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Tocotrienol and Liver Functions and Its Hepatoprotetive Effects Against CCL_4 Induced Liver Fibrosis in Rats.

KEYWORDS: Liver fibrosis, CCl_4, Tocotrienol

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ABSTRACT *Background: Although hepatic problems are responsible for a significant number of liver transplantations and deaths in Egypt, successful therapeutic options are limited and there is a great demand for the development of new effective treatment. So, the aim of this in vivo study is to evaluate the role of tocotrienol on liver functions. We also evaluate its hepatoprotetive effects against CCl4 induced hepatic fibrosis in rats and try to explore the underlying mechanisms.*

Materials and Methods: Male Sprague Dawley rats were used in this study; rats were randomly divided into five groups: control group, tocotrienol group, CCl4 group, treatment group, and protection group. At the end of experimentation blood samples were collected and centrifuged then serum was used to asses liver functions in different groups. Liver was dissected and specimens were stained with haematoxylin and eosin (H&E) to examine its morphology. Masson's tichrome stain was used to assess fibrosis. Measurement of oxidative stress biomarkers, antioxidant enzymes, platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β in liver homogenate were performed.

Results: Histopathological analysis demonstrated that CCl4 injection resulted in hepatic steatosis, necrosis, and fibrosis. Tocotrienol administration, especially in protection group, significantly reduced the histopathological changes induced by CCl4. Also, tocotrienol significantly improve the deterioration in liver functions induced by CCl4 injection. Moreover, tocotrienol significantly reduced the elevation induced by CCl4 in oxidative stress biomarkers, hepatic PDGF, and TGF-β. Conclusion: Tocotrienol supplementation can have beneficial effects on liver functions and an effective role for the prevention of CCl4 induced hepatic damage in rats through increasing the activity of the antioxidant defense system, inhibition of lipid peroxidation and inhibition of HSC activation.

Introduction:
Liver fibrosis is common type of chronic liver disease (1,2) and it's possible consequences are liver cirrhosis or hepatocellular carcinoma (3). Several researches reported that free radicals and reactive oxygen species play a pivotal role in the various steps that initiate and regulate the progression of liver fibrosis independently of the agent responsible on fibrosis (4,5). Various xenobiotics are known to cause hepatotoxicity one among them is carbon tetrachloride (CCl_4) (6).

CCl_4 was previously used in several industrial applications such as metal degreasing and as dry cleaning, fabric-spotting, and fire extinguisher fluids, grain fumigant and reaction medium. These days, its uses are restricted to very limited industrial applications because of its harmful effects. High exposure to CCl4 can cause liver, kidney and central nervous system damage. Liver is especially sensitive to CCl_4 and several reports from investigators have established that CCl_4 is a potent environmental hepatotoxin (7,8). CCl_4 is usually used experimentally to induce liver injury, fibrosis, and carcinoma in rodents. A single dose of CCl_4 leads to centrizonal necrosis and steatosis (9), while repeated administration leads to liver fibrosis, cirrhosis, and hepatocellular carcinoma (10). CCl_4 impairs hepatocytes directly by alternating the permeability of the plasma, lysosomal, and mitochondrial membranes. Highly reactive free radical metabolites are also formed in hepatocytes causing sever centrilobular necrosis (11,12). In addition, CCl_4 inhibits the antioxidant enzymes of the liver specially superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) (13, 14).

Current researches are now directed towards finding naturally occurring antioxidants, which might help prevent oxidative damage. Tocotrienol, a member of vitamin E, is a potent natural antioxidant which is able to scavenge large number of free radicals(15). The natural vitamin E family is composed of 8 members equally divided into 2 classes: tocopherols (TCP) and tocotrienols (TE), each differing in the number and position of the methyl groups on the chroman ring and by the presence or absence of a double bond on the phytyl side chain (16). A growing body of evidence suggests TE possess potent biological activity not shared by TCP. In particular, TE has a neuroprotective and anticancer properties that are not exhibited by TCP (17,18). Several studies have demonstrated beneficial effects of vitamin E on fatty liver (19, 20, 21).

Although hepatic problems are responsible for a significant number of liver transplantations and deaths in Egypt, successful therapeutic options are limited and there is a great demand for the development of new effective treatment. In the present study, we evaluate the in vivo role of tocotrienol on liver functions. We also evaluate its hepatoprotetive effects against CCl_4 induced hepatic fibrosis in rats and try to explore the underlying mechanisms.

