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Role of Anise on the hepatotoxicity induced by carbon tetrachloride in adult male albino rats

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SUMMARY

Oxidative stress induced by free radicals is known to be a common cause of liver damage and hepatic fibrosis. Anise oil and its compounds have been identified to have antioxidant, anti-inflammatory and antifibrinogenic properties that may play a role in the management of hepatic disorders and promote liver regeneration. Thus, the purpose of the study was to evaluate the effects of anise oil on hepatotoxicity induced by carbon tetrachloride in adult male albino rats. Sixty male albino rats were divided into control group, CCL_4-treated group that was injected with 1 mg /kg CCL_4 intraperitoneally (ip), CCL_4+anise oil-treated group that was injected with 1 mg /kg of CCL_4 and 0.5 ml/kg of anise oil (ip), and anise oil-treated group that was injected with 0.5 ml/kg of anise oil. Animals received treatment for 4 weeks and sacrificed 24 hours after the last administration. Livers were removed and processed for light and electron microscopy analysis. The CCL_4-treated group revealed loss of normal architecture of hepatic lobules, steatosis, necrosis, cholestasis, portal congestion and progressed grading of lobular inflammation, ballooning degeneration and fibrosis. On the other hand, the CCL_4 + anise group showed reduced liver damage and increased signs of regeneration. We conclude that anise oil has a protective effect on liver damage caused by CCL_4 and promotes liver regeneration.

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INTRODUCTION

Carbon tetrachloride (CCL_4) is a manufactured toxic chemical that was used in the production of pesticides, cleaning fluids, degreasing agents, refrigeration fluids and fire extinguishers (ATSDR, 2005). Carbon tetrachloride was known to damage human liver, causing acute hepatocellular injury with centrilobular necrosis and steatosis. Since then, hepatotoxicity was induced experimentally by administration of CCL_4 (Recknagel, 1987; Etim et al., 2008).

The most common causes of hepatotoxicity are viral infection, drugs, toxicants and autoimmune diseases (Friedman, 1998). It was noticed that oxidative stress and lipid peroxidation were implicated as the common causes and links between liver damage and hepatic fibrosis (Di Sario et al., 2007). Antioxidant molecules such as glutathione (GSH) and superoxide dismutase (SOD) were known to provide hepatocytes with defense mechanisms against the continuous exposure to reactive oxygen species (Li et al., 2003). Estradiol was also found to play a protective role in liver diseases (Jarvelainen et al., 2001).

The fruits of anise plant, *pimpinellaanisum L.*, are locally known as aniseed and yansoon. In traditional medicine, aniseed has been used for the treatment of nausea, abdominal colic, insomnia and epilepsy (Said et al., 1996 and Kreydiyyeh et al., 2003). The principal constituents of *pimpinellaanisum* are the anise oil. The major

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component of anise oil, trans-anethole (75-90%), is considered to be an active estrogenic agent. Other constituents include coumarins, lipids, antioxidant polyphenolic compounds, protein, carbohydrate and minerals (calcium and phosphorus) (Leung, 1980; DerMarderosian and Beutler, 2002). Anise aqueous suspension was discovered to have antioxidant, anti-inflammatory, cytoprotective, antibacterial and anti-ulcer activities (Ibrahim et al., 2007; Al-Bayati, 2008). So the aim of the present study was to evaluate the effects of anise oil on hepatotoxicity induced by carbon tetrachloride in adult male albino rats.

MATERIALS AND METHODS

Animals: Sixty adult male albino rats (weighing 150 to 200 grams and aged more than 12 weeks) were obtained from the animal house of the Faculty of Veterinary medicine, Suez Canal University, and used in the present study. Rats were left at the experimental animal house for 2 weeks to allow them to acclimatize to the new environment. Rats were housed in spacious wire mesh cages in a good ventilated room at room temperature. They were fed a standard laboratory pelleted food and water ad libitum. All experiments were carried out in accordance with the guidelines of Institutional Animals Ethics Committee. The animals were divided randomly into 4 groups, 15 rats each:

Group (I) [control] The animals were received intraperitoneal injection of 1.5 ml of sterile distilled water twice a week for 4 consecutive weeks. Animals were sacrificed 24 hours after the last administration.

Group (II) [carbon tetrachloride-treated] The animals were injected intraperitoneally with carbon tetrachloride (CCL_4) (1mg /Kg B.W) twice a week (3 days apart from each other) for 4 consecutive weeks (Wong et al., 2003; Xu et al., 2010).

Group (III): [anise oil + carbon tetrachloride-treated] The animals received CCL_4 as group II and anise oil (0.5 ml/kg B.W) 4 days a week (day after day including the days of CCL_4 injection) for 4 weeks intraperitoneally (Sahraei et al., 2002).

Group (IV) [anise oil-treated] The animals were injected with anise oil (0.5 ml/kg BW) 4 days a week (day after day) for 4 weeks intraperitoneally (Sahraei et al., 2002).

Carbon tetrachloride was obtained from ADWIC Co, Egypt. Anise oil was obtained from CAP PHARM Co, Egypt.

