Hepatoprotective Effect of Bee Propolis in Rat Model of Streptozotocin-Induced Diabetic Hepatotoxicity: Light and Electron Microscopic Study

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Abstract: Liver disease is one of the dealing causes of death in persons with type 2 diabetes. The present study was carried out to evaluate the hepatoprotective effect of bee propolis against streptozotocin induced liver injury in rats, from histological and ultrastructural points of view. Thirty adult male albino rats were used in the present study. They were allocated into 3 groups. Rats of the first group served as control, and were injected interaperitonealy (i.p.) with saline solution. Animals of the second group were injected (i.p.) with a single dose of streptozotocin (60 mg / kg b. wt.), while, after the animals of the third group had been injected with the previous single dose of streptozotocin, they were given orally daily 300 mg / kg b. wt / day bee propolis for two weeks. All the animals were sacrificed and liver samples were obtained and processed for histological and ultrastructural examination. Histological examination of liver sections of diabetic rats showed fatty changes in the cytoplasm of the hepatocytes, inflammatory lymphocytic infiltration, and proliferation of Küpffer cells. The portal area showed hyperplastic bile ducts and congested branches of the portal vein. On the other hand, the liver sections of diabetic rats treated with bee propolis showed minimizing the toxic effects of streptozotocin. Electron microscopic investigation of the hepatic tissue of diabetic rats revealed conspicuous alterations, represented by aggregations of polymorphic mitochondria with apparent loss of their cristae and condensed matrices. Besides, the rough endoplasmic reticulum was proliferating and fragmenting into smaller stacks. The cytoplasm of hepatocytes exhibited vacuolation and displayed a large number of lipid droplets of different sizes. The ultrastructural results revealed that treatment of diabetic rats with bee propolis led to apparent repair of the injured hepatocytes. **Conclusion:** This study showed that bee propolis, in early stages of diabetes induction, can decrease the destructive progress of diabetes and cause hepatoprotection against damage resulting from streptozotocin induced hyperglycemia.
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Keywords: Bee propolis, Diabetic rats, Histology, Mammalian liver, Streptozotocin, Ultrastructure.

1. Introduction

In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus, which is a syndrome resulting from a variable interaction of hereditary and environmental factors and characterized by abnormal insulin secretion or insulin receptor or post-receptor events affecting metabolism involving carbohydrates, proteins and fats in addition to damaging β-cells of pancreas, liver and kidney in some cases **(Ghosh, 2001).**

Liver disease is one of the dealing causes of death in persons with type 2 diabetes. The standardized mortality rate death from liver disease is greater than that cardiovascular disease. The spectrum of liver disease in type 2 diabetes ranges from nonalcoholic fatty liver disease to cirrhosis and hepatocellular carcinoma **(Keith *et al.*, 2004).**

Experimental type 1 diabetes induced with streptozotocin or alloxan in rats displays many features seen in human subjects with uncontrolled diabetes mellitus **(Chattopadhyay *et al.*, 1997*).*** Streptozotocin induced diabetes mellitus in many animals species has been reported to resemble human hyperglycemic nonketonic diabetes mellitus **(Weir *et al.*, 1981).** This effect has been extensively studied and appears to be mediated through a lowering of beta cell nicotinamide adenine dinucleotide (NAD^+) and results in histopathological alteration of pancreatic islet beta cells **(Karunanayake *et al.*, 1974).** Hepatomegaly is also associated with streptozotocin-induced diabetes **(Kume *et al.*, 1994). Mir *et al.* (2008)** reported that streptozotocin-induced diabetes causes disturbances in biochemical and histological features in rabbits and serves as a model in studying the various complications arising due to this illness. **Zafar *et al.* (2009)** in their study on altered liver morphology and enzymes in streptozotocin-induced diabetes rats, they found that the liver showed accumulation of lipid droplets, lymphocytic infiltration, increased fibrous content, dilatation and congestion of portal vessels and proliferation of bile ducts. **Monika *et al.* (2007)** reported that the histopathological evaluation of the liver revealed that propolis reduced the incidence of liver lesions including hepatocyte swelling and lymphocytic infiltrations induced by carbon tetrachloride CC14.

