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Research Article

Carbon tetrachloride-induced liver disease in rats: the potential effect of supplement oils with vitamins E and C on the nutritional status

Leberschädigung durch Tetrachlorkohlenstoff bei Ratten: der Einfluss von Nahrungsöl sowie Vitamin E und C auf den Ernährungsstatus

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Abstract

The aim of the present investigation was to study the effects of olive oil (OO), corn oil (CO), and flaxseed oil (FO), with or without supplementation of vitamins E and C, on food intake, body weight gain %, liver weight to body weight %, total lipids, liver functions, and liver histology in male rats intoxicated with carbon tetrachloride (CCl_4).

Forty-two rats were divided into two main groups. The first main group was fed on basal diet (BD) as a negative control group (NC). The second main group received subcutaneous injections of CCl_4 in paraffin oil (50% v/v 2ml/kg) twice a week to induce chronic damage in the liver. The group was then divided into six subgroups, three of which were fed on 4% unsupplemented oils (CO, FO, and OO) as positive control for the three oils used. The rest of the groups were fed on 4% of the same oils supplemented with vitamins E and C.

The results of the flaxseed oil rat group indicate that supplementing vitamin E and C led to a significant reduction in the mean values of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and liver alanine amino transferase enzyme (ALT). Moreover, it caused an increase of the mean value of high-density lipoprotein cholesterol (HDL-C) as compared to the negative control group (NC). The olive oil group supplemented with the same vitamins showed a significant decrease in the mean value of serum TC and significant ($P<0.05$) increase in the mean value of serum HDL-C as compared to NC. The results of the corn oil group supplemented with vitamins showed a significant increase in the mean value of serum HDL-C as compared to the negative control group. The histology results confirmed that the group hepatically injured with CCl_4 treatment and fed on supplemented FO or OO showed apparently normal hepatocytes.

Conclusion: The most effective treatment was observed with oils supplemented with vitamins E and C. Hierarchically FO achieved the best results compared to other additives, followed by OO and finally CO showing the least effective treatment among the observed groups.

Keywords: chronic liver disease, rats, vitamin E, vitamin C, lipid profile, liver functions

Zusammenfassung

Das Ziel der vorliegenden Studie war es, den Einfluss von Olivenöl (OO), Maisöl (CO) und Leinsamenöl (FO) mit und ohne Zusatz von Vitamin E und C auf die Nahrungsaufnahme, auf den Anstieg des Körpergewichts, auf das Verhältnis Lebergewicht zu Körpergewicht (%) sowie auf die Konzentration von Serumlipide, die Leberfunktion und die Leberhistologie in Ratten, die mit Tetrachlormethan vergiftet wurden, zu untersuchen.

42 männliche Ratten wurden in zwei Gruppen unterteilt; die 1. Gruppe wurde mit einer Basaldiät ernährt und diente als negative Kontrollgruppe (NC). Die 2. Gruppe erhielt Tetrachlorkohlenstoff in Paraffinöl (50% v/v, 2 ml/kg) zweimal pro Woche subkutan injiziert, um eine chronische Lebererkrankung zu erzeugen. Diese Gruppe wurde anschließend in 6 Subgruppen unterteilt, 3 von ihnen wurden jeweils mit 4% Öl (CO, FO und OO) ohne Zusatz ernährt und dienten als Positivkontrollen. Der Rest der Gruppe wurde mit den gleichen Ölen, ergänzt mit Vitamin E und C, ernährt.
Die Ergebnisse zeigen, dass Ratten, die mit Leinsamenöl unter Zusatz von Vitamin E und C ernährt wurden, eine signifikant niedrigere Konzentration an Gesamtcholesterin (TC)-, LDL-Cholesterin (LDL-C)- und Alaninaminotransferase (ALT)-Aktivität aufwiesen. Darüber hinaus hatten diese Ratten ein höheres HDL-Cholesterin verglichen mit den Negativkontrollgruppen (NC). Die Rattengruppe, die Olivenöl, ergänzt mit den Vitaminen E und C erhielt, hatte eine verminderte Konzentration an Serumcholesterin, einen signifikanten Anstieg von HDL-Cholesterin im Vergleich zu den Negativkontrollgruppen (NC). Die Ergebnisse in der Rattengruppe, die mit Maisöl mit den Vitaminen E und C ernährt wurde, zeigten einen signifikanten Anstieg von HDL-Cholesterin im Serum im Vergleich zu der Negativkontrollgruppe. Die histologischen Befunde bestätigten, dass die mit Tetrachlorkohlenstoff geschädigten Ratten ernährt mit Leinsamenöl und Olivenöl, eine normale Hepatozytenmorphologie aufwiesen.
Schlussfolgerung: Die wirksamste Behandlung der lebergeschädigten Ratten wurde mit den Ölen, die mit Vitamin E und C ergänzt wurden, beobachtet. In der Abstufung erzielte Leinsamenöl die besten Resultate, verglichen mit den anderen Zusätzen, und Olivenöl (OO) und Maisöl (CO) hatten die geringste Wirksamkeit unter den behandelten Gruppen.