Histological examination of the liver. The animals were sacrificed 24 hours after the last administration. The livers were removed and carefully examined. The right half of each liver was prepared and processed for light microscopic examination. They were fixed in 10% neutral buffered formalin solution for 24 hours, dehydrated in a graded ethanol series and processed for paraffin embedding for light microscopic study. Serial sections (4 µm thick) were prepared and stained with Hematoxylin and Eosin (H&E) and Masson's trichrome stains. Sections were studied using Olympus light microscope and the histopathological changes were recorded and photographed using a Canon PC1200 power stone camera under different magnification. The degree of liver damage was assessed using a grading system according to Kleiner et al. (2005) as follows:

a) Lobular inflammation: grade 0: none; grade 1, 1-2 foci /field; grade 2, 3-4 foci /field; grade 3, more than 4 foci /field.

b) Ballooning degeneration of the hepatocytes: grade 0, none; grade 1, few cells and grade 2, many cells/ prominent.

c) Fibrosis: grade 0, none; grade 1, perisinusoidal or periportal; grade 2, perisinusoidal and periportal; grade 3, bridging fibrosis and grade 4, cirrhosis.

Electron microscopic analysis. The left part of each liver was processed for electron microscopic examination. It was divided into small pieces that were fixed in buffered glutaraldehyde 2.5% for two hours and fixed in 1% osmic tetroxide for electron microscopic studies. Ultrathin sections were cut using MT 600-XL RMC ultratome and stained with uranyl acetate, lead citrate. They were examined with JEOL-1010 (Japan) transmission electron microscope, at the regional cen-

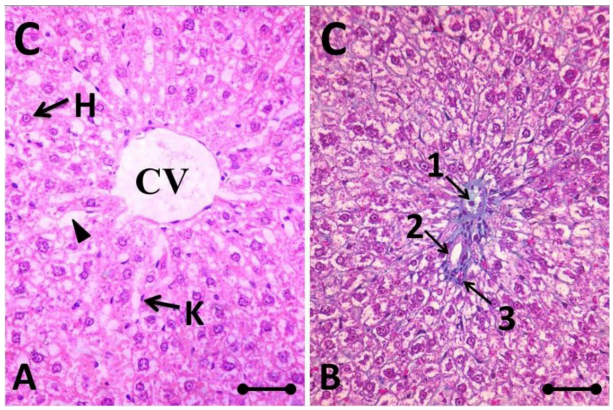


Fig. 1. Sections of the rat liver in control group. **(A)** normal architecture of hepatic lobule, with regularly arranged hepatocytes (H) forming a network of hepatic cords around a central vein (CV), blood sinusoid (head arrow) and phagocytic Küppfer cell (K). (H & E, x 400, Scale bar, 25 µm). **(B)** Fibrous tissues around the portal triad which consists of a branch of the portal vein (1), bile duct (2) and a branch of the hepatic artery (3). (Masson's trichrome, x 400, Scale bar, 25 µm).

ter for mycology and biotechnology transmitting electron unit, Alazhar University, Cairo and photographed under different magnification.

Statistical analysis. Data of all groups were studied using the statistical program of social science (SPSS) version 8. Differences between experimental groups were tested using chi-square tests. The statistical significance of the data was determined by P value ($P< 0.05$ was considered significant).

RESULTS

Light microscopy

Group I (control) (Fig. 1). Light microscopic examination of the liver showed normal architecture of hepatic lobules. Each hepatic lobule was formed of radiating cords of hepatocytes, forming a network around a central vein. The hepatic cords were alternating with blood sinusoids which were defined as narrow blood spaces surrounded and supported by a delicate sheath of reticular fibers and lined with a discontinuous layer of endothelial and phagocytic cells (Von Kuppfer cells). Hepatocytes appeared cuboidal to polyhedral in shape with relatively large size separated with fine bile canaliculi. The cytoplasm of hepatic cells possessed finely basophilic granules and a prominent centrally located nucleus. Angles of hepatic lobules showed the portal triad, which was clearly surrounded by connective tissue, each enclosed a branch of portal vein, a branch of hepatic artery and a bile ductule. Portal venules were comparatively the largest of the portal triad, whereas the lumina of hepatic artery and bile ductules were much narrower (Fig. 1).

Group II (carbon tetrachloride) (Figs. 2,3). The liver of CCL_4-treated group revealed loss of normal architecture of hepatic lobules, hemorrhage, steatosis, hepatic necrosis and Küppfer cell hyperplasia (Figs. 3,4). The portal venules and blood sinusoids showed dilatation and congestion. The bile canaliculi showed dilatation and rupture leading to the appearance of bile stasis (Fig. 2). According to Kleiner et al. (2005) scoring system, lobular inflammation, ballooning degeneration and fibrosis were significantly increased compared with other groups (Table 1 and Fig. 3).

Group III (carbon tetrachloride + anise) (Figs. 4,5). Light examination of the liver of CCL_4 + anise-treated group revealed normal architecture of hepatic lobules with mild steatosis, bile stasis, portal venule congestion and Küppfer cell hyperplasia. Newly formed and dividing hepatocytes were observed, indicating regeneration process. Lobular inflammation, ballooning degeneration and fibrosis were decreased compared with CCL_4 -treated group (Table 1 and Figs. 4 and 5).