Materials and Methods
A fifty healthy male Sprague Dawley rats with an average weight of 180 ± 50 grams were used in this study. They were obtained from Medical Experimental Research Center of Mansoura University, Egypt. The experimental protocol was approved by the Institutional Animal Ethics Committee of Mansura University. All rats were fed with chow diet and housed in policarbon cages and were exposed to a 12h

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

light-dark cycle at a room temperature of 21-24°C and 50 - 60% relative humidity. Rats were randomly divided into five groups, each consists of 10 rats. Group1**;** was the vehicle control in which rats were intraperitoneally (i.p.) injected with the vehicle olive oil twice a week for a period of six weeks. Group 2; was the tocotrienol (TE) control in which rats were orally given TE in a dose of 60 mg/kg diluted in olive oil (22) daily for 6 weeks. Group 3; was the CCl_4 group in which rats were i.p. injected with CCl_4 (0.5 ml/ kg) (23) and olive oil [1:1 (v/v)] twice a week for a period of six weeks. Group 4; was the treatment group in which rats were injected with CCl_4 twice a week and treated with tocotrienol daily for 6 weeks in a dose of 60 mg/ kg by a needle gavage . Group 5; was the protection group in which rats were orally given TE (60 mg/kg) daily for a period of two months then CCl_4 were injected i.p. twice weekly for six weeks in association with daily oral TE. Carbon tetrachloride was purchased from Sigma–Aldrich, St. Louis, MO while TE was obtained from Cairo North Mill Co., Cairo Egypt.

At the end of experimentation, the body weight was measured then the animals were anesthetized with pentobarbital [0.6 ml/ kg] and the blood collected by heart puncture and allowed to clot for 30 min. Serum was separated by centrifugation at 2500rpm for 15 min and used for biochemical estimations. After that rats were sacrificed by cervical dislocation and the abdomen was open quickly and the liver was immediately removed and washed thoroughly with ice-cold 0.9 % sodium chloride solution [saline] and dried with filter paper and weighted. Then portion of the liver was used to assess biochemical parameters and the other was fixed in 10 % formalin for histological examinations.

Liver functions assay:
Biochemical analysis were carried out to determine the serum concentrations of total protein, albumin, total bilirubin, cholesterol, triglycerides (TG) and the activities of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline Phosphatase (ALP) by colorimetric method. Biochemical analysis was done by using commercial kits provided by Biomerieux, Egypt and Uvikon 930 spectrophotometer (Kontron Instroment, Milan, Italy). The absorbance of the test samples were read at 505nm for AST and ALT and at 510 nm for ALP. Total protein was determined by the Biuret method (24), albumin by the bromocresol green method (25), and total bilirubin was estimated by the method described by Jendrassik and Grof (26). TG and total cholesterol concentrations were determined using enzymatic methods as described in the instructions provided with the kits (Analyticon, Biotechnologies AG, Germany). The absorbance of the test samples for TG and cholesterol were read at 546 nm against blank.

Liver homogenate preparation
Samples of liver tissue were homogenized (1:10, w/v) in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%).The homogenate was centrifuged at 8000 rpm for 5 min at 4°C to separate the nuclear debris and supernatant was collected .The centrifuged homogenates were stored at -25°C until they were analyzed.

Measurement of hepatic reduced glutathione (GSH) concentration, superoxide dismutase (SOD), and catalase (CAT) activity

Liver homogenate was subjected for the assay of free radical metabolizing enzymes by using a spectrophotometer and commercially available colorimetric kits (Bio Diagnostics, Dokki, Giza, Egypt). and according to the protocol provided by the manufacturer. The absorbance of the test samples were read at 412nm for GSH, 560nm for SOD and at 240 nm for CAT.

Measurement of oxidative stress biomarkers
Malondialdehyde (MDA) was measured in liver homogenate for assay of lipid peroxidation of the liver and according to the modified method of Iqbal et al. (27) Serum advanced oxidation protein products (AOPP) was measured in the serum according to the procedure of Witko-Sarsat (28) and according to the protocol provided by the kits. The absorbance was measured at 535 nm for MDA and at 340 nm for AOPP against blank. Kits used were commercially available colorimetric kits (BioDiagnostics, Dokki, Giza, Egypt).

