

Antioxidant, hepatoprotective and immuno-stimulant effects of nutraceutical compounds from carotenoid origin in rat treated with carbon tetrachloride

Enayat A. M. Omara^{1,3}, Somaia A. Nada², Hanan G. Zahran³

¹Pathology Dept., ²Pharmacology Dept., ³Clinical Chemistry Dept.,
National Research Centre, Dokki, Cairo, Egypt

Abstract

Aim of the work: the present study was conducted to evaluate antioxidant, hepatoprotective, and immuno-stimulant properties of carotenes derived from food byproducts (tomato peels (TPW), mango peels (MP), corn gluten (CG), and agriculture dill waste (DW)), they were selected for their high superoxide dismutase activity (SOD).

Material and methods: antioxidant and hepatoprotective effects were investigated in carbon tetrachloride (CCl₄) damaged liver. Rats treated with oral doses of each carotene (25 mg /Kg b.wt.) for 15 days prior CCl₄ administration and 4 days post- CCl₄-treatment.

Results: all tested carotenes significantly reduced the elevated values of liver function tests (GGT, ALT & AST) in hepatic damaged groups as well as, they had an immuno-stimulate property. They increased IgG levels in normal and liver damaged rats treated with the tested carotenoids. Whereas, IgG level reduced significantly by CCl₄-treatment. Histopathological examination of the liver tissues exposed to CCl₄ showed inflammatory cell infiltration, necrosis and fibrosis. Glycogen and total protein contents also recorded. Treatments with carotenoids led to an improvement in the histological and histochemical alterations induced by CCl₄.

Conclusion, carotenes may play an important role as nutraceutical preparation, specially, when obtained from wastes of food byproducts in which economically of low cost production.

Keywords: Antioxidant, hepatoprotective, histopathology, nutraceutical, carotenoids.

Introduction

Antioxidants are used to preserve foods by retarding discoloration, rancidity or deterioration (Yen and Chuang, 2000). However, currently used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been suspected to cause or promote toxic and carcinogenic effects (Koleva *et al.*, 2002 and Tepe *et al.*, 2005). Therefore, the interest for cheap, renewable and abundant sources of natural antioxidants has grown due to safety concerns; especially the toxicological data about synthetic antioxidants were deteriorating the health effect (Garrote *et al.*, 2004). Antioxidant action plays an important role in protection against CCl₄-induced liver injury. Protective effects of various natural products in CCl₄ hepatotoxicity have been reported (Jeong and Yun 1995 and Hsiao *et al.*, 2003). The administration of antioxidants such as Vitamin E, selenium, Vitamin C, carotenoids and others may protect against xenobiotic-

induced damage (Antunes *et al.*, 2000; Atessahin *et al.*, 2005 and El-Demerdash *et al.*, 2004). The dietary necessity of the carotenoid beta-carotene, the precursor of vitamin A, has been recognized for many decades (Levy, 2004). Lycopene may have various benefits for human health. As a major carotenoid in human blood, lycopene protects against oxidative damage to lipids, proteins, DNA and specific inhibitor of cancer cell proliferation and had a potent quencher of singlet oxygen (a reactive form of oxygen), which suggests that it may have comparatively stronger antioxidant properties than the other major plasma carotenoids (DiMascio *et al.*, 1989 and Levy *et al.*, 1995). Lutein is one of the most prominent carotenoids in human serum and in foods and has been used for pigmentation of animal tissues, for coloration of foods, drugs and cosmetics (White *et al.*, 1988). Lutein and zeaxanthin are found in the eye and have been

associated with reduced risk of cataract development and age-related macular degeneration (Nahum *et al.*, 2001 and Seddon *et al.*, 1994). β -carotene has been associated with enhanced immune response by increasing the percentage of leukocytes found in the peripheral blood and blocking suppression of lymphocytes and helper T lymphocytes caused by UV exposure (Bendich and Shapiro, 1986). Numerous studies have

demonstrated that carotenoids, such as lutein and β -carotene possess antioxidant activity and thus may enhance LDL degradation and prevent cardiovascular disease (Rao *et al.*, 1998). Various carotenoids such as lycopene have been reported to exhibit the highest antioxidant activity, followed by β -cryptoxanthin, β -carotene, lutein and zeaxanthin (Miller *et al.*, 1996). Carbon tetrachloride (CCl₄) has been used in animal model to induce liver damage similar to that of acute viral hepatitis in human patients (Kumar *et al.*, 2009).

Material and Methods

Chemicals

Carbon tetra chloride (CCl₄) (ADWEIC) *Diagnostic kits*: commercial diagnostic kits from Biomerieux, Laboratory reagents and products, Maary L'Etoile, France were used for the determination of serum gamma glutamyl transferase (GGT), Super oxide desmutase (SOD) and activities of transaminases (ALT & AST), Cholesterol and Triglycerides .

Animals

The Sprague Dawley rats approved by the committee of ethics and bio-security at NRC, Cairo, Egypt. Male rats weighing 130-150 gm were used and purchased from the animal house colony. Animals were divided into equal groups (12 rats each) housed under standard environmental conditions (23 \pm 1°C, 55 \pm 5% humidity and a 12-h light: 12-h dark cycle) and fed on a standard laboratory diet *ad libitum* with free access water.

Experimental design:

Toxicity study: rats were used to evaluate the toxic effects of TPW, MP, CG and DW

after oral administration of different doses up to 5 g / Kg b.wt. Animals were observed for any toxic symptoms and mortality 24- 72 hr post-treatment.