Electron microscopic observations also showed improvement in ultrastructure of liver. Also, **Alqasoumi *et al.* (2008)** showed similar hepatoprotective effect of bee propolis against the toxic effect of carbon tetrachloride. The authors noted that the liver of rats treated with propolis showed good protection against the toxic effect of carbon tetrachloride. The present study was designed to investigate the histopathological and ultrastructural alterations that occur in liver of rats after the evaluation of streptozotocin-induced diabetes and the role of bee propolis in minimizing these hepatic alterations.

2. Materials and Methods

Experimental animals

Thirty adult male Sprague Dawley albino rats weighing 150 ± 15g, obtained from the Egyptian Organization for Biological Products and Vaccines, were used in the present investigation. The animals were housed in wire meshed cages and were feed (commercial rat diet) and water *ad libitum.* All diets were prepared weekly and stored at 4ºC.

The applied drug

Streptozotocin (Batch No. T1829656) was purchased from Sisco Research laboratories Pvt. Ltd. Mumbai, India and was freshly dissolved in saline solution (pH = 4.5) and bee propolis was purchased in the form of white powder from Faculty of Agriculture Ain Shams University

Experimental design

The animals were allocated into three groups, each of 10 animals. The first group served as control and was injected intraperitonealy with a saline solution. The rats of the second group were injected intraperitonealy with a single dose of streptozotocin (60 mg/kg b. wt.) following 12 hours fasting. This dose was determined in accordance to the dose utilized in previous researches of experiment **(Daisy and Eliza, 2007)** while, after the animals of the third group had been injected with the previous single dose of streptozotocin, they were given orally daily 300 mg / kg b. wt / day bee propolis for two weeks..

For light microscopic examination small pieces of the liver were immediately fixed for 24 hours in aqueous Bouin's solution and then preserved in 70% alcohol. The specimens were then dehydrated, cleared in terpineol and embedded in paraffin wax. Sections of 5µm thickness were stained with haematoxylin and eosin **(Bancroft and Gamble, 2002)**, microscopically examined and photomicrographs were made as required.

For ultrastructural evaluation by transmission electron microscopy as described previously by **Dykstra *et al.* (2002),** livers were cut into small pieces and fixed in 2.5% glutraldehyde for 4 hours and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). The samples were post-fixed in a buffered solution of 1% osmium tetroxide at 4ºC for one hour. This was followed by dehydration in ascending series of ethyl alcohol, clearing in two changes of propylene oxide, 5 min each, and embedding in Epon-epoxy-resin **(Weakley, 1981).** Semi-thin sections of 1 µm thickness were cut, picked up on glass slides, stained with toluidine blue and examined for general orientation under a bright field-light microscope. Specimens were then retrimmed to the detected region and ultra-thin sections, 60 nm thickness were cut and picked up on copper grads. Sections mounted on grids were double stained using uranyl acetate and lead citrate **(Reynolds, 1963).** Sections were examined and photographed on a Joel transmission electron microscope at the Faculty of Science, Ain Shams University.

3.Results

1- Light microscopical studies:

Group I: The control rats

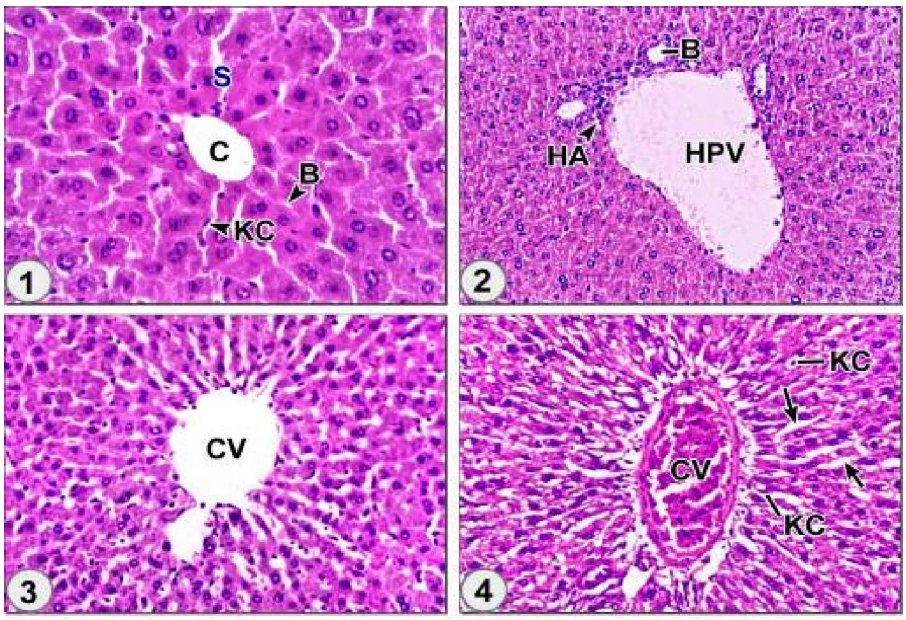
The liver of control rats showed the common characteristic lobular organization of the mammalian liver. Each lobule is formed of cordes of hepatocytes radiating from a central vein. The hepatic cords are separated from each other by blood sinusoids lined with endothelial cells interspersed by Küpffer cells (Fig. 1). The hepatic lobules are separated by loose connective tissue containing at certain angles the portal triads including branches of the portal vein, hepatic artery and a narrow bile ductule (Fig. 2).

Group II: Streptozotocin-treated rats (diabetic rats)

The liver of streptozotocin-treated rats exhibited dilatation and congestion of the central veins as illustrated in Figures 3-7. The markedly dilated central vein possessed irregular lining formed of damaged and detached endothelial cells (Fig. 3). Intact and haemolysed blood cells were occupying the severely dilated central vein (Figs. 4 & 5). There was an inflammatory infiltrate in the portal tract (Fig. 5). The inflammatory infiltrate varied in intensity from one tract to other. There was a proliferation of bile ducts in the portal tracts in this group (Fig. 5). Küpffer cells were actively proliferating, markedly increased in size and number and some of them were pushed into the lumina of sinusoids (Figs. 3-7). Fatty changes were observed in hepatocytes (Fig. 7).

Group III: Streptozotocin and bee propolis-treated rats.

The histological structure of the liver of most diabetic rats treated with bee propolis for two weeks revealed little pathological change when compared with diabetic rats only. The hepatic cords were well organized and the cytoplasmic vacuolation disappeared. Most nuclei exhibited normal shape, being spherical and centrally located (Fig. 8), except for a few pyknotic ones (Fig. 8).



Figures 1&2: Photomicrographs of sections of the liver of a control rat.

Fig. (1): Showing hepatic cords radiating from a central vein (CV), blood sinusoids (S) and Küpffer cells (KC). Notice, the bile canaliculi (B) found between the hepatic cells. X. 400

Fig. (2): Showing a portal area containing the hepatic portal vein (HPV), hepatic artery (HA) and bile ductule (B). X. 400

Figures 3-7: Photomicrographs of liver sections of diabetic rats after treated with streptozotocin.