Group IV (Anise group): The histological exami-

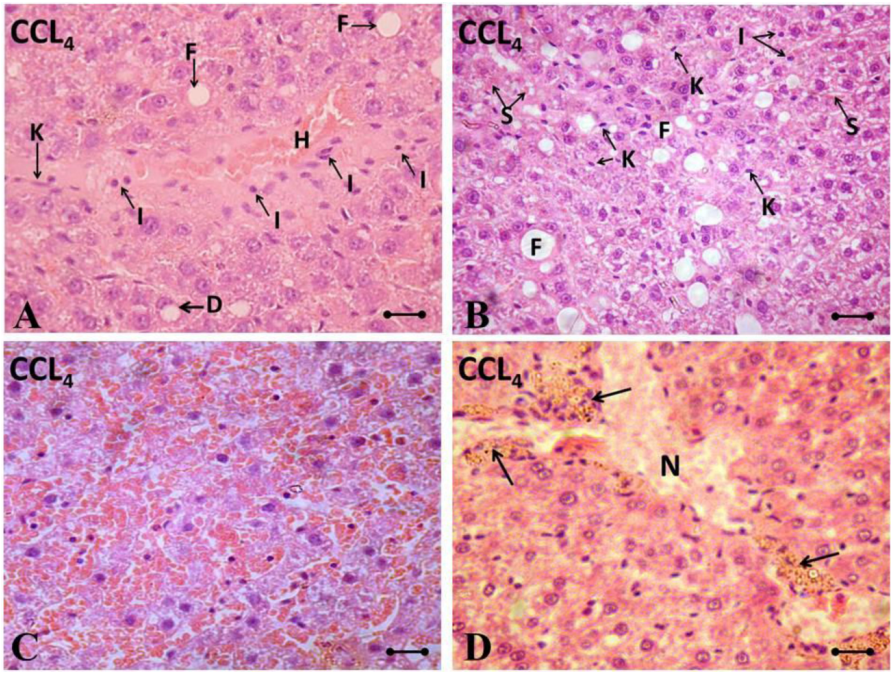


Fig. 2. H & E-stained liver sections in CCL_4-treated group. **(A)** Hemorrhage (H), ballooning degeneration of hepatocytes (D), Küppfer cell hyperplasia (K), steatosis (F) and inflammatory cells infiltration (I). (x 400, Scale bar, 25 µm). **(B)** Sinusoidal congestion (S), Küppfer cell hyperplasia (K), inflammatory cells infiltration (I), steatosis (F) and prominent ballooning degeneration of hepatocytes. (x 400, Scale bar, 25 µm). **(C)** Massive sinusoidal congestion, hemorrhage and inflammatory infiltration. (x 200, Scale bar, 50 µm). **(D)** Hepatic necrosis replaced with fibrous tissue (N), Küppfer cell hyperplasia, bile stasis (arrows) and inflammatory infiltration. (x 400, Scale bar, 25 µm).

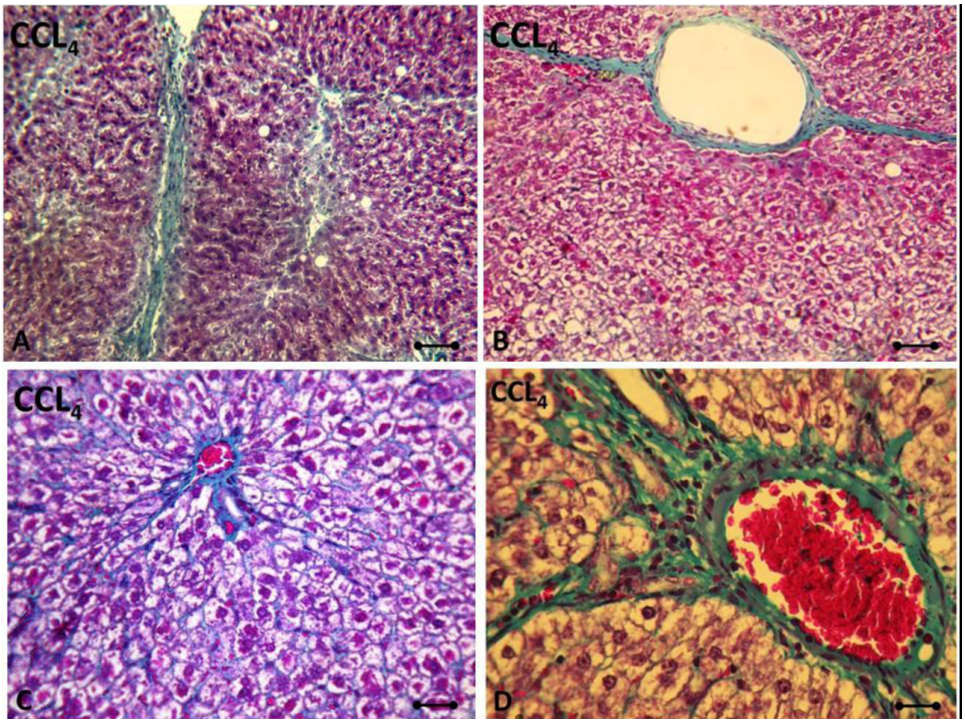


Fig. 3. Masson's trichrome-stained liver sections in CCL_4-treated group. **(A)** Perisinusoidal, periportal and bridging fibrosis (grade 3). (x 200, Scale bar, 50 µm). **(B)** Dilated central vein, centrilobular and bridging fibrosis (grade 3) and hemorrhage. (x 200, Scale bar, 50 µm). **(C)** Congested portal venule, periportal and perisinusoidal fibrosis (grade 2) and ballooning degeneration of hepatocytes. (x 200, Scale bar, 50 µm). **(D)** Ballooning degeneration of hepatocytes, periportal, portal and bridging fibrosis (grade 3), portal venous congestion and hemorrhage. (x 400; Scale bar, 25 µm).