Hepatoprotective study:

Ten groups of rats (12 rats each) were submitted to the following treatments : groups 1, 2,3 and 4 were treated daily with the TPW, MP CG and DW for 15 days (25 mg / Kg b.w. as a suspension in 5 % w/w gum accacia). While, the control group was administered the vehicle (10 ml/ Kg 5 % w/w gum accacia). On the 16th day of treatment, half numbers from all treated groups (6 rats/ group) were given 50% CCl₄ v/v in liquid paraffin (1.5 ml/Kg b.w., orally) to induce hepatic injury according to the method of Yadav and Dixit (2003) and continuing the treatments with different tested carotenoids for another 4 days.

At the end of experimental time, blood samples were collected from retro-orbital venus plexus from all animals in plain test tubes. Serum was prepared for biochemical analysis of superoxide dismutase (SOD) spectrophotometrically determined according to the procedure of Suttle (1986), γ -glutamyl transferase (GGT) (Rosalki *et al.*, 1970), aspartate and alanine aminotransferase (AST and ALT) activities according to the method of Reitman and Frankel (1957) and IgG determined according to the method's of Kricka (1999).

Histopathological and histochemical studies:

Rat livers were removed from all groups and immediately fixed in 10% neutral buffered formalin, washed in water, dehydrated in gradual series of ethanol (50-100%), cleared in xylene and embedded in paraffin. 4-5 μ m thick sections were prepared and stained with hematoxylin and eosin (H&E) for photomicroscopic observation (Drury and Wallington, 1980). Glycogen was demonstrated using periodic acid - Schiff's

technique PAS (Hockiss 1948) and mercuric-bromophenol blue stain for total proteins content (Mazia *et al.*, 1953).

IgG was determined according to the method's of Kricka (1999).

Statistical analysis: The obtained results were analyzed by ANOVA (one or two-way) using Excel 2003 Microsoft Corp (11.5612.5606), Redmond, WA software package.

Results

Hepatoprotective activity

In the toxicity study no toxic effect was noticed after administration of the four tested materials (up to 5 g / Kg b. wt., orally) in rats. The activity of serum super oxide dismutase (SOD) was increased significantly in normal groups treated with TPW, MP, DW and CG comparing with the control values (Table 1). SOD activity significantly was inhibited by CCl₄ administration. In contrast, the increase in SOD value was normalized in CCl₄- hepatic-damaged rats treated with TPW, MP and CG. While, hepatic damaged rats when treated with DW showed a significant elevation than CCl₄- alone and it still lower than normal control. Data in table (1) indicated that GGT values in groups treated with TPW, CG and DW were not differed than control, only MP -treatment caused a significant reduction in GGT values when compared with the control. CCl₄- treatment alone caused a significant elevation in ALT, AST and GGT activities as well as there was severe decrease in SOD values when compared with control values. The elevated ALT values decreased significantly in hepatic-damaged groups which treated with TPW and MP, while, ALT activity exhibited nonsignificant decrease in hepatic damaged group and treated with CG and DW when compared with CCl₄ treated group. All studied carotenoid extracts (TPW, MP, CG and DW) showed a significant inhibitory effect on AST activity in normal rats before CCl₄ administration.

Moreover, AST activity significantly decreased and returned back to the normal values by the administration of MP, CG and D to hepatic-damaged rats. TPW-treatment caused a significant reduction in AST serum level than the control group. All treatments non-significantly differ from each other in AST values pre- or post- CCl₄ treatment. Generally, IgG in serum level was mostly higher in all treatments than the normal control and its level was lower by the treatment with CCl₄ alone.

Histopathological changes in the liver tissues were in good correlation with the biochemical parameters. The liver histology of control rats showed central vein with radiating cords of liver cells. The liver cells had vesicular nuclei and granular cytoplasm. Blood sinusoids were evident between the cords of liver cells (Fig.1a).

Examination of sections of groups treated with TPW, MP, CG and DW revealed that the histological pictures were apparently similar to that of the control group. Group 5 administrated CCl₄ revealed disruption in hepatocytes, necrosis, widespread of fatty degeneration (Fig.1b). Dilated and congested portal vein with lymphocytic infiltration were observed around the portal tract (Fig. 1c). Hydropic degeneration and pyknotic nuclei in hepatocytes were also noticed in CCl₄-treated group (Fig. 1d). Liver of rats treated with CCl₄ and TPW (25 mg/kg. b.wt.) showed good recovery from CCl₄ - induced liver injury. This was evident from the well defined hepatic cords and polyhedral hepatocytes with round nuclei observed in these liver sections (Fig. 2a). The carotenoids of MP-treated group (25 mg/kg. b.wt.) were more effective in controlling the toxic effect of CCl₄. The necrotic areas were absent with less inflammatory cells. Hepatic architecture was nearly intact (Fig. 2b). The liver of rats treated with the CG1 (25 mg/kg.b.wt.)

showed definite signs of protection against CCl₄ injury, but the recovery was less than that observed with MP (Fig. 2c). The liver of rats treated with DW (25 mg/kg b.wt.) exhibited a noticeable difference in hepatic architecture (Fig.2d) in comparison to CCl₄.The normal architecture was well preserved. Only mild infiltrations and dilated blood sinusoids were seen. Binucleated cells were also seen in all rats treated with carotenoids (TPW, MP, GG and DW).