Fig. (3): Showing marked deterioration of the central vein (CV) with apparent erosion of its endothelial lining, besides its marked dilatation. X. 400

Fig. (4): Showing dilated and congested central vein (CV) and dilatation of hepatic sinusoids (arrows) with Küpffer cells (KC) proliferation in between the hepatocytes. X. 400

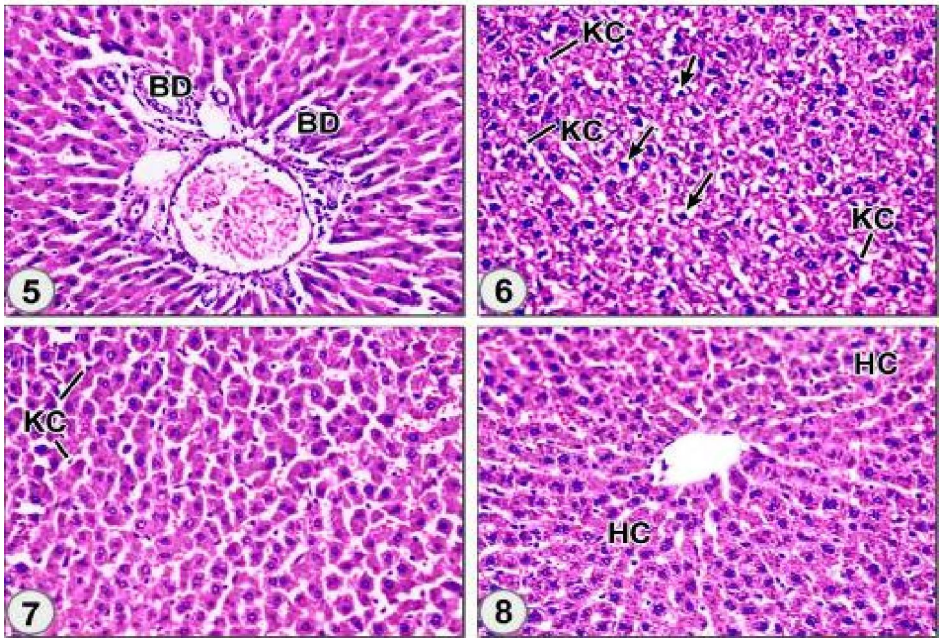


Fig. (5): Showing hyperplasia of the bile duct (BD), dilated branch of portal vein and inflammatory cells infiltration (INF) in the portal area. X. 400

Fig. (6): Showing fatty change (arrow) of most hepatocytes, deteriorated nuclei of the hepatocytes and activated Küpffer cells (KC) pushed into the lumina of the sinusoids. X. 200

Fig. (7): Showing necrotic hepatocytes with loss regular arrangement of hepatic configuration and dilatation of some hepatic sinusoids with activated Küpffer cells (KC) pushed into the lumina of the sinusoids. X. 400

Fig. (8): Photomicrograph of liver section of a diabetic rat treated daily with bee propolis showing that the hepatocytes (HC) partly restored their normal configuration X. 400