Measurement of liver platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β (pg/ml)
Levels of hepatic PDGF, and TGF- β1 in rats were determined by using a corresponding ELISA kit purchased from Sigma-Aldrich St. Louis, MO , USA and according to the protocol provided by the manufacturer. The absorbance was read at 450 nm, with reference wave length at 570 nm.

Histopathological Examination of Liver
Liver were dissected out and fixed in 10% formal saline for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5µm thickness were prepared and stained with haematoxylin and eosin (H&E) to examine liver morphology. Masson's tichrome stain was used to assess liver fibrosis. Grading and scoring of liver injury was done according to the presence and the degree of fatty change, hepatocellular ballooning, inflammation, necrosis and fibrosis. The liver pathology was scored as described by French et al. (29), while hepatic fibrosis was graded according to the method of Ruwart et al. (30).

Statistical Analysis
Values were expressed in the form of mean (+/-) SD which are done by using excel program for figures and SPSS (SPSS, Sigma Plot Software, Inc, Chicago, IL) program statistical package for social science version 16.The level of significance was taken as $p<0.05$

Results:
Effects on body weight, liver weight and relative liver weight
Body weight of the experimental animals was not affected by CCl_4 or TE. However, a significant elevation of liver weight and its relative weight was seen in CCl_4 group (table 1), indicating that CCl_4 resulted in liver hypertrophy. By contrast, TE in combination with CCl_4, both in treatment and protection groups, significantly reduced the elevated weight of the liver, suggesting the possibility that TE has a protective role against CCl_4 induced liver injury.

Table 1: Body weight, liver weight and relative liver weight in different studied groups.

Parameters	Group1 (Control)	Group2 (TE)	Group3 (CCl_4)	Group4 (Treatment)	Group5 (Protection)
Body weight (g)	25.9 ± 1.4	24.9±1.1	21.6 ± 4.1	26.0± 1.3	25.2± 2.9
Liver weight (g)	0.56 ±0.1	0.54±0.1	0.85 ± 0.1#	0.65± 0.1*	0.59± 0.1*

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Relative liver weight (g/g body weight, %)	2.16 ±0.1	2.17±0.1	3.94 ± 0.1#	2.5± 0.1*	2.34± 0.1#*

Values are expressed as mean ± S.D. (n = 10). * means significantly different from the group treated with $CC1_4$ (group3). # means significantly different from the control (group1).

Effects on liver functions

CCl_4 administration produced significant decrease in the serum concentrations of total protein and albumin and significant elevation of serum triglycerides, cholesterol, bilirubin, and liver enzymes (ALT, AST, and ALP) as compared to normal control group (table 2). However, TE administration attenuated the elevation of serum triglycerides, cholesterol, bilirubin, and liver enzymes in the rats treated with CCl_4 and increased the serum level of total protein and albumin toward control values. TE administration in the protection group resulted in more significant effect than TE administration in the treatment group. There was no significant alternation in rats treated solely with TE except for AST enzyme it resulted in significant reduction.

Table 2: Liver function tests in different studied groups.

Parameters	Group1 (Control)	Group2 (TE)	Group3 (CCl_4)	Group4 (Treatment)	Group5 (Protection)
Total protein(g/L)	13.6 ±1.1	14.01±2.1	6.71±1.12#	11.5± 2.1#*	14.06±3.1*$
Albumin (g/L)	1.81 ± 0.1	1.97±0.1	0.51 ±0.12#	0.83± 0.1*	1.01±0.1*$
TG(mg/dl)	1.86± 1	1.70±1	6.32± 2#	3.54± 1#*	2.12± 2*$
Cholesterol (mg/dl)	3.63 ± 1.1	3.14±1.1	10.10±2.12#	6.40± 1.14#*	4.10±2.1#*$
Total bilirubin (mg/dl)	1.29± 1	1.17 ±1	3.71 ± 2#	2.70± 1#*	1.83± 2*$
ALP(IU/L)	17.18± 1.7	16.1 ±1	103.13±2.9#	29.2± 1#*	18.06± 2*$
ALT(IU/L)	24.71± 1.7	22.18 ±1	121.67±2.9#	64.9± 1#*	29.90±2*$
AST(IU/L)	45.15±7.7	39.75± 6.4#	210.08± 12.9#	90.53± 11.5#*	52.35±12*$

Values are expressed as mean ± S.D. (n = 10). * means significant difference from $CC1_4$ group (group3). # means significant difference from the control (group1). $ means significant difference between treatment group (group4) and protection group (group5). Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate amino aminotransferase (AST), triglycerides (TG).