Figure 3 showed the distribution of glycogen in liver tissue stained by PAS reaction, characterized in normal rats by deeply stained reddish granules and flakes in cytoplasm of hepatocytes (Fig. 3a). The glycogen content was reduced in CCl₄ - treated control rats (Fig. 3b). Hepatocytes of carotenoids treated groups showed marked increase in glycogen content with variable compound with those treated with CCl₄ -treated group. (Figs. 3c, d, e and f).

In addition, bromophenol blue staining was used to demonstrate total proteins content in liver sections. Examination of liver sections in the control group showed a strong bromophenol blue reaction in the cytoplasm and in the nuclear membrane of the hepatocytes of the control rats (Fig. 4a). CCl₄-treated rats showed marked decrease in protein content in the necrotic periportal zones and the damaged hepatocytes (Fig. 4b). Obvious improvement was detected in protein content in hepatocytes of groups treated with carotenoids prior CCl₄-administration (Fig. 4c, d, e and f).

Discussion

In the present study, carotenoids extracted from wastes of tomato, mango, corn gluten and dill were very safe, and showed no death among the different treatments, whereas all animals looked healthy and no specific symptoms appeared over the observation period (72 hr) post oral administration up to 5 g/ Kg b.wt. Effects of different treatments on SOD activity were discussed in table (1). Treatment with CCl₄

significantly inhibited SOD activity. The decrease in the activity of SOD in serum of rats treated with CCl₄ may be due to the increased lipid peroxidation or inactivation of enzyme by cross linking with malondialdehyde. This increased accumulation of free radicals, which could further stimulate lipid peroxidation.

It has been hypothesized that one of the principal causes of CCl₄-induced liver injury is formation of lipid peroxides by free radical derivatives of CCl₄ (CCl₃[•]). Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl₄-induced hepatopathy (**Morrow et al., 1992**).

Carotenoids administration restored SOD activity and liver enzymes (GGT, ALT and AST) nearly to the normal values comparing with the corresponding CCl₄-administrated group. However, these groups of carotenoids act as immune stimulant agents. The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, catalase and GPX. These enzymes constitute a mutually supportive team of defense against reactive oxygen species (**Venukumar and Latha, 2002**).

The reduced activities of SOD and elevation of enzyme markers (GGT, ALT and AST) indicating the hepatic damage in the rats administered CCl₄ (**Altug et al., 2007**). They added that, after the administration of lycopene lipid peroxidation level has decreased. The strong antioxidant effect is because of tomato's content of lycopene being responsible for the increase of GSH levels and increases SOD activity As well as, **Kim et al., (2004)** who observed that tomato extract partially inhibits the activity of AST and sorbitol dehydrogenase in hepatic CCl₄ - damaged liver in rats.

Histopathological changes observed in liver of rats administrated-CCl₄ revealed that hepatocytes were disrupted, vacuolated and lost their polyhedral shape. Vacuolization were severe especially in the centrilobular region which showed widespread of necrosis and fatty degeneration. Previous experimental studies have shown that CCl₄ administration caused increase serum levels

of AST, ALT and ALP (**Teocharis *et al.*, 2001**). The hepatotoxicity induced by CCl₄ was confirmed in our study by significant elevation in serum AST, ALT and GGT levels. It has been reported that CCl₄ caused necrosis (**Naziroglu *et al.*, 1999** and **Ashok-Sheno *et al.*, 2002**), fibrosis (**Natsume *et al.*, 1999**) and foamy degeneration of hepatocytes (**Teocharis *et al.*, 2001**) in liver. Therefore, our histopathological findings in the liver due to CCl₄ administration are in agreement with the previous studies.

The histochemical investigations of the present study revealed that CCl₄ induced highly decreased glycogen and protein contents, this is in agreement with the results of **Lockard *et al.*, (1983)** who reported that rats treated with CCl₄ showed sever decrease in the percentage of glycogen within the hepatocytes. CCl₄ was found to reduce the quantity of liver glycogen (**Bernacchi *et al.*, 1988**) and the quantity of blood glucose (**Dubale and Bais 1982**). **Hickenbottom and Hornbrook (1971)** reported that depletion of hepatic glycogen, in response to CCl₄ treatment, has been linked to changes in activities of glycogen transferase and glycogen phosphorylase. Also the decrease in protein content may be due to the oxidative damage occurred to the cellular proteins subsequently caused alteration in cellular function (**Timbrell and Waterfield, 1996**; **Sundari and Ramakrishna 1997** and **Ohta *et al.*, 2000**).

In the present work the administration of tomato, mango, corn gluten and dill after exposure to CCl₄ the fibrosis, necrosis and inflammatory cell infiltration were less. β -carotene showed protective activity against CCl₄-induced hepatotoxicity in rats (**Khorshid *et al.*, 2008**), hepatic inflammation, fibrosis, and attenuating cirrhosis in rats (**Seifert *et al.*, 1995**). Consistent with these reports, we found that fibrosis, necrosis and inflammatory cell infiltration in the liver central areas were less in carotenoids -treated animals. Most of these beneficial effects are supposed that β -carotene acts as antioxidant, antifibrotic and anti-inflammatory agent. The protective mechanism of β -carotene may also involve enhanced immunity and down regulation of key cytokines (**He *et al.*, 2004**).

Lycopene had a potent quencher of singlet oxygen (reactive form of oxygen), which

suggests that it may have comparatively stronger antioxidant properties than other major plasma carotenoids (**Di-Mascio *et al.*, 1989**; **Levy *et al.*, 1995**; and **Nahum *et al.*, 2001**).