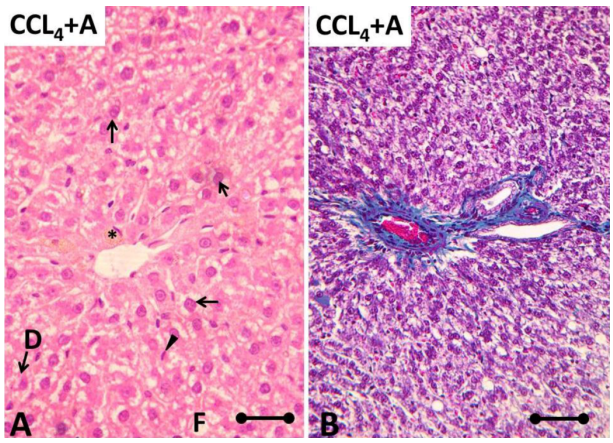


Fig. 4. Liver sections in CCL_4 + A-treated group. **(A)** Regularly arranged hepatic cords, few number of hepatocytes showing ballooning degeneration (D), few Küppfer cells (arrow head), mild steatosis (F), mild bile stasis (*), inflammatory infiltration and regenerating hepatocytes (arrows). (H & E, x 400; Scale bar, 25 µm). **(B)** Grade 1 fibrosis, mild steatosis and portal congestion. (Masson's trichrome, x 200; Scale bar, 50 µm).

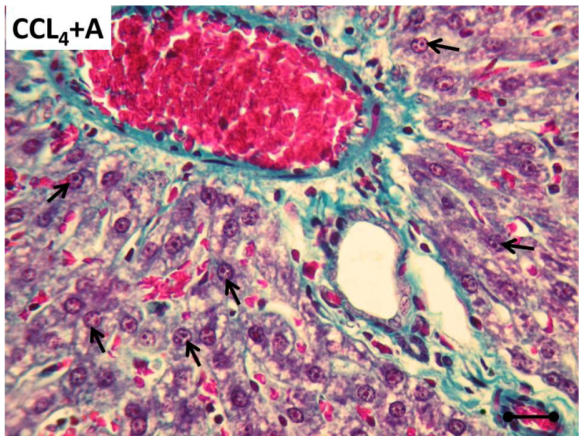


Fig. 5. Section in CCL_4 + A-treated group rat liver showing regularly arranged hepatic cords with dividing hepatocytes (arrows) and mild periportal fibrosis. (Masson's trichrome, x 400; Scale bar, 25 µm).

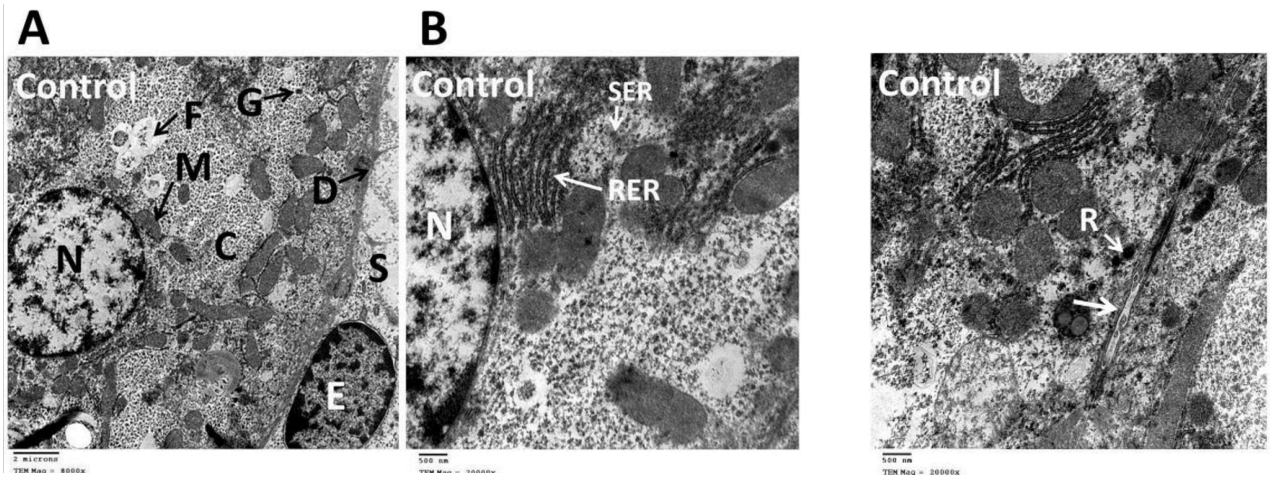


Fig. 6 (Left). Electron photomicrographs of hepatocytes from control group rat liver. **(A)** A central nucleus (N), abundant cytoplasm (C), normal mitochondrial distribution (M), Fat droplets (F), glycogen (G), space of Disse (D), endothelial cell (E) and blood sinusoid (S). (Uranyl acetate and Lead citrate; x 8000; Scale bar, 2 µm). **(B)** A part from a nucleus (N), rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER). (Uranyl acetate and Lead citrate; x 20,000; Scale bar, 500 nm).

