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Antioxidant Activity and Hepatoprotective Potential of Black Seed, Honey and Silymarin on Experimental Liver Injuries Induced by CCl_4 in Rats

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Abstract: The possible antioxidant activity and hepatoprotective potential of black seed honey and silymarin on CCl_4 induced liver injuries in rats was investigated. Fifty male rats were used in this study and divided into five groups, 10 rats each. Group 1 served as a control; group 2 injected 1 mL kg^{-1} day^{-1} CCl_4 intraperitoneally 3 times a week for 4 week, groups 3, 4 and 5 subjected to the same injection of CCl_4 and co-treatment with black seed, honey and silymarin (50 mg kg^{-1} b.wt.), respectively, daily by stomach tube for 4 weeks. Blood and tissue samples were taken for biochemical and histopathological studies. The results revealed that CCl_4 administration caused significant elevations in the levels of MDA, NO, MMP-2, AST and ALT. Histopathological observations showed severe damage in the liver. Its fibrotic areas were measured using Image Analyzer. Combined treatment with CCl_4 and black seed, honey and silymarin showed marked improvement in antioxidant status and in histopathological findings as well as reductions in the fibrotic areas. These results concluded that black seed, honey and silymarin have protective characteristics against CCl_4-induced rat liver injury through potentiation of antioxidant capacity of liver cells and prevention of oxidative stress that accompanied with CCl_4 hepatotoxicity. The protective effect was higher in silymarin followed by black seed then honey.

Key words: Liver, antioxidants, black seed, honey, silymarin, CCl_4, rats

INTRODUCTION

Liver is the main organ involved in the metabolism of biological toxins and medicinal agents. Such metabolism always associated with the disturbance of hepatocyte biochemistry and generation of Reactive Oxygen Spices (ROS) (Fernandez-Checa and Kaplowitz, 2005). Lots of liver damages ranging from subclinical icteric hepatitis to necroinflammatory hepatitis, cirrhosis and carcinoma have been proved to associate with the redox imbalance and oxidative stress (Vrba and Modriansky, 2002). Therefore, a potential noval approach, namely developing antioxidant drugs to treat and protect liver injury and liver disease, has been proposed by Bansal *et al.* (2005). One of these drugs is silymarin, which was chosen in the present study in addition to black seed and honey.

Black seed (*Nigella sativa*) is an herbaceous plant known to have many properties in traditional medicine and used as a natural remedy for a variety of complications including liver diseases (El-Dakhakhny *et al.*, 2002). Black seed with its active principle Nigellone found to have an antioxidant activity and may reduce the hepatotoxicity resulted from many insults (Mabrouk *et al.*, 2002). It was reported as hepatoprotective agent against CCl_4-induced liver fibrosis (Turkdogan *et al.*, 2001, 2003), possess antiviral effect in viral infected model (Salem and Hossain, 2000), have anticestode and antinematode action (Mahmoud *et al.*, 2002), prevent lipid peroxidation through the decrease in MDA, increase in antioxidants, prevent liver damage (Ramadan *et al.*, 2003) and have anti-inflammatory activity (Arifah *et al.*, 2004).

Honey is one of honeybees' products which are used in medicine in many cultures since ancients' times. Honey is the main source of concentrated sweetness in the diet of many people and contained about 80% carbohydrate, 20% water and traces of protein and ash (Mahdy and Morsy, 2001). Honey is known to exhibit a broad spectrum of activities including antiviral, antibacterial and immunostimulant (Molan, 2002; Mato *et al.*, 2003). It was found to have antioxidant activity due to its high content of flavonoids (Mabrouk *et al.*, 2004; Aljadi and Kamaruddin, 2004).

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Silymarin is among the drugs that are used in the treatment of hepatic dysfunction. Silymarin is a potential mixture of antioxidant flavonolignans, extracted from the seed of *Silybum marianum* (Shalan *et al*., 2005). It is used as hepatoprotective agent against hepatic injury caused by many toxic substances such as CCl_4 (Muriel *et al*., 2005), aflatoxin B_1 (Mekala *et al*., 2006) and galactosamine (Dhanabal *et al*., 2006).

Carbon tetrachloride (CCl_4) is widely used as hepatotoxic compound for screening the anti-hepatotoxic/hepatoprotective activity of drugs in experimental model systems, because CCl_4-induced hepatotoxicity is regarded as an analogue of liver injury caused by a variety of hepatotoxicity in man. It has been generally reported and accepted that CCl_4-induced hepatotoxicity due to its hepatotoxic metabolites and trichloromethyl free radicals ($\bullet CCl_3$) induced lipid peroxidation (Basu, 2003; Lee *et al*., 2005). Therefore, one of the therapeutic strategies against liver injury is to find antioxidant compounds that are able to block liver injury through scavenging of trichloromethyl free radical generated by CCl_4 (Lee *et al*., 2005). Accordingly the present work was designed to elucidate the antioxidant activity and hepatoprotective potential of black seed, honey and silymarin (a standard drug for liver fibrosis) on experimental liver injuries induced by CCl_4 in rats to known the possible mechanisms(s) targeted by these natural antioxidants in hepatoprotection against CCl_4 toxicity.