Kim (1995) suggested that β -carotene, lycopene and lutein have protective effects on oxidant-induced liver injury, improved the cell viability of hepatocytes, increased catalase activities and glutathione levels in hepatocytes from chronically ethanol-fed rats (**Suh-Ching *et al.*, 2004**). Moreover, β -carotene suppressed lipid peroxidation in mouse and rat tissues induced by CCl₄ -injection and ultraviolet exposure (**Lomnitski *et al.*, 1997**).

Carotenoids exert their mode of action as antioxidants due to one of the following hypothesis: (1) radical addition; (2) electron transfer; or (3) allylic hydrogen abstraction. It has been proposed that a lipid peroxy radical (ROO[•]) might add at any place across polyene chain of carotenoids, resulting in the formation of a resonance-stabilized carbon-centered radicals (ROO-CAR). Since this radical should be quite stable, it would interfere with the propagating step in lipid peroxidation and would explain the antioxidant effect of carotenoids (**Liebler and McClure 1996** and **Krinsky and Johnson 2005**).

Conclusions, the tested carotenoids extract appear to have definite protective effect by way of preventing deleterious effects of CCl₄ in liver.

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Table(1): Hepatoprotective and Immuno-stimulant effects of carotenoid extracted from TP, MP, CG and D wastes industrial food products (25 mg / Kg b. wt., orally) for 15 days on serum superoxide dismutase (SOD), gamma glutamyle transferase (GGT), amino-transferases (ALT and AST) as indication of liver function , then followed by CCl₄ (1.5 ml /Kg b. wt. of 50 % concentration) (Means ± SE, n = 6 rats / group).

Parameters Groups	Normal groups					CCl ₄ - hepatic damaged rats					LSD
	Control	TPW	MP	CG	D	CCl ₄	TPW1	MP1	CG1	D1	
SOD U/ml	A 304.80 ± 6.08	B 511.14 ± 3.33	C 447.31 ± 11.34	D 346.93 ± 5.36	C 430.75 ± 3.31	E 129.62 ± 2.10	A 327.55 ± 2.30	A 297.77 ± 3.50	F 293.61 ± 12.67	G 261.38 ± 2.78	18.1
GGT IU/ml	A 1.47 ± 0.18	AB 1.10 ± 0.11	B 1.02 ± 0.06	AB 1.45 ± 0.15	AB 1.09 ± 0.09	C 6.16 ± 0.27	D 2.26 ± 0.18	E 2.99 ± 0.13	E 3.24 ± 0.10	E 2.92 ± 0.17	0.44
ALT IU/ml	AC 32.50 ± 1.01	A 28.60 ± 2.00	A 30.65 ± 0.91	A 30.88 ± 3.26	A 31.20 ± 1.61	B 44.84 ± 3.20	A 29.90 ± 2.20	AC 34.49 ± 2.27	BC 38.96 ± 2.96	BD 41.74 ± 2.01	6.48
AST IU/ml	A 85.11 ± 2.73	B 71.81 ± 1.60	B 73.72 ± 1.55	B 74.25 ± 1.42	B 74.69 ± 1.67	C 100.93 ± 2.53	D 79.88 ± 1.54	AD 81.45 ± 1.39	AD 82.17 ± 1.58	AD 84.93 ± 1.52	5.14
IgG (ng/ ml)	A 1005.94 ± 2.50	B 1202.93 ± 5.40	C 1159.00 ± 10.68	D 1059.41 ± 3.91	D 1052.48 ± 13.55	E 954.92 ± 17.33	C 1158.73 ± 4.92	F 1113.84 ± 2.97	F 1119.42 ± 2.29	A 1028.75 ± 2.32	23.63

One –way ANOVA

In each column different capital letters are significantly different at P<0.05.