Schlüsselwörter: chronische Lebererkrankung, Tetrachlorkohlenstoff, Ratten, Nahrungsöl, Vitamin E, Vitamin C, Leberfunktion

Introduction

Liver diseases have a variety of causes such as infections, parasites, nutrition deficiency, inborn errors, toxic substances and malignancy. Viral hepatitis is the major cause of liver disease in tropical areas including Egypt [1].
Nutrition and the liver interrelate in many ways and the possible effect of slowing down liver damage has been considered [2]. Several studies have emphasized on the importance of dietary composition in the treatment of fatty liver. Dietary fat affects the fatty acid composition, lipid peroxidation and antioxidants defence system of the body [3].
Liver cell injury induced by carbon tetrachloride involves initially the metabolism of carbon tetrachloride to trichloromethyl free-radical by the mixed function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanisms link carbon tetrachloride metabolism to the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from carbon tetrachloride metabolism or from peroxidative degeneration of membrane lipids. The possible involvement of radical species such as trichloromethyl (CCl_3), trichloromethylperoxy ($OOCCl_3$), and chlorine (Cl) free radicals, as well as phosgene and aldehydic products of lipid peroxidation, as toxic intermediates is discussed. Data do not support the view that an increase in cytosolic free calcium is important in the toxic action of carbon tetrachloride or bromotrichloromethane. In addition, carbon tetrachloride-induced inhibition of very low density lipoprotein secretion by hepatocytes is not a result of elevated levels of cytosolic free calcium [4].
Sufficient data are not yet available to decide whether lipid peroxidation is a major or minor consequence of the metabolism of carbon tetrachloride. It has been reported that the development of hepatic steatosis results from an imbalance in the rates of entry, synthesis, or clearance of fat from the liver [5].
More specific, the hepatic uptake of fatty acids and triacylglycerol and their rates of synthesis, the secretion of these compounds via plasma, bile, hydrolysis of triacylglycerol or oxidation of fatty acids may be altered. Non-alcoholic steatohepatitis (NASH) pathogenesis includes increased oxidative stress and increased lipid peroxidation may be altered [6].
It has been reported that steatosis and starvation may act synergistically on the depletion of antioxidants, predisposing fatty liver to a reduced tolerance to oxidative injury [7].

It has been suggested that disturbances in antioxidants parameters in blood of patients with chronic liver disease may be due to peroxidative damage of cells [8]. A study conducted by Aguilera and his co-workers [9] showed that treatment with virgin olive, sunflower and fish oils enhances hepatic antioxidant defence system.
A study demonstrated that α-tocopherol is the main antioxidant involved in the protection of unsaturated lipids. Clinical and experimental conditions characterized by decrease in α-tocopherol are associated with enhanced peroxidation of lipids and lipoprotein. The redox of α-tocopherol depends on the availability of reduced ascorbate, which is decreased markedly in rat steatotic liver [10].
The investigated oils are commonly used in Egyptian recipes. Supplementation with vitamin E and C was conducted to maximize the beneficial effects of vitamins in order to combat the toxic effects of CCl_4 as model of chronic liver disease. These oils are different in their chemical composition and tocopherol content according to Table 1.

Table 1: Fatty acid composition of dietary oils %

Fatty acid	Olive oil*	Flaxseed oil**	Corn oil***
10:0	–	–	–
12:0	–	–	–
14:0	–	–	–
16:0	12	3	11
18:0	3	7	2
18:1	71	21	28
18:2 (ω–6)	10	16	58
18:3 (ω–3)	1	53	1
total	97	100	100

Values are the area percentages of total methyl esters fatty acid concentration. <1% are not included. Elaidic acid [C18:1 (ω-9t)] not determined. Percentages may not add to 100% in some oils due to rounding and other constituents not listed. Table was modified from [44].
* Indicates tocopherol content in oils 14.4 mg/100g,
** 9.3 mg/100g, *** 21mg/100g [35, 36, 45].

The aim of this study was to compare the effect of dietary flaxseed, olive, and corn oils supplemented or not with vitamins E and C on the nutritional status, serum lipid fractions, liver functions and liver damage, in rats suffering from chronic liver disease.