MATERIALS AND METHODS

Black seed, honey and silymarin used: Black seed and honey were purchased from the local market at Cairo, Egypt. The black seed was washed, dried in sun and ground and then suspended in water before use. The honey used is a cotton flower honey, was diluted with water (1/1 V/V) before use. Silymarin was obtained from the pharmacy as 10 sachets (instant) produced by SEDICO pharmaceutical Co., 6 October City, Egypt. Each sachet contains 140 mg silymarin (calculated as silybin). It was prepared by dissolving the content of each sachet in water (50 mL) and administered immediately. All the treatments were given to rats by oral using stomach tube.

Animals and diets: Fifty male albino rats of the Sprague-Dawley strains, weighing 90-130 g each, were left under normal healthy conditions at the Animal House of the National Research Centre. Animal were fed on basal diets (Reeves *et al*., 1993; NRC, 1995) and water was supplied *ad libitum*.

Experimental design: The animals were segregated into five groups each of 10 rats as follows:

- Normal controls.
- **CCl_4 intoxicated group:** Rats received 1 mL kg^{-1} CCl_4 (10% v/v olive oil) intraperitoneally three times a week for 4 week.
- **CCl_4 and black seed supplemented group:** Rats treated with 1 mL kg^{-1} CCl_4 and received orally 50 mg kg^{-1} black seed daily for 4 week.
- CCl_4 and honey supplemented group: Rats treated with 1 mL kg^{-1} CCl_4 and received orally 50 mg kg^{-1} honey daily for 4 week.
- **CCl_4 and silymarin supplemented group:** Rats treated with 1 mL kg^{-1} CCl_4 and received orally 50 mg kg^{-1} silymarin daily for 4 week.

Collection of blood samples: At the end of the experiment, blood samples were collected after 16 h fasting using the orbital sinus technique of Sanford (1954). Blood samples were left to clot in clean dry test tubes and then centrifuged at 3000 rpm for 10 min. The clear supernatant serum was then frozen at -20°C for the biochemical analysis.

Biochemical methods: Serum alpha Glutathione-S-Transferase (α-GST), was estimated by the enzymatic immunoassay method using kit produced by Biotrin International – Ireland, according to the method described by Meister (1985). Serum Malonedialdehyde (MDA) was estimated by the enzymatic immunoassay method using kit of Oxis Research, Inc. USA, according to the method described by Liu *et al*. (1991). Nitric Oxide (NO) and Matrix Metalloproteinase-2 (MMP-2) were estimated in serum by the immunoassay technique, using kit of R and D systems, Inc. USA, according to the method of Conner and Grisham (1995) and Parks and Mecham (1999), respectively. Serum liver function tests, AST and ALT were estimated using the kit of Sentinel-Italy, according to the method described by Reitman and Frankel (1957).

Histological studies: The liver of rats of different groups were removed and fixed in 10% formal saline, 5 μm thick paraffin sections were stained with haematoxylin and eosin (Drury and Wallington, 1980) and examined by light microscope. Quantitative measurement of fibrotic areas was achieved by using computerized image analysis (Leica Qwin 500 Image) in Image Analyzer Unit, Pathology

Department, National Research Center. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Ten fields were chosen in each specimen and the mean values were obtained.

Statistical analysis: The data obtained in the present work are represented as average (mean)±standard error. Statistical analysis was evaluated using the student t-test. $p<0.05$ were treated as statistically significant (Armitage, 1971).

RESULTS

Biochemical results: The results obtained indicated that, CCl_4 treated rats exhibited significant increases in MDA, NO, MMP-2, AST and ALT levels in comparing with control group. The group of rats co-administered with CCl_4 and black seed revealed significant increase in serum α-GST as compared with control group and significant decreases in the levels of MDA, NO, MMP-2, AST and ALT when compared with the group of rats treated with CCl_4 only. The group of rats co-administrated with CCl_4 and honey exhibited significant increase in serum α-GST as compared with control group and significant decreases in the levels of MDA, AST and ALT when compared with the group of rats treated with CCl_4 only. The co-administered rats with CCl_4 and silymarin exhibited significant increase in serum α-GST as compared with control or CCl_4 groups and significant decreases in the levels of serum MDA, NO, MMP-2, AST and ALT when compared with CCl_4 treated group (Table 1).

Histological results: The liver of control rats revealed normal characteristic hepatic architecture as presented in (Fig. 1A). The treatment rats with CCl_4 showed moderate fibrosis, massive vacuolar degeneration; minute fatty changes and many pyknotic nuclei was also founded. The dilated blood sinusoids are filled with red blood cells (Fig. 1B, C). The liver of CCl_4 administered rats that protected by black seed for 4 week showed some protective effects as compared to CCl_4-administered group. Examination of liver sections showed moderate fibrosis and minute fatty change. Focal necrosis and some pyknotic nuclei could be noticed (Fig. 1D). The liver of CCl_4 administered rats that protected by honey for 4 week showed more improvement in the pathological changes in the form of diminution of vacuolar degeneration and fibrosis as compared to CCl_4-treated group. However, small haemorrhagic areas, many pyknotic nuclei and cellular infiltration were still present (Fig. 1E). The liver of CCl_4 administered rats that protected by silymarin for 4 week showed some obvious pathological changes, but these changes were some what less than those of CCl_4-administered group. Examination of liver sections showed that dilated blood sinusoids are filled with red blood cells, focal necrosis and hydropic degeneration. Large haemorrhagic areas were also showed (Fig. 1F).