2- Ultrastructural observations

Group I: The control rats

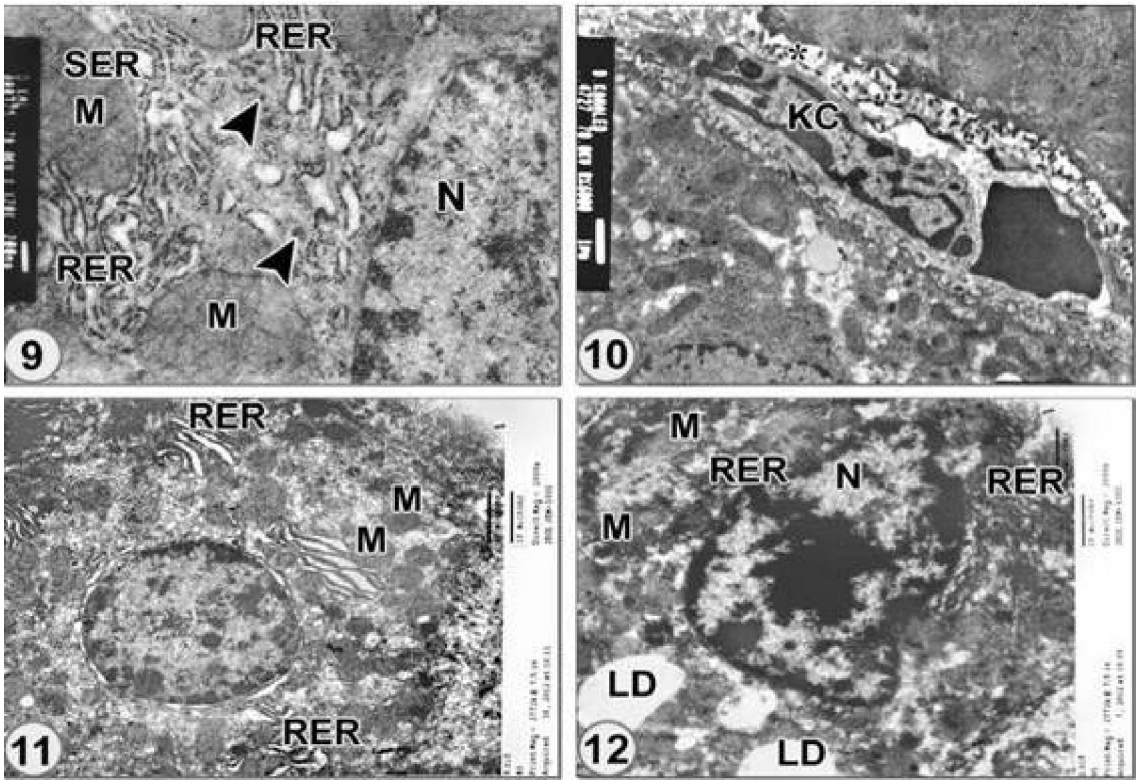
The ultrastructure of the liver of the control rat is shown in Figures 9 and 10. The cytoplasmic organells as well as the nuclei of the hepatocytes exhibited the normal ultrastructural appearance. The cytoplasm contained numerous mitochondria dispersed all over the cytoplasm. The mitochondria are spherical or ovoid in shape with well developed cristae. The rough endoplasmic reticulum consisted of closely packed parallel and flattened cisternae studded with ribosome's (Fig. 9). Considerable electron-dense glycogen rosettes or granules are clearly detected. The nucleus is spherical with a distinct nuclear envelope, and the nucleoplasm showed aggregations of euchromatin and heterochromatin materials (Fig. 9). Hepatic sinusoidal localized between the hepatocytes and lined with a Küpffer cell is observed in Figure 10.

Group II: Streptozotocin-treated rats (diabetic rats)

The electron micrographs of the liver cells of rats treated with streptozotocin revealed marked cytopathological alterations (Figs. 11 & 12). The mitochondria underwent swelling with obvious condensation of their matrices by materials that displayed high electron density and most of them lost their cristae. There was abundant rough endoplasmic reticulum that was usually localized near the mitochondria. The cisternae of the rough endoplasmic reticulum were fragmented into smaller stacks. The hepatic sinusoids appeared markedly dilated and contained unusual aggregations of red blood corpuscles in their lumina (Fig. 13).

Group III: Streptozotocin and bee propolis-treated rats

Electron microscopic examination of the liver of these rats revealed marked improvement of the cytoplasmic organelles following bee propolis treatment. The hepatic cells contained numerous mitochondria exhibiting an almost normal appearance (Fig. 14). The hepatic cells revealed well developed rough endoplasmic reticulum in the form of parallel and flattened cisternae studded with ribosomes (Figs. 14 & 15). A few lipid droplets were seen.



Figures 9-10: Electron micrographs of liver sections of a control rat.

Fig. (9): Showing the cytoplasm of a hepatocyte occupied by rough endoplasmic reticulum (RER), mitochondria (M), a few portion of smooth endoplasmic reticulum (SER), glycogen deposits (arrow heads) and the nucleus (N). X. 40000

Fig. (10): Showing a hepatic sinusoid separated from the adjacent hepatocyte by space of Disse (*) and Küpffer cell (KC). X. 6000

Figures 11-13: Electron micrographs of liver sections of diabetic rats.