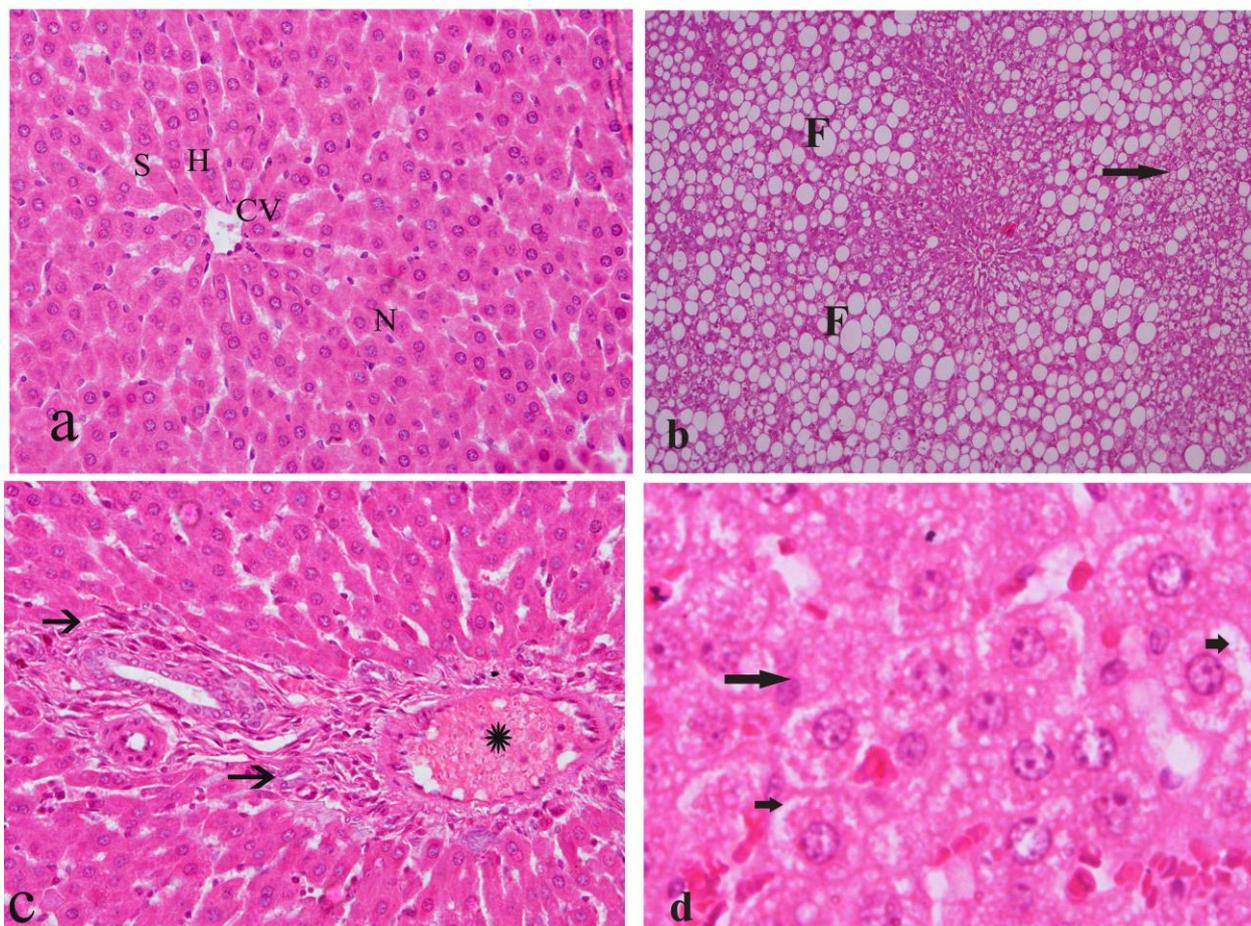


Fig 1: (a)-Photomicrograph of section of liver from control rats showing normal structure of liver, central vein (CV) and hepatic cords of hepatocytes (H) with prominent nucleus (N) separated with blood sinusoids (S). (H & E X 400).

(b) Section of liver of rats treated with CCL4 showing abnormal architecture of liver tissue and remarkable fatty degeneration (F) with necrotic cells (➡). (H & E X 200).

(c) Section of liver of rats treated with CCL4 showing dilated and congested portal vein (*) accompanied with lymphocytic infiltration. (➡) (H & E X 400).

(d) Section of liver of rats treated with CCL4 showing hydropic degeneration (➡) and pyknotic cells (➡). (H & E X 1000).