Materials and methods

Materials

Carbon tetrachloride (CCl_4), Vitamin E α-tocopherol acetate, dehydroascorbic acid, casein, vitamins, minerals, cellulose, choline chloride were obtained from El-Gomhorya company, Cairo, Egypt.
Kits used to determine serum cholesterol triacylglycerol, HDL-C, VLDL-C (very low-density lipoprotein cholesterol), LDL-C were obtained from Egyptian American Company for Laboratory Service and Supplied by Alkan Company.

Rats

Rats were obtained from the laboratory animal colony, Ministry of Health and Population, Helwan, Cairo, Egypt.

Experimental design

Forty-two male Sprague Dawley rats weighing (220±5 g) were kept in individual stainless steel cages under hygienic condition and were fed for one week on basal diet for adaptation and supplied with water *add libitum*. The basal diet (BD) in the preliminary experiment is formulated according to Ain 93 [11] (Table 2).

Table 2: Basal diet, Ain-93M

Ingredient	g/kg Diet
Cornstarch	465.692
Casein	140.000
Dextrinized cornstarch	155.000
Sucrose	100.000
*Corn oil	40.000
Fiber	50.000
Mineral mix	35.000
Vitamin mix	10.000
L-cytesine	1.800
Cholin bitartarate	2.500
Tert-butylhydroquinone	0.008

*Soybean oil was replaced by corn oil

After a period of adaptation on BD (one week), the rats were divided into two main groups. The first main group (6 rats) was fed on BD as a negative control group (NC), corn oil being the main source of fat. The second main group (36 rats) was treated with CCl_4 in paraffin oil (50% v/v 2ml/kg) twice a week by subcutaneous injection to induce chronic damage in the liver, according to the method [12]. After injection with CCl_4 blood samples were collected from the orbital plexus by means of fine capillary glass tubes. Aspartate aminotransferase (AST) and alanine amino transferase (ALT) liver functions were deter-

mined in each serum sample, in order to estimate the initial experiment period. This group was divided into six subgroups (6 rats each). The first subgroup was fed on BD containing 4% CO as a positive control group (PC). The second subgroup received BD containing 4% corn oil supplemented with 250 mg vitamin E and 40 mg vitamin C/kg diet (COEC). The third subgroup was fed on BD containing 4% flaxseed oil (FO). The fourth subgroup was fed on BD containing 4% FO supplemented with 250 mg vitamin E and 40 mg vitamin C/kg diet (FOEC). The fifth subgroup was fed on BD containing 4% OO, while the sixth subgroup received BD containing 4% OO supplemented with 250 mg vitamin E and 40 mg vitamin C/kg diet (OOEC). During the experimental period (28 days), diet consumed and body weights were recorded twice weekly. Body weight and food consumption were measured and total food intake of the experimental period (4 weeks) was calculated. Body weight gain % (BWG%) was determined according to [13].

Biochemical analysis

At the end of the experiment, the animals were fasted overnight, and then the rats were anaesthetized and sacrificed to obtain blood samples. Each blood sample was placed in dry clean centrifuge tube, and then centrifuged for 10 minutes at 3000 round per minute (rpm) to separate the serum. Serum was carefully separated into clean dry Wassermann tubes by using a Pasteur pipette and kept frozen until analyses.

Determination of serum HDL-C was carried out according to the method described by Lopes-Virelia et al. [14]. Serum VLDL-C and LDL-C were determined according to [15], [16], aspartate aminotransferase (AST) and alanine amino transferase (ALT) according to [17].

The liver was separated from each rat and weighed to calculate liver to body weight percentage.

Histological examination

Specimens from liver tissues were taken immediately after sacrificing animals, and fixed in 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in bedded in paraffin, cut in sections of 46 microns thickness and stained with haematoxylin and cosin stain [18].

Biostatistics

The data analysis was carried out with SPSS Inc. software (version 15.0). One-way ANOVA was used to study a significant difference between means of the dietary groups with a significance level of $P<0.05$. Duncan's test was used to compare the significance among the rat groups. All data are presented as ± standard deviation of means (SD) [19].

Results

Food intake (g/day for each rat) decreased in the PC group as compared to the NC group (12.7±2.1 vs. 18.2±1.3 g/day respectively). Rats injected with CCl_4 and fed on BD containing 4% FO (supplemented or not with vitamin E and C) recorded higher food intake values than other intoxicated groups (15.1, 14.7), followed by the group that received 4% OO supplemented with vitamin E and C (14.3). However, all groups injected with CCl_4 and treated with different dietary oils showed significant increase in food intake as compared to the PC group ($P<0.05$).