Fig. (11): Showing a hepatocyte illustrating devastated mitochondria (M) and fragmented rough endoplasmic reticulum (RER). X. 2772

Fig. (12): Showing part of a hepatocyte illustrating mitochondria (M) with electron dense matrices, fragmented rough endoplasmic reticulum (RER) and presence of large lipid droplets (LD). Notice the nucleus (N) with irregular nuclear envelope (arrow). X. 2772

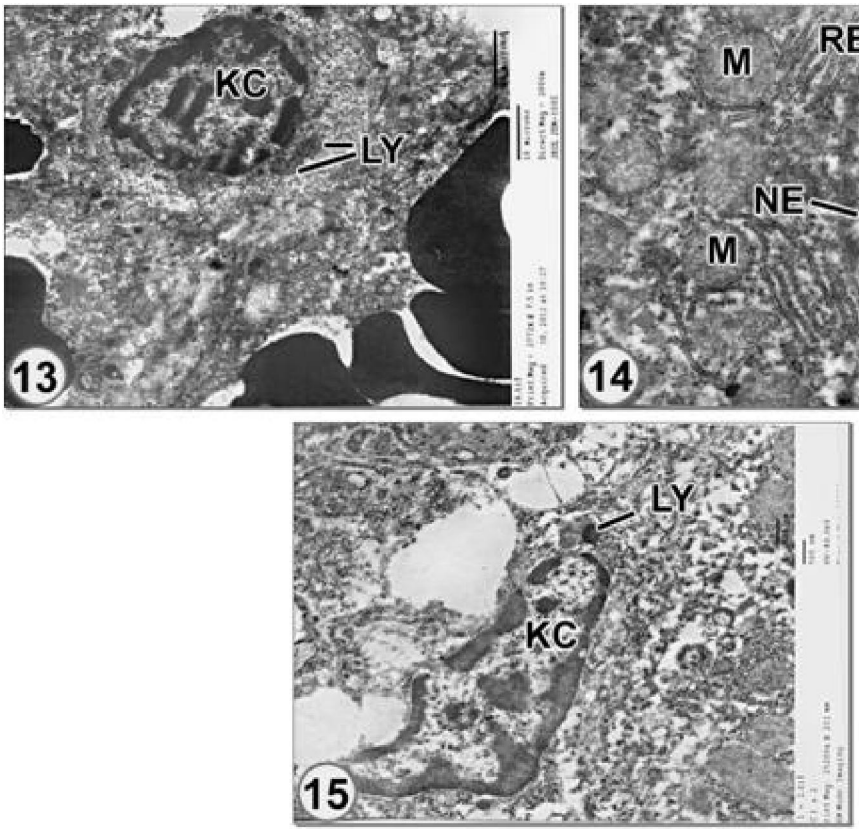


Fig. (13): Showing congested hepatic sinusoid with stagnant blood cells, activated Küpffer cell (KC) having pyknotic nucleus with irregular nuclear envelope and presence of many lysosomes (LY). X. 2772

Figures 14-15: Electron micrographs of liver sections of diabetic rats treated daily with bee propolis.

Fig. (14): Showing part of a hepatocyte with mitochondria (M) in the form of rounded configuration, rough endoplasmic reticulum (RER) in the form of parallel cisternal localization near the nuclear envelope or scattered into the cytoplasm. The nucleus (N) with distinct nuclear envelope (NE) and nucleoplasm with euchromatin and heterochromatin. X. 36600

Fig. (15): Showing Küpffer cell (KC), with a few number of lysosomes (LY). X. 25200

4. Discussion

The liver is an organ of prime importance and plays a significant role not only in metabolism and detoxification of exogenous toxins and therapeutic agents, but also in the bioregulation of fats, carbohydrates, amino acids and proteins. A number of pharmacological and chemical agents act as hepatotoxins and produce a variety of liver ailments **(Ram, 2001).**