Fig. 7 (Right). An electron photomicrograph of hepatocytes from control group rat liver, showing cytoplasm abundant with glycogen and ribosomes (R) and a bile canaliculus (arrow). (Uranyl acetate and Lead citrate; x 20,000; Scale bar, 500 nm).

nation of liver in anise group showed the same appearance as in control group without any pathological changes.

Ultrastructure

Group I (control) (Figs. 6,7). The ultrastructure of the liver revealed hepatocytes with central nuclei with plenty chromatin and cytoplasm rich in glycogen particles and lipid droplets. Cytoplasmic organelles, such as rough and smooth endoplasmic reticulum, ribosomes and mitochondria were located around the nucleus. Various cytoplasmic organelles were found as follows: cisternae of rough endoplasmic reticulum were arranged in typical parallel arrays in the perinuclear cytoplasm, and were abundant in active hepatocytes engaged in high rates of protein synthesis. Mitochondria were scattered in the cytoplasm and contained randomly distributed shelf-like cristae and a less dense granular matrix internally. Blood sinusoids were separated from hepatocytes with spaces of Disse (Figs. 6 and 7).

Table 1. Frequency of histopathological features in the liver of control and other treated groups

Histopathological features	Grade #	Control	CCL_4	CCL_4 + anise	Anise
Lobular inflammation	0	99%	6.25%[a,b,c]	61.4%	98.8%
	1	1%	18.75%	33%	1.2%
	2	0%	50%[a,b,c]	4%	0%
	3	0%	25%	1.6%	0%
Ballooning degeneration	0	99.3%	0%	40%	99.1%
	1	0.7%	5%	55%	0.9%
	2	0%	95%[a,b,c]	5%	0%
Fibrosis	0	100%	8.7%	25%	100%
	1	0%	13%	55%	0%
	2	0%	26.1%	10%	0%
	3	0%	52.2% [a,b,c]	10%	0%
	4	0%	0%	0%	0%

Chi square test: a) $P<0.05$ compared to control group, b) $P<0.05$ compared to anise group, c) $P<0.05$ compared to CCL_4 + anise group.
Scoring system according to Kleiner et al. (2005).

Group II (carbon tetrachloride) (Figs. 8-10). The electron microscopic appearance of the liver revealed hepatocytic shrinkage and vacuolation. Most of the hepatocytes exhibited compressed rough endoplasmic reticulum. Mitochondria were swelled, aggregated, compressed and their matrices were highly electron dense. The intercristal spaces appeared shrunken due to mitochondrial swelling. The nuclei showed chromatin clumping, fragmentation and areas of absent chromatin. Some hepatocytes showed nuclear membrane lysis causing leak of chromatin into the cytoplasm. There were sites of cytoplasmic protrusions (blebbing) toward the surrounding structures. The cytoplasm was filled with coalesced fat globules and devoid of glycogen particles (Figs. 8 and 9). The bile canaliculi were dilated and contained finely granular and electron-dense material aggregations (bile) and the blood sinusoids were congested and filled with aggregated RBCs (Fig. 10).