There was a significant decrease ($P<0.05$) in body weight gain (BWG%) for PC as compared to the NC group (7.9 vs. 37.7). On the other hand, all groups injected with CCl_4 and fed on BD containing different oils recorded a significant decrease in BWG% compared to the NC group, while showing a significant increase in BWG compared to the PC group. The group fed on FO or OO oils supplemented with vitamins E and C recorded the best result of BWG% as compared to PC group (17.1, 12.4 vs. 7.9 respectively) (Table 3).

Weight of liver changes by injection of CCl_4, consequently the mean value of liver/body weight % for the PC group showed significant increase ($P<0.05$) as compared to the NC group (3.8 vs. 2.9 respectively). These results were in accordance with those of Hashimoto et al. [20], showing that treatment with CCl_4 and phenobarbital causes liver deformity and high percentage of liver weight in the group injected with CCl_4 and fed on BD. The group fed on 4% FO supplemented with vitamins E and C recorded the least mean value of liver weight/body weight % as compared to the PC group (3.1 vs. 3.8 respectively), followed by the group with BD containing 4% OO supplemented with vitamin E and C which recorded mean liver weight/body weight % of 3.4 vs. 3.8 respectively. On the other hand, all treated sub-groups recorded significant decrease in liver weight/body weight % ($P<0.05$) as compared to the positive control group (Table 3).

The effects of supplemented BD with 4% FO, CO, OO, with or without supplementation with vitamins E and C, on serum levels of total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), low and very low density lipoprotein cholesterol (LDL-C and VLDL-C), in rats suffering from chronic liver diseases is presented in Table 4.

The effects of supplemented diet with different oils with or without vitamins E and C as useful indicator for liver functions in rats suffering from chronic liver disease are presented in Table 5.

Table 3: Effect of different dietary oils supplemented or not with vitamin E & C on food intake, body weight gain % and liver/ body weight % of rats suffering from chronic liver diseases

Parameters / Groups	Food intake (g/day for each rat)	Body weight gain %	Liver weight / Body weight %
NC	18.2 ± 1.3^{a}	37.7 ± 3.2^{a}	2.9 ± 0.2^{d}
PC	12.7 ± 2.1^{de}	7.9 ± 1.4^{d}	3.8 ± 0.3^{a}
COEC	13.2 ± 0.6^{cd}	9.3 ± 1.0^{d}	3.4 ± 0.1^{b}
FO	14.7 ± 0.4^{b}	12.2 ± 2.7^{c}	3.4 ± 0.1^{b}
FOEC	15.1 ± 0.6^{b}	17.1 ± 1.7^{b}	3.1 ± 0.1^{c}
OO	13.1 ± 1.8^{ce}	9.3 ± 1.3^{d}	3.6 ± 0.2^{b}
OOEC	14.3 ± 0.8^{bc}	12.4 ± 1.0^{c}	3.4 ± 0.1^{b}

NC = negative control, PC = positive control, COEC = corn oil supplemented with vitamin E and C, FO = flaxseed oil, FOEC = flaxseed oil supplemented with vitamin E and C, OO = olive oil, OOEC = olive oil supplemented with vitamin E. Values are expressed as mean ± SD. Levels of significance: a, de, cd, b, ce, bc, c, d. Values which do not share the same letter in each column are significantly different. Significance at $P<0.05$.

Table 4: Effect of studied dietary oils supplemented or not with vitamins E & C on lipid profile of rats suffering from chronic liver disease

Parameters / Groups	Lipid profile (mg/dL)				
	Cholesterol	Triacylglycerol	HDL-C	LDL-C	VLDL-C
NC	68.4 ± 4.5^{e}	52.7 ± 3.4^{d}	40.8± 5.2^{a}	17.1 ± 1.3^{f}	10.5 ± 0.7^{d}
PC	111.3 ±7.2^{a}	80.4 ± 4.4^{a}	29.3 ± 5.7^{d}	66.7 ± 2.2^{a}	16.1± 0.9^{a}
COEC	87.9 ± 6.4^{b}	69.6 ± 6.2^{b}	36.3 ± 2.8^{ac}	37.6 ± 2.6^{b}	13.9 ± 1.2^{b}
FO	75.9 ± 4.1^{cd}	65.4 ± 4.0^{bc}	34.0 ± 2.6^{bc}	28.8 ± 2.3^{d}	13.1 ± 0.8^{bc}
FOEC	67.7 ± 3.5^{e}	63.1 ± 2.4^{c}	38.9 ± 2.3^{a}	15.3 ± 1.8^{f}	13.4 ± 0.8^{b}
OO	79.2 ± 4.5^{c}	68.6 ± 1.8^{b}	33.7 ± 4.0^{bcd}	31.7 ± 1.0^{c}	13.7 ± 0.4^{b}
OOEC	71.9 ± 2.4^{de}	61.7 ± 1.8^{c}	38.2 ± 3.0^{ab}	21.3 ± 1.1^{e}	12.3 ± 0.4^{c}

