Investigation of the effect of vitamin E against carbon tetrachlorur hepatotoxicity and alterations of glutathione, glutathione peroxidase

Karbon tetraklorür hepatotoksisitesine karşı E vitamini etkisinin ve glutatyon, glutatyon peroksidaz değişikliklerinin araştırılması

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ÖZET: *Ratlarda E vitamininin karbon tetraklorür (CCl4) hepatotoksisitesine karşı etkileri ile glutatyon (GSH) ve glutatyon peroxidaz (GSH-Px) değişiklikleri incelendi. Bu amaçla, karaciğerde hücresel hasarın göstergesi olan lipid peroxidasyonu ile beraber, GSH, GSH-Px ve hepatotoksisitesinin biyokimyasal göstergeleri olan serum enzim aktiviteleri de değerlendirildi. Toplam 32 rat eşit ayrıldı: Kontrol (Grubu I); CCl4 (Grup II), E vitamini+CCl4 (Grup III) ve E vitamini (Grup IV). Grup II'deki ratlara tek doz (0.5 ml/kg) intraperitoneal (i.p.) olarak CCl4 enjekte edildi. Grup III'deki ratlara tek doz (0.5 ml/kg) CCl4 enjeksiyonundan hemen sonra 30 mg E vitamini (dl-alfa tokoferol asetat) i.p. olarak uygulandı. 24 saat sonra, serum transaminaz (AST, ALT) düzeylerini belirlemek içinde kan örnekleri; lipid peroxidi, GSH ve GSH-Px düzeylerini ölçmek için doku örnekleri alındı. Bütün gruplar kontrol grubuyla karşılaştırıldığında; Grup II ve III'de karaciğer lipid peroxid düzeyleri (P<0.001) ile serum AST (P<0.001) ve ALT (P<0.001) aktivitelerinin anlamlı şekilde yüksek olduğu saptandı. Grup IV'de, karaciğer lipid peroxid, GSH, GSH-Px ile serum transaminazlarında anlamlı bir değişiklik saptanmadı (P>0.05).*

Karbon tetraklorür grubuyla karşılaştırıldığında; Grup III'de, karaciğer lipid peroxid (P<0.02) düzeyleri ile ALT (P<0.005) düzeylerinin anlamlı olarak düştüğü, GSH, GSH-Px ve AST düzeylerinin ise değişmediği gözlendi (P>0.05). Grup IV'de, karaciğer lipid peroxid, GSH, AST ve ALT aktivitelerinin anlamlı olarak düştüğü (P<0.001), GSH-Px (P<0.02) aktivitelerinin ise yüksek olduğu saptandı.

Sonuç olarak, E vitaminin CCl4'ün bilinen hepatotoksik etkisini önemli derecede etkilemediği, E vitamininin protektif etkisinin genel kanının aksine henüz tartışmalı sayılabileceği ve bu konuda daha ileri çalışmalara gereksinim olduğu görüşüne varıldı.

Anahtar Kelimeler: **Karbon tetraklorür, Hepatotoksisite, Lipid peroxidasyonu, Glutatyon, Glutatyon peroxidaz, E vitamini**

SUMMARY: *The protective effect of vitamin E against carbon tetrachloride (CCl4) hepatotoxicity, glutathione (GSH) and glutathione peroxidase (GSH-Px) alterations were investigated in rats. Lipid peroxidation, which is an indicator of cellular injury in the liver, GSH, GSH-Px and serum enzyme activities (biochemical indicators of hepatotoxicty) were evaluated to this end. Totally, 32 rats were divided into four equal groups: Control (Group I), CCl4 (Group II), vitamin E + CCl4 (Group III), and vitamin E (Group IV). A single dose (0.5 ml/kg) of CCl4 was injected intraperitoneally (i.p.) to the rats in group II, Thirty mg of vitamin E (dl-alpha tocopherol) was intraperitoneally administered following a single dose (0.5 ml/kg) of CCl4 injection to the rats in the group III. After 24 hours, blood samples were taken for ALT and AST determinations. Tissue samples were taken to measure lipid peroxidation, GSH and GSH-Px levels. When all groups were compared to the control group, it was found that liver lipid peroxide levels (P<0.001), and serum AST (P<0.001) and ALP (P<0.001) activities were significantly higher in group II and group III. In addition, it was found that liver GSH (P<0.001) levels descreased, while GSH-Px activity was unchanged. No significant alteration was detected in liver lipid peroxide, GSH, GSH-Px and serum transaminases in group IV, compared to the control group (P>0.05). When compared to the carbon tetrachloride group, it was observed that liver lipid peroxide (P<0.02) levels and ALT (P<0.005) levels significantly decreased, while GSH, GSH-Px and AST levels remained unchanged in group III (P>0.05). In group IV, it was determined that liver lipid peroxide, GSH, AST and ALT activities were significantly lower, (P<0.001), while GSH-Px (P<0.02) activities were higher.*