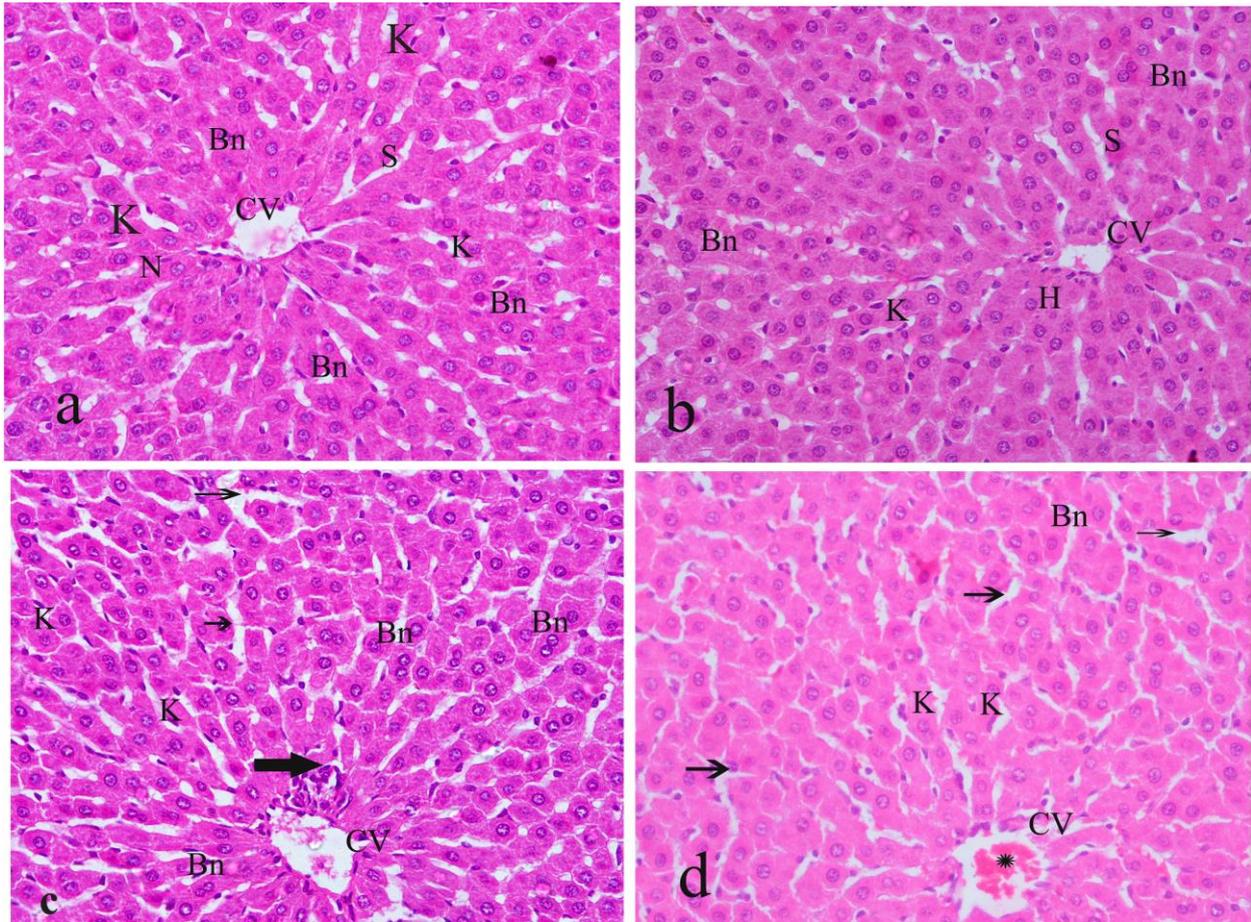
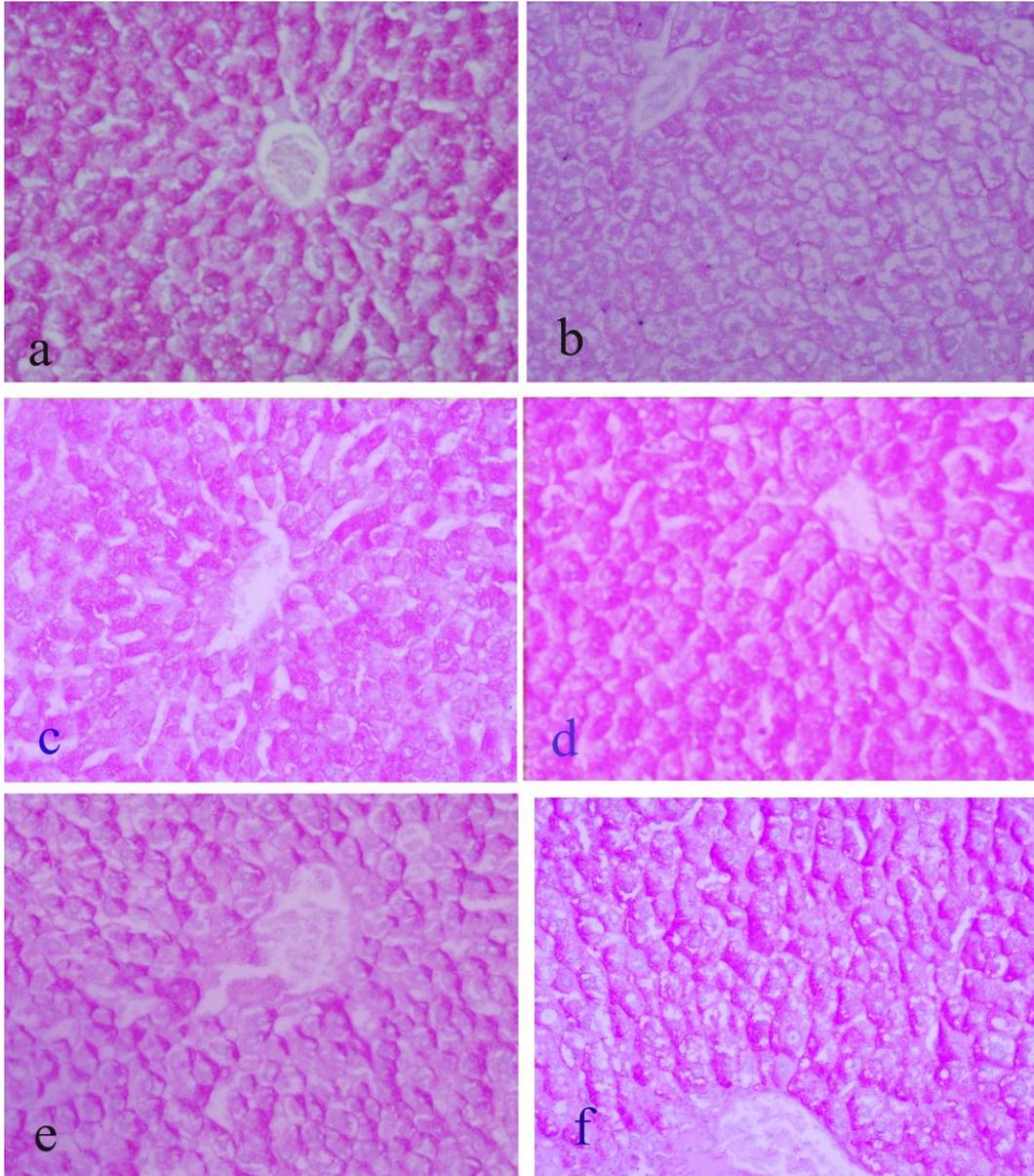


Fig 2: Effect of cartenoids (TPW, MP, CG1 and DW) on hepatic histological damage induced by CCL4.

- (a) Section of liver of rats treated with TPW & CCL4 showing normal architecture of hepatocytes, central vein (CV), nucleus, (N), binucleated cells (Bn), blood sinusoid (S), and Kupffer cells (K). (H & E X 400).
- (b) Section of liver of rats treated with MP & CCL4 showing the liver tissue is restoring its normal architecture to great extent, central vein (CV), hepatocytes (H), binucleated cells (Bn), blood sinusoid (S) and Kupffer cells (K). . (H & E X 400)
- (c) Section of liver of rats treated with CG1 & CCL4 showing an almost normal histological architecture of the liver with central vein (CV), binucleated cells (Bn), and Kupffer cells (K), few inflammatory cells (➡) and dilated blood sinusoids (➡). (H & E X 400)
- (d) Section of liver of rats treated with DW & CCL4 showing an almost normal histological architecture of the liver with central vein (CV), binucleated cells (Bn), Kupffer cells (K), dilated blood sinusoids (➡). (H & E X 400) .