Image analysis of liver fibrosis: Areas of liver fibrosis were assessed by hepatic morphometric analysis which has been considered as the gold standard for quantitative of fibrosis. Significant increase in the area of fibrosis was observed in the group of rats treated with CCl_4 only as compared with control group. The liver of CCl_4 administered rats that protected by black seed, honey and silymarin showed significant decreases ($p<0.05$) in the area of fibrosis as compared with CCl_4 treated group (Table 2).

Table 1: Serum levels of fibrosis markers and liver function tests in control, CCl_4-exposed rats with or without black seed, honey and silymarin treatment for 4 week

Serum levels	Control group n = (10)	CCl_4 treatment group (n=10)	CCl_4+ black seed treatment group (n=10)	CCl_4 + honey treatment group (n=10)	CCl_4 + silymarin treatment group (n=10)
α-GST (μg L^{-1})	9.11± 0.46	10.16±0.60	11.36±0.82[a]	11.23±0.73[a]	12.32±0.70[ab]
MDA (μg L^{-1})	3.10±0.02	4.19±0.03[a]	3.17±0.01[b]	3.20±0.01[b]	3.12±0.03[b]
NO (μM L^{-1})	63.80± 2.37	101.50±5.32[a]	84.40±3.20[ab]	99.10±2.97[a]	88.90±3.75[ab]
MMP-2 (ng mL^{-1})	108.10±2.81	126.40±2.59[a]	118.90±2.73[ab]	125.40±2.09[a]	119.20±2.91[ab]
AST (μ L^{-1})	98.90±1.35	119.30±1.08[a]	110.60±1.99[ab]	113.60±1.16[ab]	99.40±2.35[b]
ALT (μ L^{-1})	65.60±1.47	94.80±0.92[a]	79.30±1.03[ab]	85.10±1.52[ab]	65.40±1.39[b]

[a]: Significant differences vs control group ($p<0.05$), [b]: Significant differences vs CCl_4 group ($p<0.05$)

Table 2: Mean values of fibrotic areas in liver of CCl_4-exposed rats with or without black seed, honey and silymarin treatment for 4 weeks

Parameters	CCl_4 treatment group	CCl_4 + black seed treatment group	CCl_4 + honey treatment group	CCl_4 + silymarin treatment group
Area (μm^2)	518.37±44.46	203.10±40.68[a]	345.88±61.94[a]	171.78±17.70[a]
Area fraction	0.28±0.025	0.10±0.02[a]	0.16±0.03[a]	0.10±0.01[a]
Area (%)	28.00±2.49	9.11±1.83	15.69±2.71	10.11±1.15

[a]: Significant difference vs CCl_4 group ($p<0.05$)

