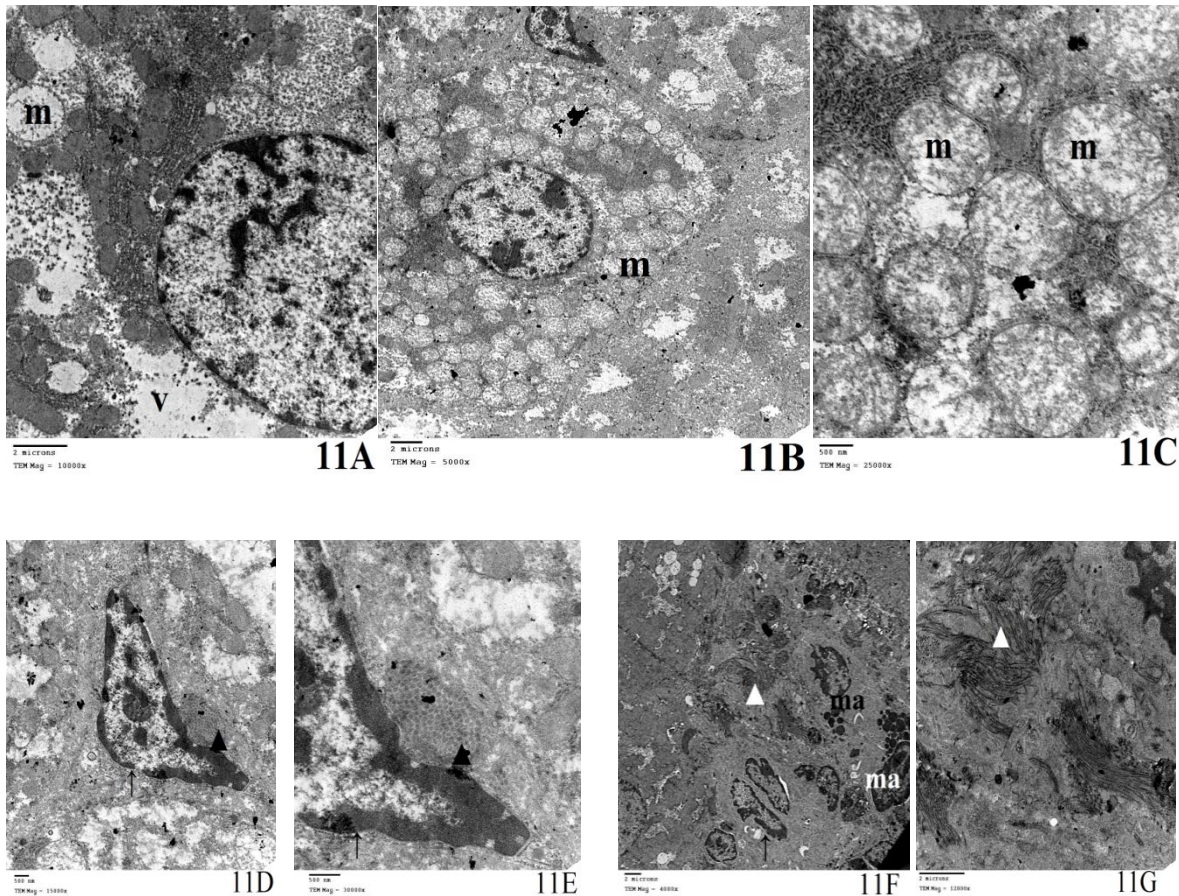


Figure 11: Shows liver sections of rats of group III (**HFD group**). **A:** Shows hepatocyte with vacuolated cytoplasm (v) and loss of cristae of some mitochondria (m). **B&C:** Show hepatocyte containing swollen mitochondria with disrupted cristae (m). **D&E:** Show HSC that appears as elongated cell (↑) with large euchromatic nucleus in between hepatocytes associated with collagen fibrils (▲). **F&G:** Show numerous collagen fibrils (▲) in between hepatocytes in close proximity to HSC (↑) and macrophages (ma) with many lysosomes. TEM Ax10000 Bx5000 Cx25000 Dx15000 Ex30000 Fx4000 Gx12000

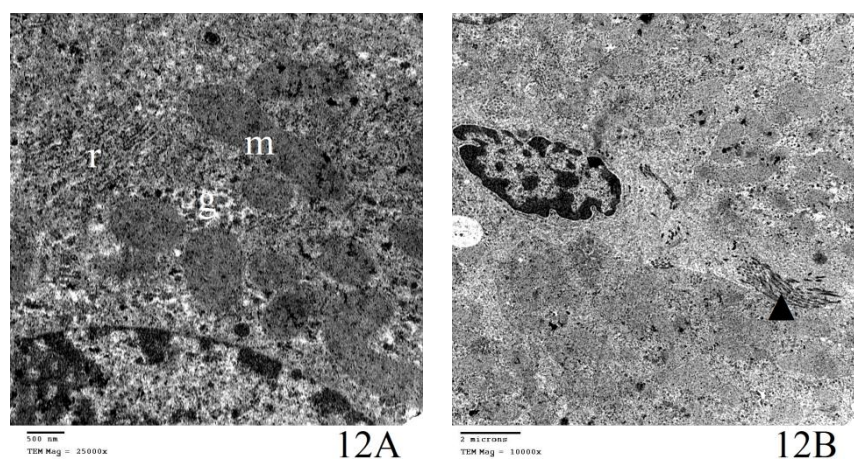


Figure 12: Shows different liver sections of rats of group IV (**HFD and cinnamon group**). **A:** Shows many intact mitochondria(m), r.ER (r) and glycogen rosettes(g) in the cytoplasm of a hepatocyte. **B:** Shows few collagen fibrils (▲) and HSC in between the hepatocytes. TEM Ax25000 – Bx10000.