Effects on hepatic antioxidant enzyme and oxidative stress biomarkers levels

The results in table (3)show that CCl_4 administration resulted in significant reduction in hepatic GSH concentration and SOD and CAT activity compared to control group. TE administration in treatment and protection groups prevented the decrease induced by CCl_4 and restored the activity of SOD and CAT and the normal concentration of GSH. There was no significant difference between the treatment and protection group except SOD which was elevated more with protection group . In group 2 TE administrations resulted in significant elevation of hepatic GSH concentration, and hepatic SOD and CAT activity compared to control group.

Table (4) show that CCl_4 administration resulted in significant elevation in hepatic MDA and serum AOPP in group 3 compared to control group. By contrast, TE administration prevented the elevation induced by CCl_4 and restored the near normal level of hepatic MDA and serum AOPP. TE administration in protection group had more significant effect than TE administration in treatment group. Also, TE administration in group 2 resulted in significant reduction of hepatic MDA and serum AOPP as compared to control group.

Table 3: Hepatic reduced glutathione (GSH) concentration, superoxide dismutase (SOD), and catalase (CAT) activity in different studied groups.

Parameters	Group1 (Control)	Group2 (TE)	Group3 (CCl_4)	Group4 (Treatment)	Group5 (Protection)
GSH (µmol/gm)	9.71± 2.7	13.55± 1.4#	7.51± 1.9#	10.57± 1.5*	11.37±2#*
SOD (U/mg)	12.45 ± 2	18.08± 1.4#	4.18± 1#	9.57± 2.5#*	11.97±2*$
CAT (U/mg)	10.23± 2	19.16± 1.45#	6.55± 1.3#	9.88± 2.5*	10.57± 2.1*

Values are expressed as mean ± S.D. (n = 10). * means significant difference from $CC1_4$ group (group3). # means significant difference from the control (group1). $ means significant difference between treatment group (group4) and protection group (group5).

Table 4: Hepatic lipid peroxidation (MDA) and serum advanced oxidation protein products (AOPPs) levels in different studied groups.

Parameters	Group1 (Control)	Group2 (TE)	Group3 (CCl_4)	Group4 (Treatment)	Group5 (Protection)
MDA (nmol /h/g)	19.03 ± 2	13.54 ±1.45#	68.89 ±1.3#	33.54 ±2.5#*	21.02±2.1*$
AOPP (µmol/L)	25.92 ± 2	14.32 ±1.4#	49.68 ±0.3#	32.50 ±2.5#*	25.70±2.1*$

Values are expressed as mean ± S.D. (n = 10). * means significant difference from $CC1_4$ group (group3). # means significant difference from the control (group1). $ means significant difference between treatment group (group4) and protection group (group5).

Effects on hepatic PDGF and TGF-β

Table (5) show that hepatic PDGF and TGF-β content were significantly higher in CCl_4 treated group while, TE administration in group 4 and 5 significantly reduced hepatic PDGF and TGF-β content to near their normal levels. TE administration in protection group had more significant effect than its administration in treatment group. There was no significant alternation in rats treated solely with TE.

Table 5: Effect of WGO on hepatic platelet-derived growth factor (PDGF) and hepatic transforming growth factor (TGF)-β contents in different studied groups.

Parameters	Group1 (Conrol)	Group2 (TE)	Group3 (CCl_4)	Group4 (Treatment)	Group5 (Protection)
PDGFC (pg/ml)	346 ±2 2	335±19.4	1784±41#	834±28.5#*	573±33#*$
TGF- β (pg/ml)	226 ± 0	213±19.4	1583±29.3#	702±28.5#*	410±19.1#*$

Values are expressed as mean ± S.D. (n = 10). * means significant difference from $CC1_4$ group (group3). # means significant difference from the control (group1). $ means significant difference between treatment group (group4) and protection group (group5).