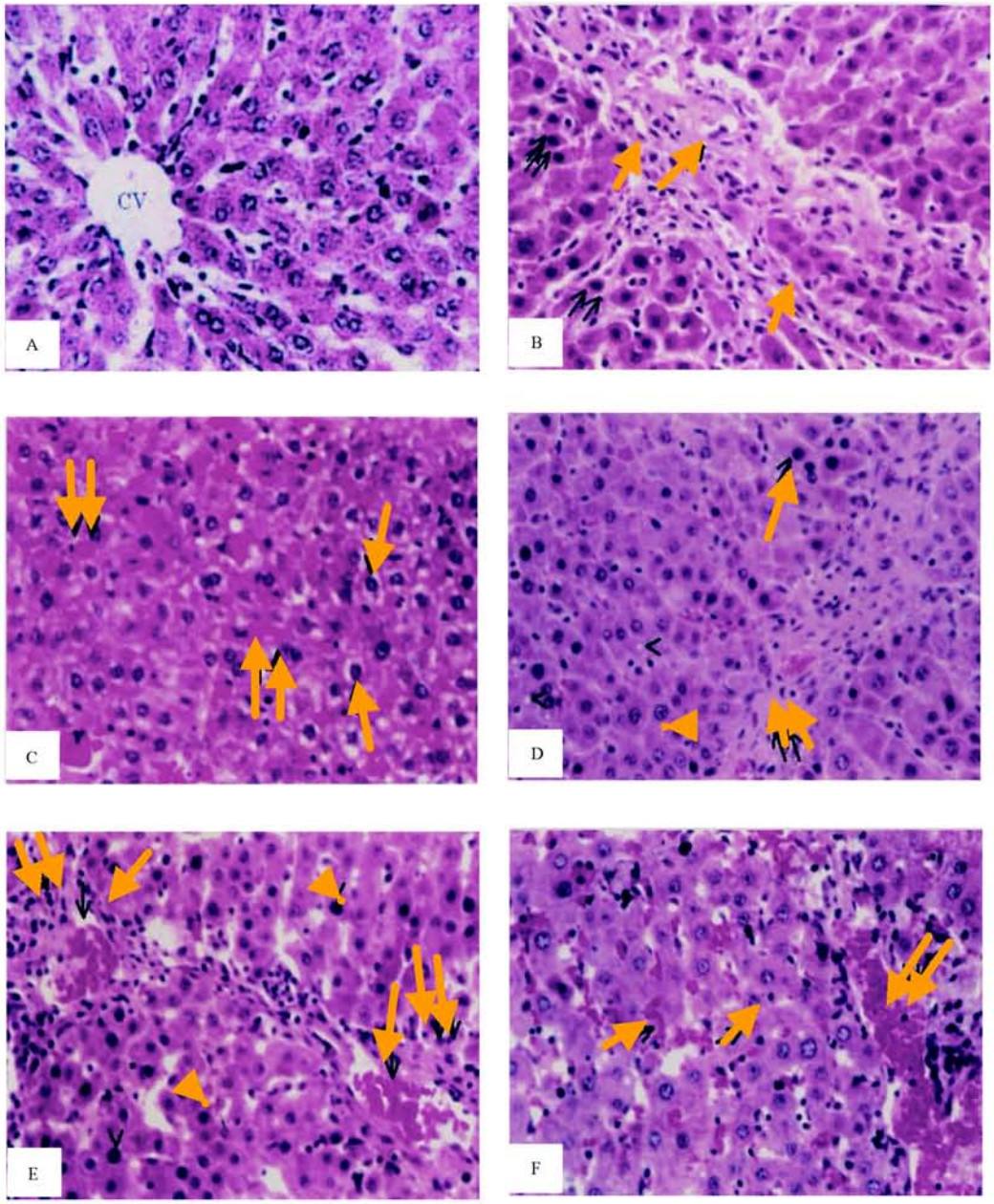

Fig. 1: Sections in the liver of (A) Control rat showing normal histological structure of hepatic lobules and central vein (CV), (B) Rat administered with CCl_4 for 4 weeks showed moderate fibrosis (arrow), (C) Rat administered with CCl_4 for 4 weeks showing massive vacuolar degeneration (arrow) and the dilated blood sinusoids are filled with red blood cells (double arrows), (D) Rat administered with CCl_4 and protected by Black seed showing pyknosis in some hepatocytes (arrow) and moderate fibrosis (double arrows) and minute vacuolar degeneration (arrowhead), (E) Rat administered with CCl_4 and protected by honey showing small haemorrhagic area (arrow) and cellular infiltration (double arrows). Many pyknotic nuclei could be noticed (arrow head) and (F) Rat administered with CCl_4 and protected by Silymarin showing dilated blood sinusoids are filled with red blood cells (arrow). Large haemorrhagic areas are noticed (double arrows) (Hx and E x 200)

DISCUSSION

In this study we investigated the possible protective effects of black seed, honey and silymarin on liver injury induced by CCl_4 to know the possible mechanisms(s) targeted by these natural antioxidants in hepatoprotection and prevention of the oxidative stress that accompanied with hepatotoxicity. We focused on the biochemical changes elicited in the liver including antioxidants capacity and oxidative damage, in addition to the histopathological changes in liver cells.

The present results showed significant increase in the levels of serum MDA in CCl_4-administered group after 4 week These results are in accordance with many reports that found increase level of MDA in hepatic cirrhosis induced by CCl_4 (Cabre *et al*., 2000; Cremonese *et al*., 2001) and in hepatoctomized cirrhotic rats (Andiran *et al*., 2003). The increase in MDA induced by CCl_4 has been explained by Socha *et al*. (1992), they postulated that the rise of MDA in liver disease may be attributed to the chronic pathology of the liver lead to disturbance in circulation and oxygenation which in turn cause lipid peroxidation and subsequently increase MDA concentration. Therefore, lipid peroxidation may cause severe damage and play a key role in pathogenesis of several human diseases. It was found that also that the elevated MDA was found in serum with staging of fibrosis and in tissue mainly around periportal area (Mahmood *et al*., 2004).

In the present study, histopathological examinations of CCl_4-exposed rats for 4 week showed a massive vacuolar degeneration and micro-fatty changes. These results are in agreement with Turkdogan *et al*. (2003) who reported that the treatment of rats with CCl_4 caused hepatocellular necrosis, vacuolar degeneration and advanced fibrosis. Conciding with Germano *et al*. (2001) and Al-Ghamdi (2003) whom reported that the administration of CCl_4 induced hepatic lesions including fatty change, ballooning infiltrate in the form of neutrophils and mononuclear cells. However, the pathological changes observed in liver of rats due to administration of CCl_4 may be attributed to lipid peroxidation and glutathione depletion (Meki and Hussein, 2001).

In the present study the treatment of rats with CCl_4 only showed significant increase in AST and ALT. These results are in parallel with the results of Lee *et al*. (2007) who reported that the treatment of rats with CCl_4 at a dose level of 0-15 mL kg^{-1} b.wt. three time a week for 8 week showed high significant increase in AST and ALT levels and with the results of Ichi *et al*. (2007) who found that the treatment of rats with CCl_4 at dose level of 4 mL kg^{-1} b.wt. showed severe elevation in plasma AST and ALT after 6 h of administration.