IV. Discussion

Sugar substitutes such as fructose had been found to offer the advantage of a better utilization in conditions of limited insulin production. It was clarified that fructose was readily absorbed and metabolized by human liver and it was a potent regulator of glycogen synthesis and liver glucose uptake. This evidence supported the use of fructose as proper sugar substitute for diabetic control [10]. Based on these observations, nutritive sweeteners which were made of fructose were considered safe by the Food and Drug Administration, but it was found that fructose intakes above 25% of total energy would cause hypertriglyceridemia and gastrointestinal symptoms [11].

For thousands of years human consumed about 16–20 grams of fructose per day, mainly from fresh fruits. Changing in the diet habits had resulted in significant increases in added fructose, leading to increase in typical daily consumptions to about 85–100 grams of fructose per day. The exposure of the liver to such large quantities of fructose led to rapid stimulation of lipogenesis and triglycerides (TG) accumulation, which in turn contributes to reduced insulin sensitivity [12]. The fructose is able to by-pass the main regulatory step of glycolysis, the conversion of glucose-6-phosphate to fructose 1,6-bisphosphate, controlled by phospho-fructokinase. Thus, while glucose metabolism is negatively regulated by phospho-fructokinase, fructose can continuously enter the glycolytic pathway. Therefore, fructose produces glucose, glycogen, lactate, and pyruvate. These particular substrates, and the resultant excess energy flux due to unregulated fructose metabolism, would promote the over-production of TG [13].

In the present study, the liver of rats of the group III (HFD group) revealed some structural changes. Hepatocytes around the central vein appeared highly vacuolated with clear areas in the cytoplasm. These changes were explained by Burkitt et al [14] and David et al [15]. They clarified that early evidence of metabolic injury to the hepatocytes was the appearance of fatty liver which was manifested by the presence of large cytoplasmic vacuoles. With more severe metabolic disruption, the hepatocytes undergo hydropic degeneration and become swollen and vacuolated, an appearance described as ballooning degeneration. This stage of ballooning degeneration was also characterized by the presence of some fibrosis which is classically found encircling hepatocytes, so called peri-cellular fibrosis [15]. The present morphometric results revealed a significant increase in the area percentage of collagen fibers that present in between the hepatocytes of group III (HFD group) as compared to that of the control group. Electron microscopic examination of the liver of group III (HFD group) revealed numerous collagen fibrils in close proximity to HSC which appeared as elongated cells with euchromatic nucleus. Macrophage were also detected in the vicinity of collagen fibrils. This finding attributed to the ability of HSCs to be activated in response to liver injury and differentiated to myofibroblasts, which greatly contributed to the fibrogenesis process [16]. The transition of stellate cells into myofibroblast-like cells is induced by reactive oxygen species which released from damaged hepatocytes and regulated by cytokines released from macrophage [17].

In the present study, the structural changes in the liver of rats of group III (HFD group) were similar to those previously described in NAFLD. Histological changes in NAFLD typically affected peri-venular regions

of the liver parenchyma and included hepatocytes ballooning associated with peri-cellular or peri-sinusoidal fibrosis [18]. So, fructose could be considered as one of the high risk factor in developing NAFLD.

The current work revealed that ultra-structural changes of hepatocytes of rats of group III (HFD group) were mainly in the form of swollen mitochondria and loss of their cristae. Similarly, it was found that high-fructose corn syrup (HFCS) induced mitochondrial dysfunction in mice [19]. Clinical study also showed that mitochondrial defects in the form of loss of cristae and paracrystalline inclusions were present in patients with NAFLD [20]. It was proved that mitochondrial abnormalities played an important role in the pathogenesis of progressive liver injury in NAFLD [21]. The influx of triglycerides, due to high fructose consumption, into hepatocytes leads to an overproduction of reactive oxygen species by beta-oxidation, which causes an anti-oxidant/oxidant imbalance. The elevation of pro-oxidant species causes membrane and DNA damage and the inactivation of some regulatory proteins, which causes tissue inflammations [22].

The current study proved that cinnamon administration in concomitant with HFD ameliorated the hazardous effect of HFD on the structure of the liver of rats of group IV. Histological profile of most of the hepatocytes were almost comparable to that of the control group. In a previous study, cinnamon could reverse a decrease in insulin sensitivity associated with the HFD in rats [23]. These results might be attributed to the antioxidant, anti-inflammatory & lipolytic activity of cinnamon [8,24].

It was found that moderate fructose consumption in mice could lead to increase intestinal translocation of bacterial endotoxin and induction of hepatic tumor necrosis factor (TNF) [25]. Cinnamon extract treatment decreased the mRNA expression of the inflammatory factors [interleukin (IL) 1 β , IL6, and TNF- α] [26]. Cinnamon might also have a direct role in lipid metabolism. Cinnamon bark powder prevented hypercholesterolaemia and hyper-triglyceridaemia and lowered the levels of free fatty acids and triglycerides in plasma of type 2 diabetic peoples by its strong lipolytic activity [27].

V. Conclusion

The present study revealed that HED had a deleterious effect on the structure of the liver. Concomitant administration of cinnamon with HFD resulted in noticeable protection against HFD induced liver injury. Daily cinnamon supplementation might be needed to guard against the hazard of the HFD. Further studies are needed to investigate the role of overconsumption of HFD on the endemic liver diseases.

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