We are of the opinion that vitamin E does not significantly affect the known hepatotoxic effect of CCl4, that the protective effect of vitamin E is still controversial, and that further studies are needed on this matter.

Keywords: **Carbon tetrachloride, Hepatotoxicity, Lipid peroxidation, glutathione, Glutathione peroxidase, Vitamin E**

IN some recent studies, it has been reported that there is a relation between vitamin E and some liver diseases (1,2). The liver microsomal membrane is an important source of free radicals due to its structure, and the liver is therefore the most vulnerable organ to tissue damage resulting from free radicals, especially that of xenobiotic metabolism (3). Oxidative stress is important in the pathogenesis of various inflammatory and degenerative events. It is thought that many pathologic events are associated with peroxidation of biologic membrane lipids. Reactive oxygen metabolites lead to changes in membrane permeability by increasing lipid peroxidation in the cell membrane, affecting cell organoids. Thus, degeneration takes place in liver and muscle cells. Radiation, hepatotoxic agents and other xenobiotics increase lipid peroxidation rapidly (4-6).

The toxic effect of carbon tetrachloride (CCl4) on the liver is via hepatic microsomal cytochrome P-

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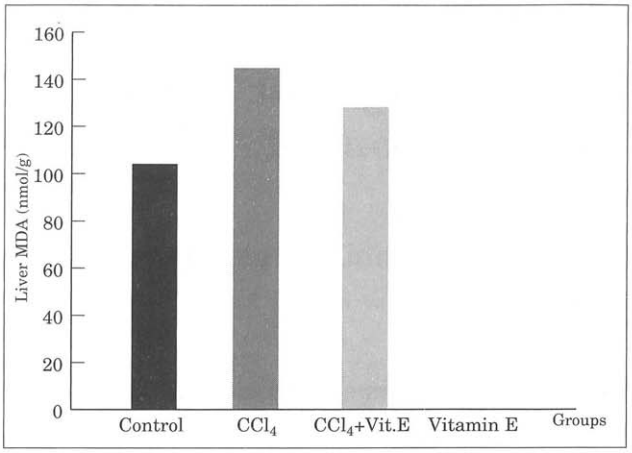
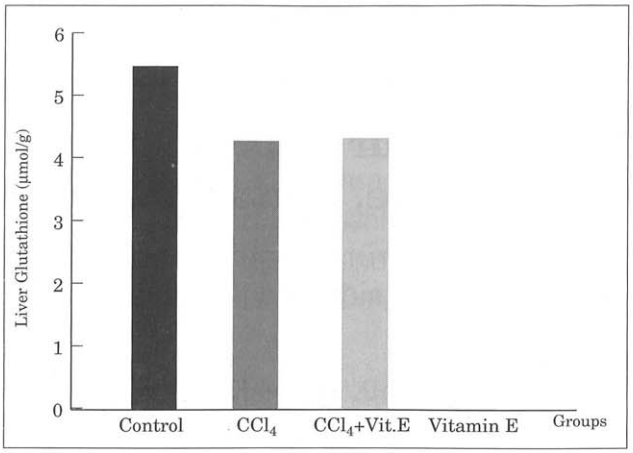


*CCl_4, *2CCl_4 + Vitamin E, cVitamin E

Figure 1. *Alterations in liver MDA levels of all groups.*
*p<0.001 (Compared to control group)
[a]p<0.02; [c]p<0.001 (Compared to CCl_4 group)



*CCl_4, bCCl_4 + Vitamin E, cVitamin E

Figure 2. *Alterations in liver glutathione levels of all groups.*
*p<0.001 (Compared to control group)
[b]p<0.05; [c]p<0.001 (Compared to CCl_4 group)

450. Cytochrome P-450 is responsible for the metabolizm of CCl4 to these reactive products of CCl4. CCl4 is metabolised and triclormethyl peroxil radicals (CCl3O2) are formed. These substances lead to CCl4 hepatotoxicity by starting lipid peroxidation in the membranes (2,7).