The present results showed significant increase in serum NO level in case of rats treated with CCl_4 alone. These results are in agreement with the previous reports that found elevation in NO level via Inducible Nitric Oxide Synthetase (INOS) in animal with advanced cirrhosis associated with endothelial dysfunction, portal hypertension and ascites after CCl_4 administration (Nelson and Eichinger, 2001). NO could protect the liver from lipid peroxidation by interacting with superoxide anion and other free radicals to produce less toxic species (Muriel, 1998). Also, NO was found to mediate pulmonary vasoreactivity observed in cirrhotic rats induced by CCl_4 (Nelson and Eichinger, 2001), inhibit Hepatic Satellite Cells (HSCs) proliferation after Dimethylnitrosamine (DMN)-induced liver fibrosis (Svegliati-Baroni *et al*., 2001) and mediate the abnormalities associated with cirrhosis in rats induced by bile duct ligation (Ortiz *et al*., 2001). The elevated levels of MDA and NO and the insignificant changes of α-GST proved that one of the mechanisms involved in the process of liver fibrogenesis induced by CCl_4 is the imbalance between antioxidants and reactive oxygen spices as well as the development of oxidative stress.

In the present histological examination, the treatment of rats with CCl_4 only showed moderate fibrosis in liver. Coinciding with the results of Luo *et al*. (2004) and Morsy *et al*. (2004) that reported that CCl_4 administration to rats for 8 week induced liver fibrosis. The liver exhibited a marked increase in the extracellular matrix content and displayed bundle of collagen surrounding the lobules, which resulted in a large fibrosis septa and distorted tissue architecture. The liver damage varied from one area to another and ranged from moderate fibrosis to cirrhosis.

In the present study, the treatment rats with CCl_4 caused significant increase in MMP-2 level. These results are in accordance with the previous reports that showed an increase in MMP-2 level during CCl_4-induced liver fibrosis in rats (McCrudden and Iredale, 2000) and in Schistosoma Mansoni-induced liver fibrosis in mice (Vaillant *et al*., 2001). MMP-2 is a very important member of MMPs family. This enzyme was synthesized by activated HSCs and involved in degrading the native form of type IV collagen, the major component of the basement membrane (Yang *et al*., 2003).

The co-treatment of CCl_4-exposed rats with black seed showed significant improvement and decreased in the levels of MDA, NO, MMP-2, AST and ALT in

comparing with group of rats treated with CCl_4 only, while significant increase in serum α-GST level was obtained, as compared with control group. According to Meral *et al.* (2001) black seed was found to prevent lipid peroxidation induced liver damage in experimentally diabetic rabbits assessed by decreased MDA and increase antioxidant defense system. Also it was found that the treatment of CCl_4 exposed rats with black seed was able to protect liver from damage by decrease MDA and increase GSH. Furthermore the histological examination, showed improvement in hepatocytes in the form of diminution of liver fibrosis and reduction in the fibrotic areas, as compared to CCl_4-administered group. These results are in agreement with Kanter *et al.* (2005) who reported that black seed prevent liver fibrosis induced by CCl_4 in experimental animals by decreasing lipid peroxidation, increasing antioxidants defense and enhancing liver enzymes. It was also reported that the treatment of rats with black seed might at least partly by successful in prevention of liver fibrosis in rabbit (Turkdogan *et al.*, 2001). Coinciding with Al-Ghamdi (2003) who reported that the treatment of rats with black seed along with CCl_4, the comparative histopathological study of liver exhibited almost normal architecture. It was reported also that the rats treated with black seed along with CCl_4 showed non of the serious histopathological findings except for sparse coagulation necrosis in periacinar regions (Turkdogan *et al.*, 2003).

Concerning the hepatoprotective activity of honey against CCl_4-induced liver fibrosis, it was observed that significant increase in serum α-GST level than control group and significant decreases in MDA, AST and ALT levels as compared to CCl_4-administered group. It was found that honey reduce lipid peroxidation and nitric oxide and greatly improved liver enzymes and lipid profile in mice implicated with carcinoma cells referred to its antioxidant activity (Antony *et al.*, 2000). Honey have a hepatoprotective activity against methyl nitrosourea (MNU)-induced oxidative stress and inflammatory response in rats by 100% via keeping normal defense system and decrease NO and MDA (Mabrouk *et al.*, 2002, 2004) and to have a hepatoprotective activity against CCl_4-induced liver damage in sheep (Al-Waili, 2003) and in mice (Resende *et al.*, 2003) by improving liver functions. Moreover, the histopathological examination showed that honey leads to some improvement in pathological changes in the form of diminution of fibrosis and vacuolar degenerations and reduction in the fibrotic areas, as compared to CCl_4-treated group. These results are in agreement with Al-Waili (2003) who reported that the intravenous injection of honey had a hepatoprotective effect against CCl_4-induced liver injury.