NC = negative control, PC = positive control, COEC = corn oil supplemented with vitamin E and C, FO = flaxseed oil, FOEC = flaxseed oil supplemented with vitamin E and C, OO = olive oil, OOEC = olive oil supplemented with vitamin E. Values are expressed as mean ± SD. Levels of significance: e, a, b, cd, de, bc, ac, bcd, ab ,f. Values which do not share the same letter in each column are significantly different. Significance at $P<0.05$.
mg/dL = milligram/deciliter, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, VLDL-C = very low-density lipoprotein cholesterol

Table 5: Effect of studied dietary oils supplemented or not with vitamins E & C on liver functions of rats suffering from chronic liver disease

Parameters / Groups	IU/L	
	AST	ALT
NC	74.3 ± 4.2^{f}	20.4 ± 3.2^{d}
PC	180.6 ± 7.1^{a}	60.7 ± 3.8^{a}
COEC	151.4 ± 6.2^{b}	41.2 ± 5.0^{b}
FO	136.9 ± 6.0^{c}	39.5 ± 3.5^{b}
FOEC	111.9 ± 5.4^{e}	23.9 ± 3.6^{d}
OO	133.2 ± 5.2^{c}	37.8 ± 2.7^{b}
OOEC	121.7 ± 5.2^{d}	28.9 ± 3.4^{c}

NC = negative control, PC = positive control, COEC = corn oil supplemented with vitamin E and C, FO = flaxseed oil, FOEC = flaxseed oil supplemented with vitamin E and C, OO = olive oil, OOEC = olive oil supplemented with vitamin E. Values are expressed as mean ± SD. Levels of significance: f, a, b, c, e, d. Values which do not share the same letter in each column are significantly different. Significance at $P<0.05$.
AST = aspartate aminotransferase
ALT = alanine amino transferase

It can be noticed that the PC group fed on BD containing 4% CO has shown a highly significant increase in the mean values of TC, TAG, LDL-C and VLDL-C compared to the NC group. Concerning the mean value of serum HDL-C, the PC group exhibited a significant decrease as compared to the NC group. These results are in agreement with those of Augusti et al. [21] who claimed that the injection with CCl_4 increased the serum and tissue lipid profile.

Replaceing CO by FO or OO in the BD of the rat groups injected with CCl_4 resulted in a significant decrease in the mean values of serum TC, TAG, LDL-C and VLDL-C, and a significant increase in the mean value of serum HDL-C as compared to the PC group.

In this respect, Maurice et al. [22] reported that n-3 family fatty acids have been shown to reduce hepatic fat and liver accumulation in poultry. Recently, Schuman et al. [23] reported that FO oil reduced hepatic fat content as well.

Histopathological results

Our results (Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figure 7) revealed that treatment of the PC group with FO or OO supplemented with vitamins E & C led to the best improvement. The histopathological investigation showed apparently normal hepatocytes.

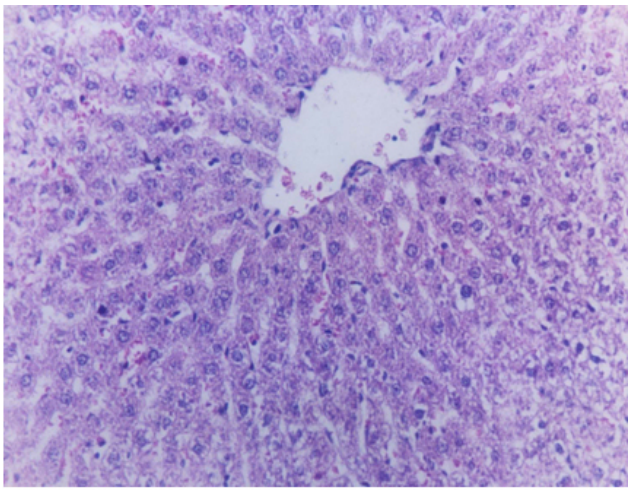

Figure 1: Liver of rat from NC group showing normal histological structure of hepatic lobule (H and E x 200)