Antioxidant , hepatoprotective and immuno-stimulant effects....



- Fig 3:** (a) - A photomicrograph of control liver of control rat shows the distribution of glycogen in hepatocytes with intense red color.
- (b) A photomicrograph of section of liver of rat treated with CCL4 showing highly decreased in glycogen content in hepatocytes. (PAS X 400).
- (c) - A photomicrograph of section of liver of rat treated with TPW & CCL4 given showing normal distribution in glycogen content in hepatocytes. (PAS X 400).
- (d) - A photomicrograph of section of liver of rat treated with MP & CCL4 given showing normal distribution in glycogen content in hepatocytes. (PAS X 400).
- (e) - A photomicrograph of section of liver of rat treated with CG1 & CCL4 given showing normal distribution in glycogen content in hepatocytes. (PAS X 400).
- (f) - A photomicrograph of section of liver of rat treated with DW & CCL4 showing normal distribution in glycogen content in hepatocytes. (PAS X 400) .

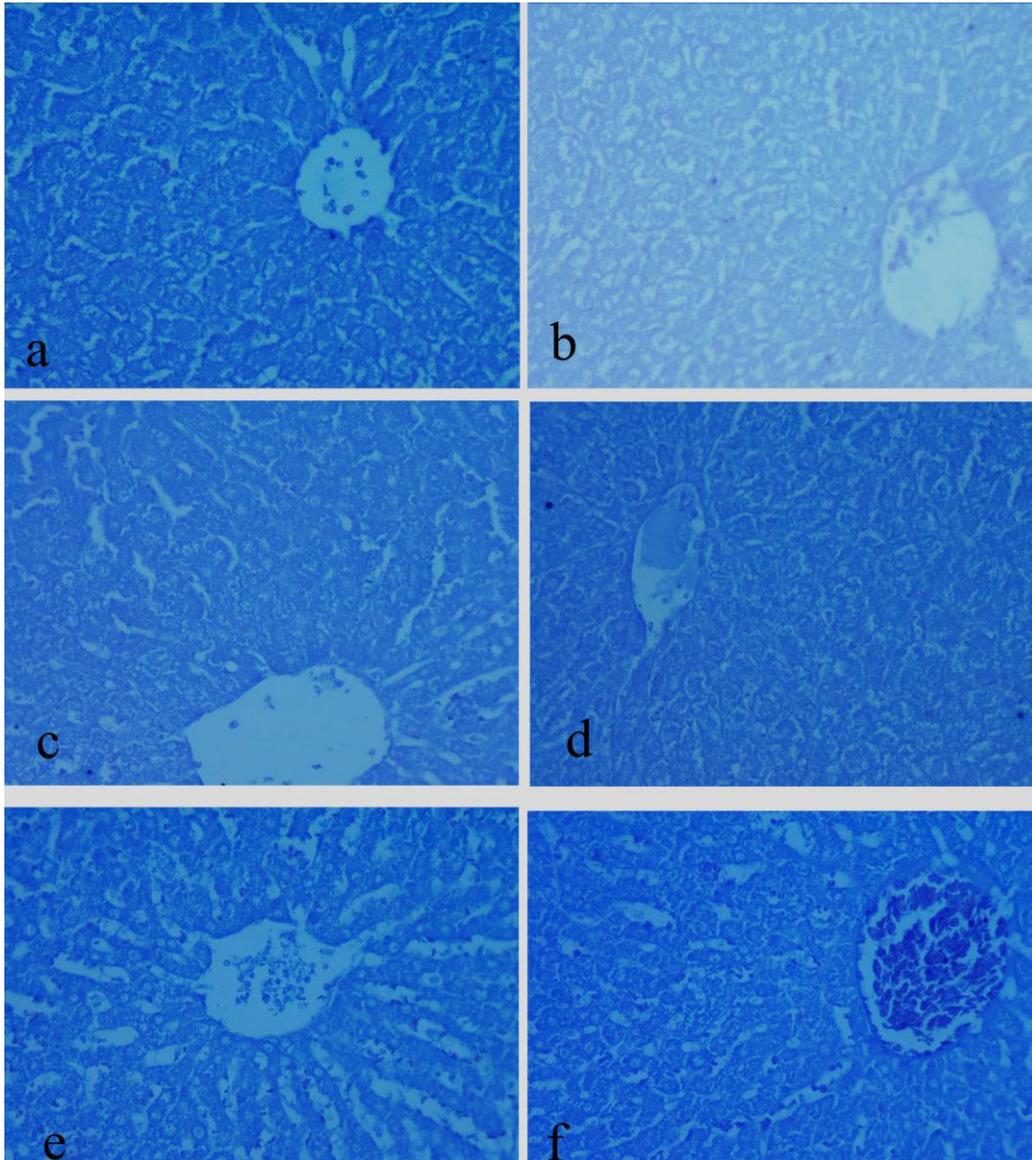


Fig 4: (a) A photomicrograph of control liver showing normal distribution of total proteins in hepatocytes. (Bromophenol blue X 400)
(b) A photomicrograph of section of liver of rat treated with CCL4 showing highly decreased in total proteins in hepatocytes. (Bromophenol blue X 400).
(c) A photomicrograph of section of liver of rat treated with TPW & CCL4 showing normal distribution of total proteins in hepatocytes. (Bromophenol blue X 400)
(d) A photomicrograph of section of liver of rat treated MP & CCL4 showing normal distribution of total proteins content in hepatocytes. (Bromophenol blue X 400)
(e) A photomicrograph of section of liver of rat treated with CG1 & CCL4 showing normal distribution of total proteins in hepatocytes. (Bromophenol blue X 400)
(f) A photomicrograph of section of liver of rat treated with DW & CCL4 showing normal distribution of total proteins in hepatocytes. (Bromophenol blue X 400)