Regarding, the hepatoprotective effect of silymarin against CCl_4-induced liver injuries in rats, It was found that silymarin leads to significant increase in serum α-GST level and prevent the elevation of MDA than control group and significant improvement and decreases were obtained in the levels of NO, MMP-2, AST and ALT. These results are in agreement with previous studies that obtained silymarin, significantly reduced lipid peroxidation, liver enzymes and increase glutathione content in rats (Muriel *et al.*, 2005) or in mice (Chrungoo *et al.*, 1996) exposed to CCl_4. The protective effect of silymarin against CCl_4 induced lipid peroxidation in experimental animals have been explained by its free radical scavenger property that prevent lipid peroxidation and making cells more resistance to osmotic lyses (Halim *et al.*, 1997). In addition silymarin has a potential antifibrotic property through inhibition of HSCs proliferation that is the central event of liver fibrosis (Shenoy *et al.*, 2001). Furthermore, the present study showed that the treatment of rats with silymarin along with CCl_4 exhibited more improvement in pathological changes in the form of diminution of fibrosis and reduction in the fibrotic areas, as compared to CCl_4-administered group. According to Jeong and co-author (Jeong *et al.*, 2005) silymarin is well known as a protective agent against hepatotoxin. Silymarin has the ability to reduce the collagen content (Muriel *et al.*, 2005).

In conclusion, the present results demonstrate that black seed, honey and silymarin acted as potent protective agents against liver toxicity induced by CCl_4 in rats through potentiation of antioxidant capacity of liver cells by increasing α-GST level, prevention of NO, MDA and MMP-2 release, as well as improvement in liver functions and reduction in the fibrotic areas. The protective effect was higher in silymarin followed by black seed then honey.

REFERENCES

Al-Ghamdi, M.S., 2003. Protective effect of *Nigella sativa* seeds against carbon tetrachloride induced liver damage. Int. J. Comp. Med. East and West, 31: 721-728.

Aljadi, A.M. and M.Y. Kamaruddin, 2004. Evaluation of phenolic contents and antioxidant capacities of two malaysian floral honeys. Food Chem., 85: 513-518.

Al-Waili, N., 2003. Intravenous and intrapulmonary administration of honey solution to healthy sheep: Effect on blood sugar, renal and liver function tests, bone marrow function, lipid profile and carbon tetrachloride-induced liver injury. J. Med. Food, 6: 231-247.

Andiran, F., K. Kilinc, N. Renda, A. Ayhan and F.C. Tanyel, 2003. Lipid peroxidation and extracellular matrix in normal and cirrhotic rat livers following 70% heaptectomy. Eur. J. Gastroenterol. Hepatol., 50: 805-808.

Antony, S.M., J.R. Rieck and R.L. Dawson, 2000. Effect of dry honey on oxidation in turkey breast meat. Poult. Sci., 79: 1846-1850.

Arifah, A.K., K.S. Cheng, M.J. Azlina and A. Zuraini, 2004. Anti-inflammatory activity of *Nigella sativa* oil in rats. J. Vet. Malaysia, 16: 15-19.

Armitage, P., 1971. Statistical Method in Medical Research. Blackwell Scientific Publications, Oxford and Edinburgh, England.

Bansal, A.K., M. Bansal, G. Soni, D. Bhatnagar, 2005. N-nitrsodiethylamine induced oxidative stress in rat liver. Chem. Biol. Interact., 156: 101-111.

Basu, S., 2003. Carbon tetrachloride-induced lipid peroxidation: Eicosanoid formation and their regulation by antioxidants nutrients. Toxicology, 189: 113-127.

Cabre, M., J. Camps, J.L. Patenain, N. Ferre and J. Joven, 2000. Time-course of change in hepatic peroxidation and glutathione metabolism in rats with carbon tetrachloride-induced cirrhosis. Clin. Exp. Pharmacol. Physiol., 27: 694-699.

Chrungoo, V.J., S. Kuldip and J. Singh, 1996. Silymarin mediated differential modulation of toxicity induced by CCl_4, paracetamol and D-galactosamine in freshly isolated rat hepatocytes. Indian J. Exp. Biol., 35: 611-617.

Conner, E.M. and M.B. Grisham, 1995. Method of Enzymology. Methods, 7: 3.

Cremonese, R.V., A.A. Pereria Filho, R. Magalhaes, A.A. de Mottos, C.A. Marroni, C.G. Zettler and N.P. Marroni, 2001. Experimental cirrhosis induced by carbon tetrachloride inhalation: Adaptation of the technique and evaluation of lipid peroxidation. Arquivos de Gastroenterol Ogia, 38: 40-47.

Dhanabal, S.P., G. Syamala, K. Elango and B. Suresh, 2006. Protective effect of galactosamine-induced liver damage. Nat. Prod. Sci., 12: 8-13.

Drury, R.A.B. and E.A. Wallington, 1980. Carleton's Histological Technique. 4th Edn. Oxford, New York, Toranto, 19: 129-140.

El-Dakhakhny, M., N.I. Mady and M.A. Halim, 2002. *Nigella sativa* L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneimittel Forschung, 50: 832-836.

Fernandez-Checa, J.C. and N. Kaplowitz, 2005. Hepatic mitochondrial glutathione: Transport and role in disease and toxicity. Toxicol. Applied Pharm., 204: 263-273.