It has been reported that vitamin E makes the invasion phase of lipid peroxidation shorter by preventing the formation of free radicals which lead to lipid peroxidation. It defends membrane lipids against peroxidation by holding free radicals; in this way, it maintains the integrity of cellular and subcellular membranes in all mammalian cells (2,8).

Both vitamin E and GSH-Px are major antioxidant agents of the organism. Decrease in antioxidant activities of selenium (Se), glutathion peroxidase (GSH-Px) and vitamin E leads to increase in oxidative stress, and then to membrane destruction (9). Futhermore, it has been reported that vitamin E also plays a key role in the suppression of the agents interacting with glutathione (10). External intake of vitamin E prevents hepatic damage resulting from hepatotoxic agents (8).

In this study, the effect of vitamin E as a powerful antioxidant agent, on CCl4 hepatotoxicity, and on GSH, GSH-Px and serum transaminase levels in CCl4 hepatitis was investigated.

MATERIALS AND METHODS

Ten week old adult male wistar albino rats, (mean 180-220 gr) provided from DÜSAM, (Research Center for Health Sciences, Dicle University) were used. Enough water and standard rat fodder were given to the rats, which were placed in separate lattices. A total of 32 rats were divided into four equal groups.

Group I. Control: Sterile normal saline (%0.9 NaCl) was intraperitonally (i.p.) injected in equal volumes into the rats (n=8).

Group II. Carbon tetrachloride: A single dose (0.5 ml/kg) of CCl4 was i.p. injected (n=8).

Group III. CCl4 + vitamin E: Following a single dose (0.5 ml/kg) injection of CCl4, 300 mg of vitamin E (dl-alpha tocopherol acetate) was i.p. injected (n=8).

Group IV. Vitamin E: 300 mg of vitamin E (dl-alpha tocopherol acetate) was i.p. injected (n=8). 24 hours after injections, blood and liver tissue samples of all the rats were obtained under anesthesia, and prepared for analysis. Lipid peroxide, GSH and GSH-Px levels in the liver tissue taken, ALT and AST activities in serum were measured.

After the liver was perfused with normal saline the malondialdehyde (MDA) level, which is the end-product of lipid peroxidation, was measured by Ohkawa's thiobarbituric acid (TBA) method (11). In the measurements, the results were expressed as nmol MDA/g tissue by using molar extinction coefficient (1.56x105 M-1 x cm-1). Liver GSH levels were measured by the dithio nitrobenzen (DTNB) method described by Ellman (12), and the results were expressed mmol GSH/g tissue. Liver GSH-Px activity was measured by the method

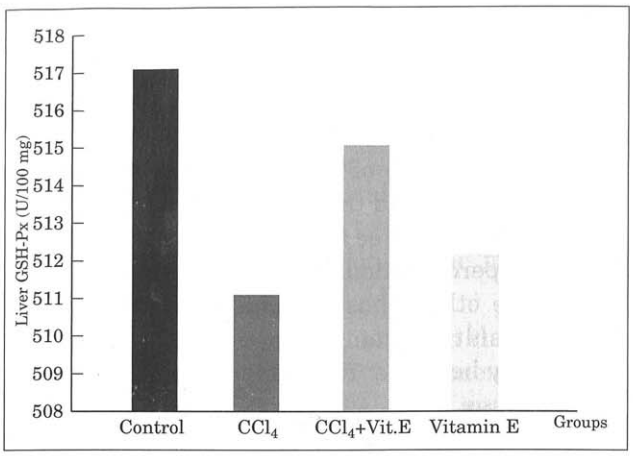


Figure 3. *Alterations in liver GSH-Px activity of all groups.*
$^ap<0.02$; $^bp<0.05$ (Compared to CCl_4 group)