استخدام الكاروتينيدات الطبيعية المستخلصة من بعض مخلفات التصنيع الغذائي كمضادات للأكسدة و حماية لخلايا الكبد فى الجرذان البيضاء المعاملة برابع كلوريد الكربون

عنايات عبد العزيز محمد عمارة , سمية احمد ندا , حنان جمال زهران
قسم الباثولوجى , قسم الفارماكولوجى , قسم الكيمياء العلاجية
المركز القومى للبحوث – الدقى - الجيزة

تهدف هذه الدراسة إلى تقييم تأثير الكاروتينيدات كمضادات للأكسدة على كبد الجرذان المصابة بتلف نتيجة للتعرض لرابع كلوريد الكربون ودراسة ذلك من الناحية الهستولوجية و البيوكيميائية وكذلك تأثيرها على المناعة. وقد استخدم فى هذا البحث ذكور الجرذان البيضاء و قسمت كالاتى: المجموعة الاولى و هى المجموعة الضابطة و قد تم معالجتها بزيت البرافين مرتين فى الأسبوع لمدة خمسة عشر يوما. المجموعات الثانية والثالثة والرابعة و الخامسة تم إعطاء 12 جرذا لكل مجموعة جرعة مقدارها 25 ملجم / كجم من مخلفات الطماطم أو مخلفات المانجو أو جلوتين الذرة أو مخلفات الشبت على التوالي. بعد 15 يوما ، تم حقن نصف المجموعات السابق ذكرها (6 جرذان / مجموعة) برابع كلوريد الكربون فى الغشاء البريتونى بجرعة مقدارها 1,5 ملجم / كجم من وزن الجسم (تركيزها 50 % مذاب فى زيت البرافين) . استكمل العلاج بهذه المستخلصات لمدة أربعة أيام أخرى من الحقن برابع كلوريد الكربون، بعد ذلك تم ذبح الجرذان وتم تجميع عينات الدم لعمل التحاليل البيوكيميائية (ALT, AST SOD and GGT,) ، ثم أعدت قطاعات شمعية وتم صباغتها بالهيماتوكسولين و الايوسين للفحص الهستولوجى وتم عمل صبغات للفحص الهستوكيميائى و هى طريقة البير ايوديك – شيف الحامضى لعديدات التسكر و طريقة البروموفينول الأزرق لتبيان كمية البروتين.

وقد أدت معالجة الجرذان برابع كلوريد الكربون إلى إرتفاع ملحوظ فى إنزيمات الكبد وكذلك حدوث بعض التغيرات الهستولوجية فى الكبد مثل تكاثر الخلايا الدهنية مما يؤدي الى فقدان الشكل المميز لخلايا الكبد وكذلك أثبت الفحص الهستوكيميائى انخفاض ملحوظ فى كمية عديدات التسكر وفى كمية البروتين.

كذلك تم تقييم الكاروتينيدات المستخلصة بيولوجيا على جرذان التجارب البيضاء كمضادات للأكسدة, وكان التأثير المضاد للأكسدة أكثر وضوحاً فى الكاروتينيدات المستخلصة من مخلفات الطماطم يليها جلوتين الذرة و مخلفات المانجو ثم مخلفات الشبت. وكذلك قامت بتنظيم انزيمات الكبد فى الجرذان الى الحد الطبيعى بعد المعاملة بمادة رابع كلوريد الكربون (هذه المادة تتلف خلايا الكبد فى الجرذان الى حد يشبه فيروس سى فى كبد الإنسان). أيضا أحدثت زيادة معنوية واضحة فى جلوبيولينات المناعة عن المجموعة الضابطة و المجموعة التى بها تلف فى الكبد و لم تأخذ علاج. كما أثبتت الدراسة المجهرية لخلايا الكبد قلة البؤر الميتة و ظهرت بعض الخلايا الملتهبة بدرجة بسيطة جدا. أظهرت المعاملة بالكاروتينيدات المستخلصة من جلوتين الذرة و مخلفات الشبت حماية و اضة لخلايا الكبد و لكنها أقل من الكاروتينيدات المستخلصة من مخلفات الطماطم و قشور المانجو. و كذلك حدث ارتفاع فى كمية عديدات التسكر و فى كمية البروتين. أظهرت النتائج أن الكاروتينيدات المستخدمة لها تأثير ايجابى فى حماية خلايا الكبد من الآثار السامة التى يحدثها رابع كلوريد الكربون حيث أدى لإنخفاض ملحوظ فى مستوى إنزيمات الكبد بالدم و تحسن هستولوجى ملحوظ . نستنتج من هذه الدراسة أنه يمكن الاستفادة من هذه المخلفات فى تصنيع أدوية أو استخدامها كمكملات غذائية لرفع كفاءة وظائف الكبد والكلى فى الحالات المرضية كما يمكن الاستفادة منها لعلاج المرضى الذين يعانون من أمراض الكلى أو الالتهاب الكبدى المزمن مثل ذلك الذى ينتج عن الإصابة بفيروس سى.