Histopathological findings

Liver sections from control rats stained with H&E showed normal liver architecture. Also, liver specimens from group

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

2 showed normal liver architecture (figures 1, 2). However, liver tissue from CCl_4 group showed centrilobular congestion, intense neutrophilic infiltration, extensive fatty changes (steatosis), increased mitotic activity, and sever centrilobular necrosis (figure 3). Hepatic congestion, fatty changes and necrosis were significantly reduced in extent in treatment group as compared to the CCl_4 group, whereas, minimal tissue necrosis and steatosis were observed at the periphery of the central vein (figure 4). In animals of the protection group, the liver showed maintained histoarchitecture almost similar to control with only weak steatosis of periportal and perilobuler hepatocytes (figure5).

Masson's tichrome stain was used to assess fibrosis. Liver sections from control rats and rats of group 2 showed normal distribution of collagen (figures 1, 2). Liver sections from CCl_4 group showed extensive fibrosis in the periportal areas (figure 3). Fibrosis was still present in liver of rats of the treatment group, but it was significantly reduced in extent as compared to the CCl_4 group (figure 4). Animals of the protection group showed only minor and sporadic pericellular fibrosis (normal liver) (figure5).

A) Masson's tichrome; ×100 B) Hx. & E.; ×100

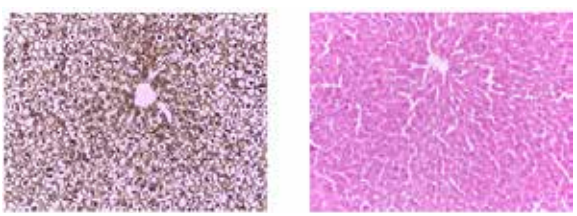

Figure 1: The photomicrograph of a section in the liver of control group. A) Masson's tichrome stain show normal distribution of collagen (no fibrosis). B) H&E show normal liver architecture.

A) Masson's tichrome; ×100 B) Hx. & E.; ×100

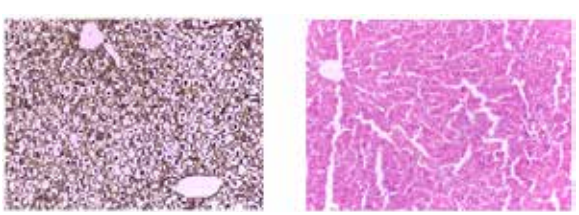

Figure 2: The photomicrograph of a section in the liver of tocotrienol group. A) Masson's tichrome stain show normal distribution of collagen (no fibrosis). B) H&E show normal liver architecture.

A) Masson's tichrome; ×100 B) Hx. & E.; ×100

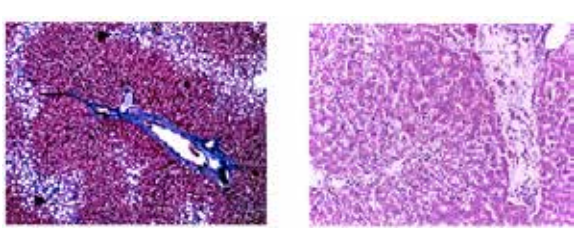

Figure 3: The photomicrograph of a section in the liver of CCl_4 group. A) Masson's tichrome stain intralobular proliferation of fibrous tissue forming thin intralobular septa with porto-portal bridging fibrosis (grade 3 fibrosis). B) H&E show intense neutrophilic infiltration, extensive fatty changes (steatosis), increased mitotic activity, and centrilobular necrosis.

A) Masson's tichrome; ×100 B) Hx. & E.; ×100

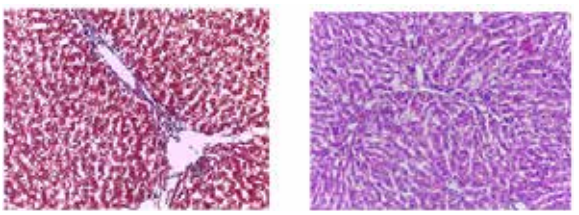

Figure 4: The photomicrograph of a section in the liver of treatment group A)Masson's tichrome stain show fibrosis is still present it is significantly reduced (grade 1 fibrosis). B) H&E show minimal tissue necrosis and steatosis at the periphery of the central vein.