Streptozotocin (STZ) is a naturally occurring nitrosourea and it is widely used to induce insulin-dependent diabetes mellitus in experimental animals because of its toxic effects on islet beta cells **(Ohno *et al.*, 2000; Merzouk *et al.*, 2000).**

In the present study, marked histological and ultrastructural alterations were observed in the liver rats treated with streptozotocin. The histological changes included disorganized hepatic cord, fatty changes in the cytoplasm of the hepatocytes and mononuclear leucocytes, inflammatory cells infiltration, as well as the diffuse proliferation of Küpffer cells. Similar observations have been reported in the liver of rats and rabbits treated with streptozotocin **(Mir *et al.*, 2008** and **Zafar *et al.*, 2009).** The hepatic cells of treated rats showed distortion of usual concentric arrangement of hepatocytes. There was also congestion of portal vessels and sinusoids and the veins were also dilated. These findings of present study are in agreement with the findings of **Das *et al.* (1996)** and **Degirmenchi *et al.* (2002)** who showed dilatation of veins, loss of usual concentric arrangement of hepatocytes and liver fibrosis.

The present study illustrated marked consequences in the hepatic vasculatures including congestion, dilatation of the hepatic central veins and

surrounding sinusoids, besides erosion of their endothelial lining cells and the activation of the phagocytic Küpffer cells in the liver of rats treated with streptozotocin. Similar observations were also obtained in the hepatic tissue of animals treated with alloxan **(Bopanna *et al.*, 1997)**, in the liver of hyperlipidemic rats **(Sakr, 2010)** and in the pancreas of rats treated with streptozotocin **(Prasad *et al.*, 2009).** On the other hand, the liver sections of rats treated with streptozotocin showed focal infiltration of inflammatory cells, these results are in agreement with those obtained by **Farokhi *et al.* (2012)** in the liver damage of diabetic rats induced by alloxan.

The ultrastructural alterations noticed in the present study include hepatocyte necrosis, aggregation of opaque mitochondria, as well as hypertrophy of the rough endoplasmic reticulum. Many lipid droplets, lysosomes and active Küpffer cells were also observed.

The mitochondria displayed gradual devastation; they manifested obvious swelling or hypertrophy as well as condensation of their matrices. The mitochondria lost their internal ridges and matrices. Similar mitochondrial injuries were obtained by **Sakr (2010)** in hepatocytes of hyperlipidemic rats.

The present results showed that the cisternae of RER were fragmented into smaller stacks in the liver of rats treated with streptozotocin. These observations are in accordance with those reported by **Sakr (2010)** who illustrated distinct changes in the endoplasmic reticulum of the hepatocytes post-application of diclofenac.

The present investigation illustrated accumulation of lipid droplets in the cytoplasm of hepatocyte. This change was reminiscent to the formation of fatty liver. It could be due to the increased influx of fatty acids into the liver induced by hypoinsulinemia and the low capacity of excretion of lipoprotein secretion from liver resulting from a deficiency of apolipoprotein β synthesis **(Ohno *et al.*, 2000).** Hyperlipidemia could be another factor for fatty liver formation. These findings of fatty liver formation are in agreement with the findings of **(Ohno *et al.*, 2000** and **Merzouk *et al.*, 2000).** Based on the above results the liver of rats treated with bee propolis revealed that bee propolis reduced the incidence of liver lesions including hepatocyte swelling and lymphocytic infiltrations induced by streptozotocin. Electron microscopic observation also showed improvement of liver of treated rats. Comparable results were obtained in a study carried out by **Alqasoumi *et al.* (2008)** in rat hepatocytes post-application of bee propolis.

In conclusion, the result of the present study indicates that the administrated bee propolis can decrease the destructive progress of diabetes and cause hepatoprotection against damage resulting from streptozotocin induced hyperglycemia. Thus, it is recommended to stat bee propolis therapy as soon as diagnosis of diabetes mellitus is established.

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