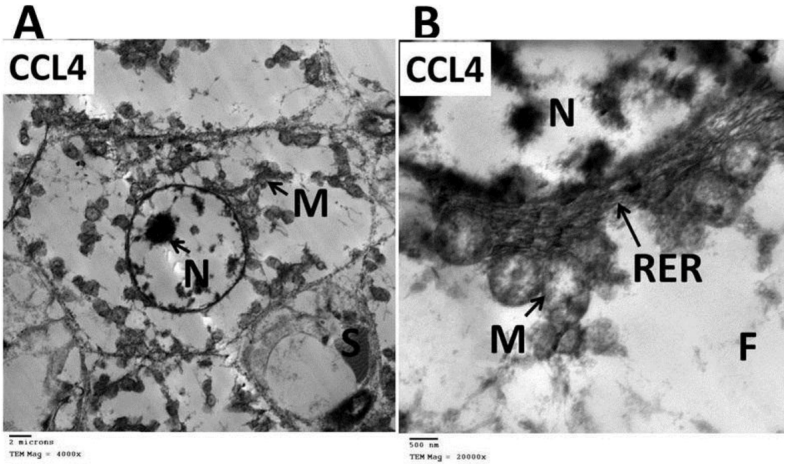


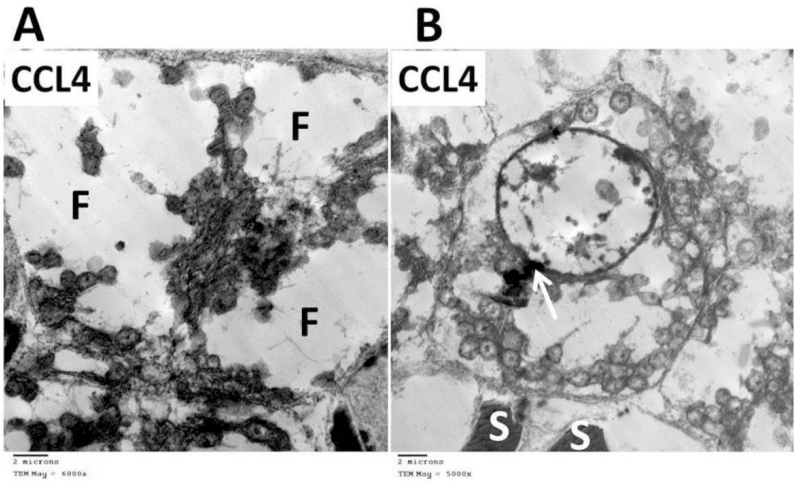
Fig. 8. Electron photomicrographs of hepatocytes from CCL_4-treated group rat liver. **(A)** Cell shrinkage and vacuolation, central nucleus with chromatin clumping (N) and areas of absent chromatin, cytoplasmic blebbing, mitochondrial aggregation (M), coalesced fat globules and congested blood sinusoid (S). (Uranyl acetate and Lead citrate; x 4000; Scale bar, 2 µm). **(B)** Chromatin clumping (N), mitochondrial swelling (M), coalesced fat globules (F) and compressed rough endoplasmic reticulum (RER). (Uranyl acetate and Lead citrate; x 20,000; Scale bar, 500 nm).

Group III (carbon tetrachloride + anise) (Figs. 11,12). The ultrastructure of liver revealed hepatocytes near control group. Few hepatocytes showed features of cell degeneration beside active regenerating and dividing ones and plenty of sinusoidal endothelial cells (Figs. 11 -12).

Group IV (anise). The electron microscopic examination of the liver in anise group showed the same appearance as in the control group without any pathological changes.

DISCUSSION

Liver sections of CCL_4-treated rats in the present study showed loss of normal architecture of hepatic lob-



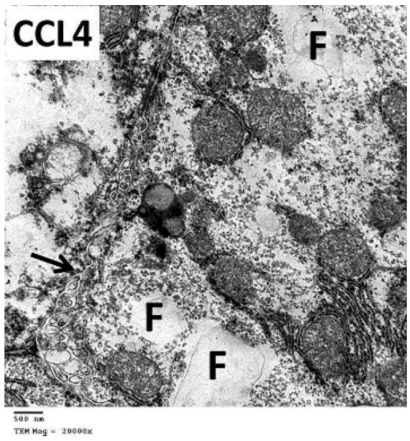


Fig. 9 (Left). Hepatocytes from CCL_4-treated group rat liver. **(A)** Cell vacuolation, large fat droplets (F) that pressed and destroyed the nucleus and organoids in the center. (Uranyl acetate and Lead citrate; x 6000; Scale bar, 2 µm). **(B)** Cell shrinkage and vacuolation, marginated nucleus, rupture nuclear membrane with leak of chromatin (arrow), chromatin clumping and fragmentation, cytoplasmic protrusions (blebbing) and blood sinusoids congested with aggregated RBCs (S). (Uranyl acetate and Lead citrate; x 5000; Scale bar ,2 µm).

Fig. 10 (Right). Rat liver in CCL4-treated group, showing a dilated bile canaliculus filled with bile (arrow) and two adjacent hepatocytes with mitochondrial swelling, fat droplets (F) and aggregated dense organelles. (Uranyl acetate and Lead citrate; x 20,000; Scale bar, 500 nm).

ules, portal and sinusoidal congestion, hemorrhage, steatosis, necrosis, bile canaliculi dilatation and cholestasis. Kuppfer cell hyperplasia, increased lobular inflammation, ballooning degeneration of hepatocytes and fibrosis were observed also in the liver specimens. These results are in accordance with those mentioned by Recknagel (1987), Lee et al. (2001), Etim et al. (2008) and Shafaq et al. (2009).

Carbon tetrachloride induced liver damage through many mechanisms including the toxic stress of free radicals and the inflammatory process mediated by the release of pro-inflammatory cytokines from activated Kupffer cells. Activated Kupffer cells and damaged hepatocytes also produced pro-fibrogenic cytokines that induced hepatic fibrosis (Tsukamoto et al., 1995). The CCL_4-toxic effect was discovered to be due to enzymatic activation of cytochrome (P450), and the conversion of CCL_4 into carbon trichloride free radical (CCL_3) within the membrane of the endoplasmic reticulum (Lee et al., 2007). Furthermore, oxidative stress aggravates liver fibrosis via hepatic stellate cells (HSCs) activation (Svegliati et al., 1998; Tahan et al., stimulation of the transcription of collagen gene (Bedossa et al., 1994). Activation of HSCs and the initiation of unbalanced synthesis of collagen are the main components in the pathogenesis of fibrosis, which is characterized by the accumulation of collagen and extracellular matrix proteins in the space of Disse (Tahan et al., 2007). Based on their explanation, kupffer cell hyperplasia and lobular inflammation that were noticed in the present study in CCL_4 -treated group, beside the toxic free radicals, were responsible for the progressive cell damage and deposition of collagen fibers centrilobular, perisinusoidal and periportal.