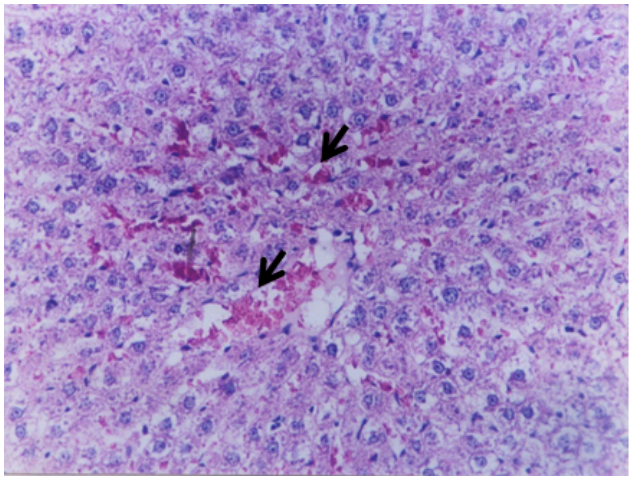

Figure 2: Liver of rat from PC group which injected CCl_4 and fed on BD showing hepatocellular vacuolization, focal hepatic necrosis, hemorrhage, fibroplasia in portal triad associated with chronic cholangitis (H and E x 200)

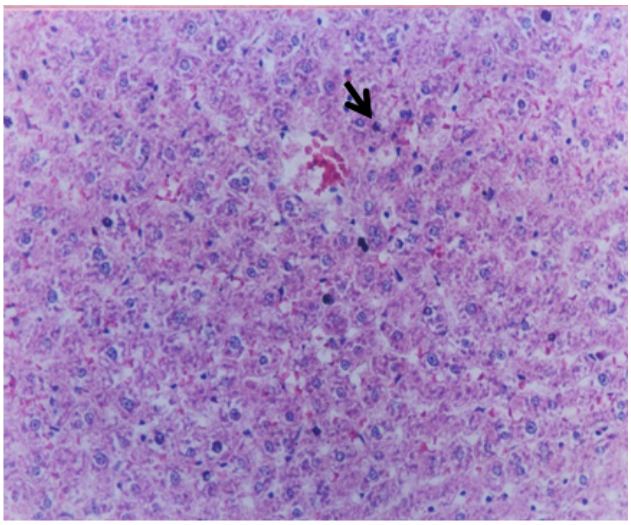

Figure 3: Liver of rat from COEC group showing slight hydropic degeneration of some hepatocytes (H and E x 200)

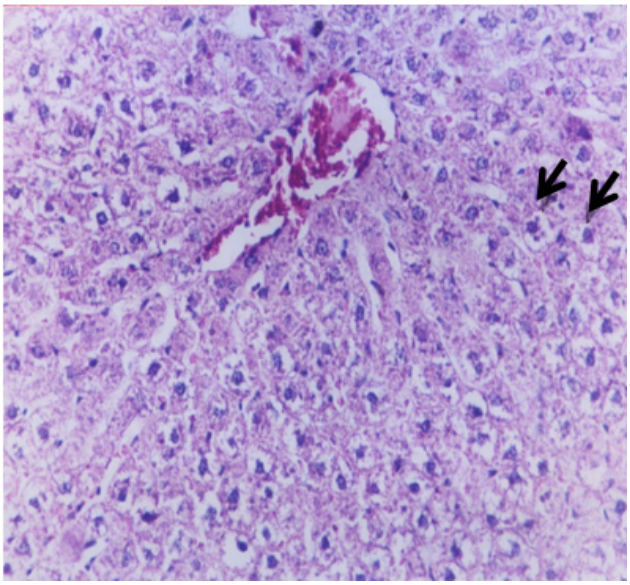

Figure 4: Liver of rat from OO group showing slight congestion of hepatic sinusoids (H and E x 200)

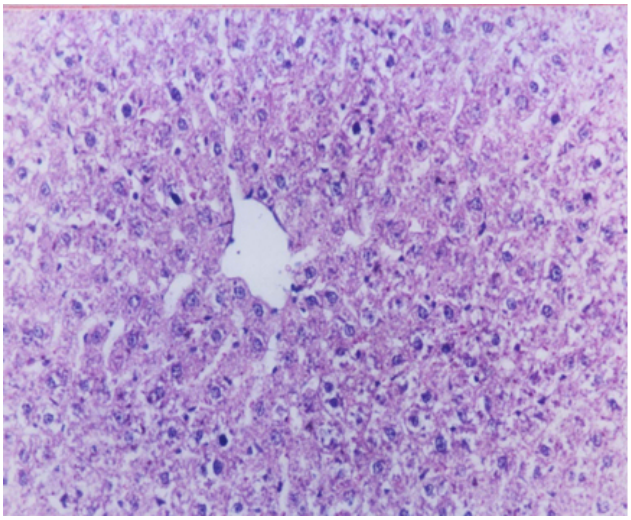

Figure 5: Liver of rat from OOEC group showing apparently normal hepatocytes (H and E x 200)