A) Masson's tichrome; ×100 B) Hx. & E.; ×100

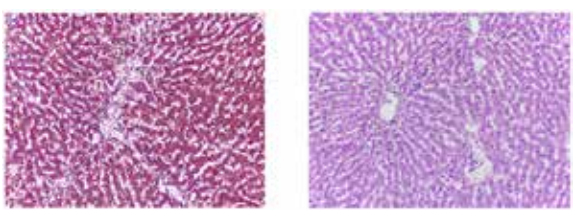

Figure 5: The photomicrograph of a section in the liver of protection group. A) Masson's tichrome stain show minor and sporadic pericellular fibrosis. B) H&E show normal liver architecture.

Table 6: Hepatic histopathology score in different studied groups.

Parameters	Group1 (Control)	Group2 (TE)	Group3 (CCl_4)	Group4 (Treatment)	Group5 (Protection)
Hepatocyte necrosis	0	0	3	1	0
Inflammation	0	0	4	1	1
Cell swelling	0	0	3	2	0
Fatty degeneration	0	0	4	2	0
Fibrosis	_	-	+++	+	_

The liver pathology was scored as described by French et al. (33) as follows: Score 0 = no visible cell damage, Score 1 = focal hepatocyte damage on less than 25 % of the tissue, Score 2 = focal hepatocyte damage on 25-50 % of the tissue, Score 3 = extensive, but focal, hepatocyte lesions, Score 4 = global hepatocyte necrosis. While hepatic fibrosis was graded according to the method of Ruwart et al. (34) as the following: Absent (-) = normal liver, Few (+) = increase of collagen without formation of septa, Mild (++) = formation of incomplete septa from portal tract to central vein, Moderate (+++) = complete but thin septa interconnecting with each other.

Discussion

This study evaluates the effects of TE on liver functions and its hepatoprotective effects against CCl_4 induced hepatic damage. Liver disease is a worldwide problem and drugs used in the treatment of liver diseases are sometimes inadequate or have serious side effects. So, it is necessary to search for an alternative natural substance to replace currently used drugs of doubtful efficacy and safety.

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Toxins and drugs are among the basic etiopathogenetic agents of acute liver failure in Western countries (31). CCl_4 is one of the environmental pollutants which mainly cause hepatotoxic effect. Experimental hepatic fibrosis induced by CCl_4 has been extensively used in many researches as the hepatic responses in rats to chronic CCl_4 administration are shown to be similar to human cirrhosis (32). CCl_4 is metabolized in hepatocytes by cytochrome P450 to form a reactive trichloromethyl radical (CCl_3) and trichloromethyl peroxyl radical (CCl_3O_2). Both radicals are capable of binding to DNA and cell membrane leading to lipid peroxidation, cell necrosis, extensive collagen deposition, and liver fibrosis (33).

injection resulted in significant increase in liver weight, serum triglycerides, total cholesterol, total bilirubin, and liver enzymes (AST, ALT, and ALP) and significant decrease in the serum concentrations of total protein and albumin (tables 1 and 2). These results confirm the hepatotoxic effects of CCl_4. Liver enzymes activities are used as important biomarkers for detection of hepatotoxicity. Oxidative stress results in cellular damage to the liver cells with destruction of their membranes and the release of the enzymes into the blood stream .The more severe the liver damage the higher the release of the liver enzymes (34). Diminution of total protein and albumin induced by CCl_4 is a further indication of liver damage (35). However, TE administration, especially in the protection group, attenuated the elevation of liver weight, serum triglycerides, cholesterol, bilirubin, and liver enzymes in the rats treated with CCl_4 and increased the serum level of total protein and albumin to control values. These results suggest that TE has a protective role against CCl_4 induced liver damage and can be considered a potential hepatoprotective agent.

The present study examined also the activities of antioxidant enzymes (SOD, CAT) in the serum and GSH content in liver tissue. In addition, oxidative stress biomarkers (hepatic MDA and serum AOPPs) were also determined (tables 3 and 4). SOD is an effective antioxidant enzyme that catalyses the conversion of superoxide anions into hydrogen peroxide (H_2O_2) (36). Catalase (CAT) is a haemeprotein present in all aerobic cells that catalyses the H_2O_2 to oxygen and water and protect the tissue from oxidative damage (37). GSH is the main non enzymatic and naturally occurring antioxidant. GSH reduces H_2O_2, hydroperoxides (ROOH), and xinobiotic toxicity and directly protects membrane proteins and lipids and preserves their stability (38). The results of this study showed that CCl_4 injection resulted in significant decrease in the activities of antioxidant enzymes and hepatic content of GSH and significant increase in stress biomarkers compared to control rats, which indicate increased oxidative damage to the liver cells. While TE administration attenuated hepatic oxidative stress induced by CCl_4. It significantly reduced lipid peroxidation of the liver (MDA) and serum AOPPs and increased hepatic GSH content and serum SOD and CAT activities. These results suggest that TE has a protective role against CCl_4 induced oxidative stress.