CCL_4-treated rat liver specimens also revealed ultrastructural changes as plasmalemma, nuclei and most of the cell organelles were destroyed. These findings may be secondary to the accumulation of the highly reactive compounds in hepatocytes that caused membrane lipid peroxidation (Lee et al., 2007) and progressive destruction of the endoplasmic reticulum membrane (Kuzu et al., 2007). Since the crucial pathways of carbohydrate metabolism, protein processing and drug detoxification occur in the endoplasmic reticulum with integration to other organelles (Csala et al., 2006), their damage lead to the reduction in glycogen, proteins and enzymes synthesis with accumulation of toxins. So these consequences can explain the observed glycogen depletion in hepatocytes in the present study.

Cholestasis that was observed in the CCL_4-treated group can be a result of obstruction of bile canaliculi induced by the hepatic fibrosis. In that case, bile was excreted from hepatocytes but ac-

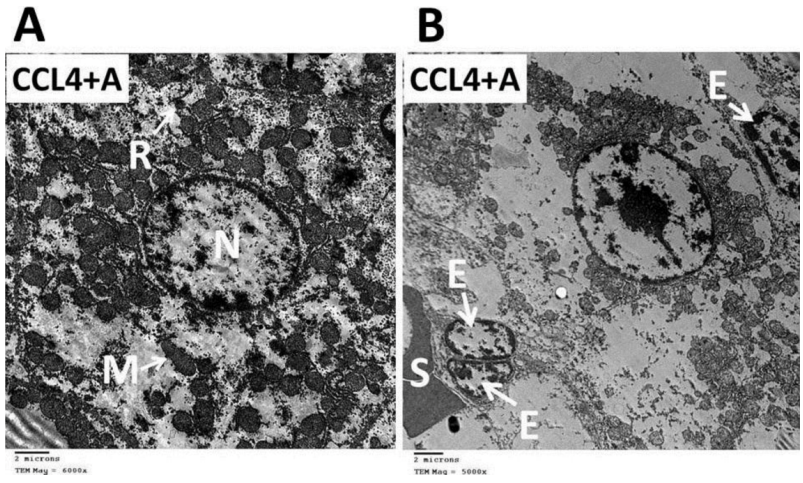


Fig. 11. Electron photomicrographs of rat liver in CCL_4 +A-treated group. **(A)** A hepatocyte, with a centralized nucleus (N) with normal chromatin distribution, cytoplasm rich in glycogen, abundant ribosomes (R) and mitochondria (M). (Uranyl acetate and Lead citrate; x 6000; Scale bar, 2 µm). **(B)** A hepatocyte with a centralized nucleus, chromatin clumping and aggregated mitochondria separated from blood sinusoid (S) by proliferated sinusoidal endothelial cells (E). (Uranyl acetate and Lead citrate; x 6000; Scale bar, 2 µm).

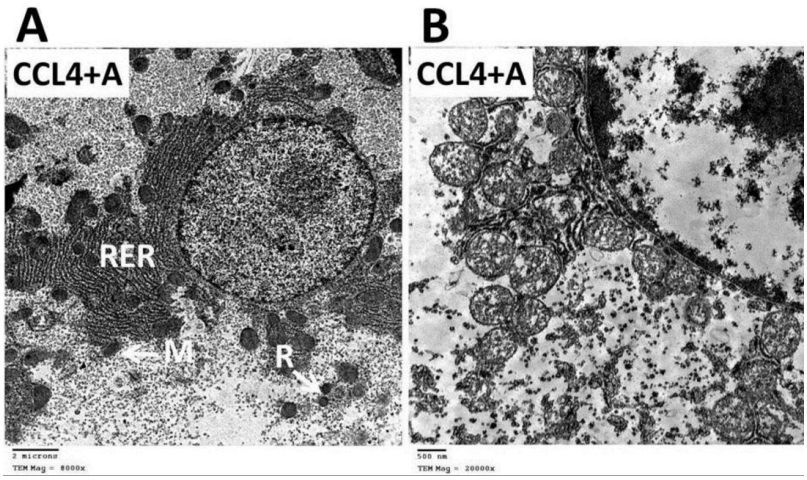


Fig. 12. Photomicrographs of rat liver in CCL_4 +A-treated group. **(A)** An active hepatocyte with plenty of rough endoplasmic reticulum (RER), ribosomes (R) and mitochondria (M). (Uranyl acetate and Lead citrate; x 5000; Scale bar, 2 µm). **(B)** A hepatocyte with cytoplasm rich in glycogen and mitochondria. (Uranyl acetate and Lead citrate; x 20,000; Scale bar, 500 nm).

cumulated inside the bile canaliculi. The over-distended bile canaliculi ruptured and spread bile into the surrounding tissues, producing bile lakes. Accumulation of reactive metabolites in hepatocytes also inhibited the hepatocellular functions such as bile salt efflux pump, with subsequent intracellular retention of its substrates. Bile retention subsequently impaired membrane integrity and permeability leading to bile leakage. These amplification mechanisms resulted in further retention of damaging substances, accelerated membrane injury, dysfunction and ultimately produced failure of the excretory mechanism for bile. These explanations were in accordance with Pauli-Magnus et al. (2005).