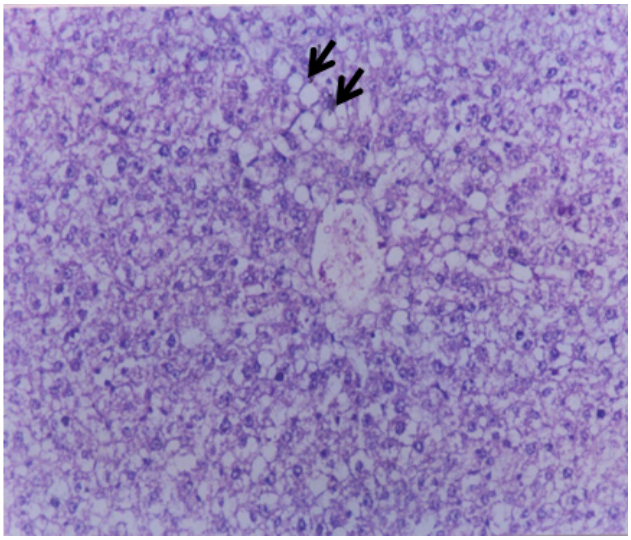

Figure 6: Liver of rat from FO group showing hepatocellular vacuolization (H and E x 200)

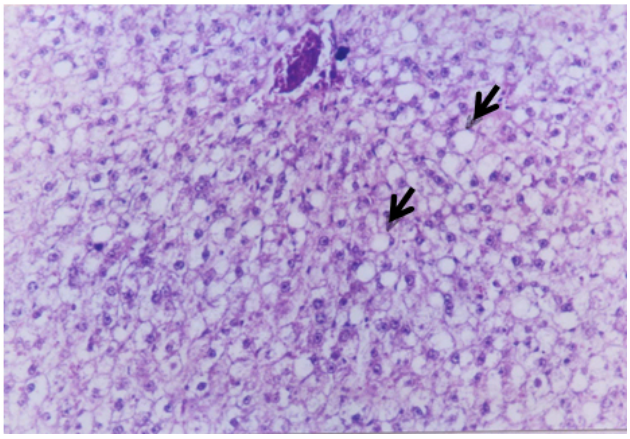

Figure 7: Liver of rat from FOEC group showing some improvements in hepatocytes

In this respect, Alwayn et al. [24] reported that supplementation of n-3 FAs can ameliorate hepatic steatosis in a murine model of parenteral nutrition as demonstrated by histology. In this concern, Cohen et al. [25] found that n-3 PUFA as found in FO has anti-inflammatory properties that are mediated by the production of anti-inflammatory eicosanoids.

On the other hand, De la Puerta Vàzquez et al. [26] and Perona et al. [27] reported that the biological effects of olive oil is more than a simple mixture of fatty acids, and that it contains other biologically active substances such as tocopherols, polyphenols and phytosterol some of which have antioxidant and anti-inflammatory activities.

In conclusion, our results revealed that oxidative damage caused by free radicals in rat livers due to injection of CCl_4 may be attenuated by dietary supplementation of FO or OO with vitamins E and C compared to NC.

Discussion

Our results clearly demonstrated the efficacy of dietary antioxidants due to supplementation with vitamins C and E that alleviated the malfunctions of livers and the elevation of lipid profile among rat groups intoxicated with CCl_4. That may be explained by increasing rats' serum with dietary antioxidants which need further investigation.

Naturally oils do not have any vitamin C according to refining and heat treatments and rat's basal diet does not contain any vitamin C as well, however rats are quite capable to synthesize it in their bodies [28]. In this regard, the supplemented diet including antioxidant vitamins C and E (OOEC, FOEC and COEC groups) had the same amounts of vitamins apart from control groups. This was assumed to indicate the effect of the quantity and the quality of supplemented diets with vitamins.

Our results are on the same line with Morise et al. [29], who explained that α-linolenic acid (ALA) rich in FO resulted in a higher cholesterol secretion into bile, leading to depletion of the intrahepatic pool of cholesterol, and thus an increase in cholesterol synthesis and turnover.

Moreover, ALA rich diet reduced hepatic lipid accumulation both by stimulating β-oxidation and by suppressing fatty acid synthesis [30]. In respect to TAG, our results are in agreement with Feoli et al. [31] and Park et al. [32] who cleared the effect of ALA in the FO that can be attributed to a reduction in the hepatic synthesis of fatty acids, which decreases the concentration of triacylglycerol in the liver.