Germano, M.P., V. D'Angelo, R. Sanogo, A. Morabito, S. Pergolizzi and R. De-Pasqule, 2001. Hepatoprotective activity of *Trichilia roka* on carbon tetrachloride induced liver damage in rats. J. Pharmacol., 53: 1569-1574.

Halim, A.B., O. El-Ahmady, S. Hassab-Allab, F. Abdel Galil, Y. Hafez and A. Darwish, 1997. Biochemical effect of antioxidants on lipids and liver function in experimentally-induced liver damage. Ann. Clin. Biochem., 34: 656-663.

Ichi, I., K. Nakahara, K. Fujii, C. Iida, Y. Miyashita and S. Kojo, 2007. Increase of ceramide in the liver and plasma after carbon tetrachloride intoxication in the rat. J. Nut. Sci. Vitaminol., 53: 53- 56.

Jeong, D.H., G.P. Lee, W.I. Jeong, D.O. Sh, H.J. Yang, D.W. Yuan, H.I. Park, K.J. Kim and K.S. Jeong, 2005. Alterations of mast cells and TGF-beta1 on the silymrain treatment for CCl_4- induced hepatic fibrosis. World J. Gastroentrol., 11: 1141-1148.

Kanter, M., O. Coskum and A. Gurel, 2005. Effect of *Nigella sativa* on cadmium-induced oxidative stress in the blood of rats. Biol. Trace Elem. Res., 107: 277-287.

Lee, G.P., W.I. Jeong, D.H. Jeong, D.O. Sh, T.H. Kim and K.S. Jeong, 2005. Diagnostic evaluation of carbon tetrachloride-induced rat hepatic cirrhosis model. Anticancer Res., 25: 1029-1038.

Lee, H.S., K.Y. Keum and S.K. Ku, 2007. Effects of Picrorrhiza rhizome water extracts on the subacute liver damages induced by carbon tetrachloride. J. Med. Food, 10: 110-117.

Liu, J., L.Y. Chang, M.A. Feldan and H.M. Levine, 1991. Assay of aldehydes from lipid peroxidation: Gas chromatography-Mass spectrometry compared to thiobarbituric acid. Biochmecal, 245: 13-15.

Luo, Y, T.P. Yu, Z.H. Shi and L. Wang, 2004. Ginko biloba extract reverse CCl_4-induced liver fibrosis in rats. World J. Gastroenterol., 1: 1037-1042.

Mabrouk, G.M., S.S. Moselhy, S.F. Zohny, E.M. Ali, T.E. Helal, A.A. Amin and A.A. Khalifa, 2002. Inhibition of Methyl Nitrosourea (MNU)-induced oxidative stress and carcinogenesis by orally administered bee honey and *Nigella grainsin* in Sprangue Dawely rats. J. Exp. Clin. Cancer Res., 21: 341-346.

Mabrouk, G.M., S.F. Zohny, E.M.M. Ali, E.F. Ismail and S.S. Moselhy, 2004. Bee honey and *Nigella sativa* inhibit nitric oxide mediated cytochrome C release and down-regulation of connex in 43 induced by methyl nitrosourea in hepatic tissues of sprague dawely rats. Egypt J. Biochem., 22: 73-87.

Mahdy, K.A. and F.A. Morsy, 2001. Hepatoprotective effects of honey on experimental liver injuries induced by some food additives. J. Egypt. Soc. Toxicol., 25: 37-46.

Mahmood, S., M. Kowanaka, A. Kamei, A. Izumi, K. Nakata and G. Yamada, 2004. Immunohistochemial evaluation of oxidative stress markers in chronic hepatitis C. Antioxide Redox Signal, 6: 19-24.

Mahmoud, M.R., H.S. El-Abhar and S. Saleh, 2002. The effect of *Nigella sativa* oil against the liver damage induced by Schistosoma mansoni infection in mice. J. Ethnopharmacol., 79: 1-11.

Mato, I., J.F. Huidobro, J. Simal-Lozano and M.T. Sancho, 2003. Significance of nonaromatic organic acids in honey. J. Food Prot., 66: 2371-2376.

McCrudden, R. and J.P. Iredale, 2000. Liver fibrosis, the hepatic stellate cell and tissue inhibitors of metalloproteinases. Histopathology, 15: 1159-1168.

Meister, A., 1985. Methods for the selective modification of glutathione transport. Method Enzymol., 113: 571-581.

Mekala, P., N. Punniamurthy and P. Hariharan, 2006. Protective effect of curcumin and silymrain against aflatoxicosis in broiler chicken. Indian Vet. J., 83: 501-503.

Meki, A.R. and A. Hussein, 2001. Comparative melatonin reduces oxidative stress induced by ochratoxin A in rat liver and kidney. Toxicol. Pharmacol., 130: 305-313.

Meral, I., Z. Yener, T. Kahraman and N. Mert, 2001. Effect of *Nigella sativa* on glucose concentration, lipid peroxidation, antioxidant defense system and liver damage in experimentally-induced diabetic rabbits. J. Vet. Med., 48: 593-599.

Molan, P.C., 2002. Revival of honey as a medicine. Honeybee Sci., 23: 153-160.