Liver, the major metabolic site, is the most sensitive organ to peroxidative damage because it is rich in oxidizable substance. When an imbalance occurs between oxidants and defense system, oxidative stress occurs. So, a correct status of the hepatic antioxidant defense system is of major importance for the maintenance of health (39). Indeed several studies have confirmed the role of oxidative stress in the process of liver fibrosis and the benefit of antioxidants to prevent or slow down the progression of oxidative stress related damage (40, 41, 42). Antioxidants antagonize the deleterious action of free radicals and protect hepatocytes from damage. Consequently, they have been emerged as potent antifibrotic agents. Attention has been developed on the natural antioxidants contained in the dietary plants for protection or treatment of oxidative damage.

A growing body of evidence suggests tocotrienol possesses potent biological activity. In particular, it has neuroprotective and anticancer properties (17,18). Previous studies have shown that tocotrienol has a potent antioxidant capacity (43, 44, 49) and also anti inflammatory effects due to its ability to suppress inflammatory mediators such as TNFα, IL-1, and IL-6 (46). Some researchers have demonstrated a beneficial role of vitamin E on fatty liver (19, 20, 21).

Histopathological analysis was also performed to assess hepatic fibrosis and to determine the effectiveness of TE on the protection of the liver from CCl_4 induced fibrosis. Results from histopathological analysis demonstrated that CCl_4 injection showed centrilobular congestion, extensive hepatic steatosis, inflammation, fibrosis, and necrosis. While TE administration, especially in protection group, significantly reduced the histopathological changes induced by CCl_4 (figures 4,5). These results provided supportive evidence for biochemical analysis and suggested that TE protected the liver from CCl_4 induced damage and fibrosis.

Quiescent hepatic stellate cells (HSC_S) are vitamin A storing cells and account for 15% of the total number of hepatocytes (47). Liver injury or inflammation results in HSC proliferation and differentiation to myofibroblast like cells, which deposit extracellular matrix and collagen. HSC_S is activated by reactive oxygen species and various cytokines, including transforming growth factor (TGF)-β, and platelet-derived growth factor (PDGF), which are released from the damaged hepatocytes (48). The activated HSC_S produce large amount of extracellular matrix components, such as laminin, fibronectins, hyaluronic acid, and collagen type IV, in an accelerated fashion, resulting in hepatic fibrosis (48). Table 5 shows that hepatic PDGF and TGF-β content were significantly higher in CCl_4 treated group while, TE administration in group 4 and group 5 significantly reduced hepatic PDGF and TGF-β content to near their near normal levels. **Diao et al.** (49) reported that TGF-β appears to be the most important inflammatory cytokines involved in liver fibrosis, because there is higher TGF-β expression in activated HSC; TGF-β has potency in upregulating extracellular matrix expression; there is higher expression of TGF-β receptors on HSC; and TGF-β1 increases the expression of tissue inhibitor of metalloproteinases-1 which plays an important role in promoting liver fibrosis and inhibiting liver regeneration (50). Consequently, many antifibrosis strategies revolve around reducing the secretion of TGF-β and blocking its signal transduction pathway to reduce TGF-β induced HSC proliferation. The results of the present study clearly demonstrated the inhibitory effect of TE on the hepatic TGF-β level in our experimental animal model of liver fibrosis.

In conclusion, the results of this study demonstrate that tocotrienol supplementation can have beneficial effects on liver functions and an effective role for the prevention of CCl_4 induced hepatic damage in rats. Our results show that the hepatoprotective effects of TE may be due to an increase in the activity of the antioxidant defense system, inhibition of lipid peroxidation and inhibition of HSC acti-

Volume : 6 | Issue : 4 | April 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

vation. So, TE might be a therapeutic anti-fibrogenic candidate for the treatment of hepatic fibrosis in the future.

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Author contribution Abeer F. Mostafa and Mohammed Adel designed and performed research. Abeer F. Mostafa also analyzed data, and wrote the paper

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