In respect to OO, our results are in agreement with Hussein et al. [33] who found that the diet supplemented with OO, but not fish oil or butter fat, led those animals to store less TAG in their liver consequently, preventing the occurrence of dietary induced severe hepatic steatosis.

The comparative effects of dietary FO, OO, and CO supplemented with vitamins E and C revealed that the most effective treatment on serum TC, HDL-C, LDL-C, was recorded for the injected CCl_4 group fed on BD containing 4% FOEC as these treatments led to significant reduction in the mean value of TC, LDL-C and significant increase in the mean value of serum HDL-C as compared to the NC group.

The present results showed that the injected CCl_4 rats fed on BD containing 4% OOEC exhibited a significant decrease ($P<0.05$) in the mean value of serum TC and a significant increase in the mean value of serum HDL-C as compared to the NC group. While COEC led to a significant increase in the mean value of serum HDL-C as compared to NC group.

Hierarchically, the current study revealed that the most effective treatment on serum lipid profile was FOEC followed by OOEC. In this respect, Grattagliano et al. [7] reported that fatty liver induced by CCl_4 is associated with a lower level of antioxidants, which results in lipid peroxidation. Steatosis may act on the depletion of antioxidants predisposing fatty livers to a reduced tolerance to oxidative.

On the other hand, Sokol et al. [10] showed that hepatic steatosis is associated with a lower antioxidant capacity of hepatic cells, characterized mainly by a reduced availability of α-tocopherol and vitamin C rather than of glutathione GSH, this condition exposes hepatic lipids to an enhanced risk of oxidation.

Vegetable oils, nuts and plant sources are good sources of vitamin E ranging between 9.3, 14.4 and 21 mg/100 g for flaxseed, olive and corn oils, respectively [34], [35], [36]. Vitamin E is absorbed from the gut with the aid of bile salts and the vitamin is not esterfied. It is transported to the blood stream via chylomicrons and distributed to the various tissues via lipoproteins [34]. We would suggest that the greatest improvement in lipid profile of FOEC or OOEC allowed a recovery from the antioxidant vitamin reduction in the liver. α-tocopherol is the main antioxidant involved in the protection of unsaturated lipids. The redox state of α-tocopherol depends on the availability of reduced ascorbate. As stated these diets were supplemented with 40mg/kg vitamin C. The low intracellular concentration of vitamin C in fatty liver was likely related to increased consumption for recycling oxidized α-tocopherol [10]. The useful effect of vitamin E in improving liver function can be attributed also to their ability, as antioxidant, to quench free radicals and reduce the increased rate of lipid peroxidation in the liver [37].

Aminotransferases are found in the liver in addition to the brain, pancreas, heart, skeletal muscle, kidneys and lungs. It is used by the liver to help make the energy-storage of molecule glycogen. In this study, AST of NC group was found to be 3 times greater than ALT that is primarily found in the liver, while AST found in all organs previously described. These results are in agreement with the results of Young et al. [38], Abdel-Moemin et al. [39], Al-Wabel et al. [40], Cengiz et al. [41], Sánchez et al. [42]. It could be noticed that the rats of PC group showed significant increase ($P<0.05$) in the mean values of AST and ALT, compared to those of NC group. In this aspect, Mansour et al. [43] reported that a single dose of CCl_4 induced hepatotoxicity manifested biochemically by significant elevation of activities of liver functions, such as ALT and AST.

The current results showed that liver function of chronic liver disease in COEC or OOEC showed significant decrease of AST and ALT values, as compared to PC. While ALT of FOEC group recorded a significant decrease ($P<0.05$) as compared to the NC group.

Little data are available on the effect of supplemented diets with vitamin E and C on intoxicated rat livers with CCl_4. The histological results confirmed that the hepatically injured rats with CCl_4 and fed on supplemented FOEC or OOEC showed apparently normal hepatocytes.

Notes

Conflicts of interest

None declared.

Acknowledgements

The author wishes to thank Helwan University for encouraging international publications with high standard of research.

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Please cite as
Ismail RS, El-Megeid AA, Abdel-Moemin AR. Carbon tetrachloride-induced liver disease in rats: the potential effect of supplement oils with vitamins E and C on the nutritional status. GMS Ger Med Sci. 2009;7:Doc05.

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http://www.egms.de/en/gms/2009-7/000064.shtml

Received: *2008-12-12*
Revised: *2009-05-12*
Published: *2009-06-30*