Morsy, F.A., A.R.H. Farrag and S.L. EL-Sharkway, 2004. L-cornitine and melatonin reverse CCl_4-induced liver fibrosis in rats. Egypt. J. Hospit. Med., 17: 70-92.

Muriel, P., 1998. Nitric oxide protection of rat liver from lipid peroxidation, collagen accumulation and liver damage induced by carbon tetrachloride. Biochem. Pharmacol., 56: 773-779.

Muriel, P., M.G. Moreno, C. Hernandez, E. Chavez and K. Alcantar, 2005. Resolution of liver fibrosis in chronic CCl_4 administration in the rat after discontinuation of treatment: Effect of silymarin, silibinin, colchicine and trimethylcolchicinic acid. Basic Clin. Pharmacol. Toxicol., 96: 375-380.

National Research Council, 1995. Nutrient Requirement of Laboratory Animals, General Considerations for Feeding and Diet Formulation. National Academy Press. Washington, DC. USA., pp: 3-50.

Nelson, R.S. and M.R. Eichinger, 2001. Role of nitric oxide in pulmonary dysfunction associated with experimental cirrhosis. Respir. Physiol., 126: 65-74.

Ortiz, M.C., L.A. Fortepiani, C. Martinez-Salgado, N. Eleno, N.M. Atucha, J.M. Lopez-Novoa and J. Garcia-Estan, 2001. Renal effects of the chronic inhibition of nitric oxide synthesis in cirrhotic rats with ascites. Nefrologia, 21: 556-564.

Parks, W.C. and R.P. Mecham, 1999. Matrix metalloproteinase. Hepatology, 15: 331-344.

Ramadan, M.F., L.W. Krok and J.T. Morsel, 2003. Radical scavenger activity of black cumin crude seed oil and oil fraction. J. Agric. Food Chem., 51: 6961-6969.

Reeves, B.G., F.H. Nielson and G.C. Fahmy, 1993. Reported of the American Institute of nutrition. Adhoc-Wrilling Committee on the reformulation of the AIN 1979. Ardent Diet. J. Nutr., 123: 1939-1951.

Reitman, S. and S. Frankel, 1957. Enzymatic assessment of liver function tests. Am. J. Clin. Pathal., 28: 56-63.

Resende, R.J., M.E. Beletti, E.R. Machado, N.M. Fonseca, A.V. Mundim, L.C. Melo, C.A. Lima and P. De, 2003. Effect of honey and amino acids containing either levulose or liver proteolysates against carbon tetrachloride-induced acute hepatotoxicity in mice. Arch. Velerinaria, 19: 104-109.

Salem, M.L. and M.S. Hossain, 2000. Protective effect of black seed oil from Nigella sativa against murine cytomegalovirus infection. Int. J. Immunopharmacol., 22: 729-740.

Sanford, H.S., 1954. Method for obtaining venous blood from the orbital sinus of the rat or mouse. Science, 119: 100.

Shalan, M.G., M.S. Mostafa, M.M. Hasuouna, S.E. Hassab El-Nabi and A. El-Refaie, 2005. Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. Toxicology, 206: 1-15.

Shenoy, K.A., S.N. Somaayaji and K.L. Bairy, 2001. Hepatoprotective effects of Ginko bilaba against CCl_4-induced hepatic injury in rats. Indian J. Pharmacol., 33: 260-266.

Socha, P., J. Rujner and J. Socha, 1992. The role of oxygen radicals and their antioxidants in pathogenesis of chronic hepatitis. Ped. Pol., 14: 139-144.

Svegliati-Baroni, G., S. Saccomanno, H. Van Goor, P. Jansen, A. Benedette and H. Moshage, 2001. Involvement of reactive oxygen species and nitric oxide radicals in activation and proliferation of rat hepatic stellate cells. Liver, 21: 1-12.

Turkdogan, M.K., Z. Agaoglu, Z. Yener, R. Sekeroglu, H.A. Akkan and M.F. Avei, 2001. The role of antioxidant vitamins (C and E), selenium and *Nigella sativa* in the prevention of liver fibrosis and cirrhosis: Dtsch. Tierarztl Wochenschr, 108: 71-73.

Turkdogan, M.K., H. Ozbek, Z. Yener, I. Tuncer, I. Uygan and E. Ceylan, 2003. The role of *Urtica dioica* and *Nigella sativa* in the prevention of carbon tetrachloride-induced hepatotoxicity in rats. Phytother. Res., 17: 942-946.

Vaillant, B., M.G. Chiaramonte, A.W. Cheever, P.D. Soloway and T.A. Wynn, 2001. Regulation of Hepatic fibrosis and extracellular matrix genes by the Th1 response: New insight into the role of tissue inhibitor of matrix metalloproteinases. J. Immunol., 16: 7017-7026.

Vrba, J. and M. Modriansky, 2002. Oxidative burst of Kupffer cells: Target for liver injury treatment. Biomed. Pap., 146: 15-20.

Yang, C., M. Zeisberg and B. Mosterman, 2003. Liver fibrosis: Insights into migration of hepatic stellate cells in response to extracellular matrix and growth factors. Gastroenterology, 124: 147-159.