Steatosis and filled cytoplasm with coalesced fat globules may be due to mitochondrial damage, as many studies concluded that the highly reactive metabolites inhibit the mitochondrial respiratory chain causing ATP depletion, increase concentrations of reactive oxygen species and inhibit beta- oxidation leading to steatosis (Vendemiale et al., 1996; Pessayre, et al., 1999; Kaufmann et al., 2005; Waldhauser et al., 2006). The interference with ATP synthesis also leads to de-energization of the sodium and potassium ATPase at the plasmalemma, followed by an increase in sodium ions accompanied by water influx and cellular swelling. The cell shrinkage is stimulated by the loss of potassium ions, increased calcium ions, and activation of calcium and potassium channels. Damage of plasma membrane may be another cause of cell shrinkage beside the ATP deficiency (Trump et al., 1997). These processes eventually result in programmed apoptotic cell death, which is characterized by cytoplasmic and nuclear condensation and fragmentation (Stefan et al., 2009). Accordingly, the hepatocytic changes that occurred in liver specimens of CCL_4- treated group were sequels in cell apoptosis and necrosis.

On the other hand, anise oil administration with CCL_4 reduced the histopathological features of liver damage, and improved the ultrastructure of hepatocytes. Most of hepatocytes regained their activity and function, as concluded from the presence of plenty of organelles, especially mitochondria and endoplasmic reticulum and glycogen particles.

These findings are in accordance with those of previous studies that concluded that other antioxidants (vitamin E, sylinmarin and acetylcysteine) ameliorate liver injury (Parola et al., 1992; Angulo et al., 2000), and prevent fibrosis (Mohamed et al., 2005; Tahan et al., 2010). Antioxidants were reported to interfere with the pathogenesis mechanisms of liver damage through HSCs inactivation, lipid peroxidation reduction (Tahan et al., 2004), and Kupffer cell suppression (Yao et al., 2002).

Anise and its compounds also have been identified as free radicals scavengers. It was reported that the aqueous extract exhibited greater antioxidant activity than the ethanol extract (Gülçin et al., 2003). Anise oil was observed to inhibit lipid peroxidation; such activity was correlated with its polyphenolic antioxidant compounds, such as lignans and flavonoids (Teissedre and Waterhouse, 2000; DerMarderosian and Beutler, 2002). Based on these explanations, the antioxidant components of anise oil played a role in the reduction of the oxidative stress and liver damage induced by CCL_4 in the current study.

Anise oil also contains trans-anethole, which forms the major component of oil (75-90%) and is responsible for the characteristic taste and smell. Trans-anethole is considered as an active estrogenic agent (Leung, 1980 and DerMarderosian and Beutler, 2002). It has been shown to play a role in suppression of inflammation and of carcinogenesis (Chainy et al., 2000).

Xu et al. (2002) observed that the fibrotic response of the female liver to CCL_4 treatment was significantly weaker than that of the male liver. On the other hand, Robert and Arnold (1979) reported that the estrogen receptors found in the liver of male and female rats were similar on the basis of physical properties, binding properties and endocrine regulation. Xu et al. (2002) suggested that the physiological levels of estrogen have an antifibrogenic effect, as liver fibrosis was increased in ovariectomized rats that received CCL_4. On the other hand, they observed that the administration of estradiol decrease the liver enzymes (ALT and AST) and preserve the structural integrity of the hepatocytes. These findings supposed that the anethole component of anise oil, which acts as an active estrogenic substance, may play an antifibrogenic role in the liver, and may improve liver fibrosis in the CCL_4+ anise-treated group in the present study.

In the current study, Figures of regeneration including newly formed hepatocytes, dividing cells and proliferation of liver endothelial cells were observed in the CCL4+ anise-treated group compared with CCL4-treated group. These findings were in agreement with that of Gershbein (1977), as he found that subcutaneous administration of the anise oil to partially hepatectomized rats stimulated liver regeneration. Liver sinusoidal endothelial cells (LSECs) were known as integral parts of the hepatic reticulo-endothelial system. The primary roles of LSECs include scavenger function and blood clearance (Elvevold et al., 2008). They may play a role in liver regeneration as normal liver regeneration requires increased expression of hepatocyte growth factor (HGF) by liver

sinusoidal endothelial cells and LSEC proliferation (Greene et al., 2003; Ding et al., 2010) but mature LSECs, like other endothelial cells, are low in HGF (Urbich et al., 2005). Previous studies suggested that LSEC-HGF expression increased after liver injury which promoted liver regeneration (Maher, 1993; Ding et al., 2010). Accordingly, anise oil may promote liver regeneration by stimulating LSEC proliferation as observed in CCL_4+ anise-treated group and absent in CCL4-treated group.

In conclusion, our results suggest that anise oil has a protective effect on liver damage caused by CCL_4 and promotes liver regeneration. These effects might be attributed to the antioxidant, anti-inflammatory, and antifibrinogenic properties of its components. Anise oil could be clinically beneficial in reducing the hepatotoxic adverse effect of CCL_4 and other drugs. Further research will be needed to ensure anise oil effects on the physiological and biochemical levels.

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