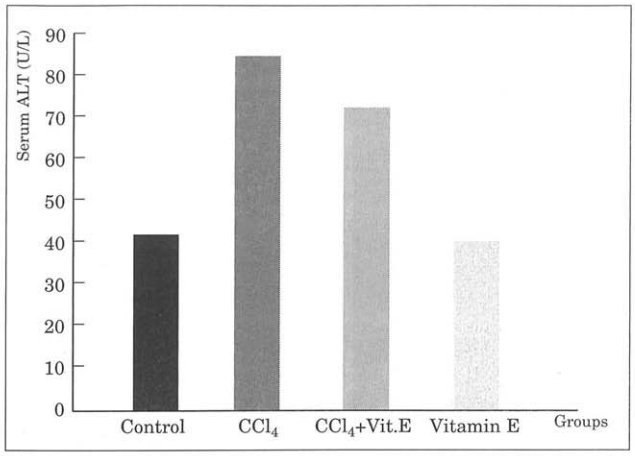


Figure 4. *Alterations in serum ALT activity of all groups.*
*p<0.001; **p>0.05 (Compared to control group)
$^bp<0.05$; $^cp<0.001$ (Compared to CCl4 group)

of Hafeman, Sunde and Hoekstra (13). When the decrease of log (GSH) per minute in enzymatic reaction was substracted from the decrease of log (GSH) per minute in non-enzymatic reaction, each 0.001 unit reduction calculated was identified as 1 enzyme unit, and results were pointed out as Unit/100 mg tissue.

AST and ALT activities were measured in Abbott spectrum autoanalyzer by the enzymatic-colorimetric method.

In the statistical evaluation of the results, the difference between averages of the two experiments series was determined by "Student's t" test.

RESULT

Liver MDA, GSH, GSH-Px levels, and serum ALT an AST enzyme activities, which were measured in the control and experiment groups, are presented in Table II.

1. Liver MDA levels (Figure-1):

Liver MDA levels were increased in the CCl4 and CCl4 + vitamin E groups when compared to controls (p<0.02). Compared to the carbon tetrachloride group; a decrease was found in liver MDA levels in both the CCl4 + vitamin E (p<0.02) and vitamin E (p<0.001) groups.

2. Liver GSH levels (Figure-2):

Compared to the control group; while GSH levels in CCl4 and CCl4 + vitamin E groups were found to be decreased (p<0.001), any alterations were not statistically determined in the vitamin E group (p>0.05).

Compared to the carbon tetrachloride group; it was determined that there were no differences in GSH levels (p>0.05) in the CCl4 + vitamin E group, while GSH levels descreased statistically significantly in the vitamin E group (p<0.001).

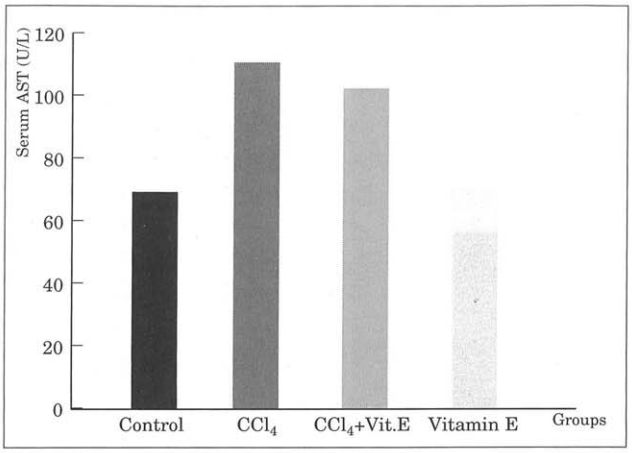
3. Activities of liver GSH-Px (Figure-3):

Compared to the control group; no statistical alterations were found in liver GSH-Px levels in all the groups (p>0.05).

Compared to carbon tetrachloride it was observed

Table 1. Experiment Plan

Experiment Group	*Rat Number*	*Treatment*	*Dose*	*Time*
I	8	Control		24 hours
II	8	CCl4	0.5 ml/kg	24 hours
III	8	CCl4+Vitamin E	0.5 ml/kg+300 mg	24 hours
IV	8	Vitamin E	300 mg	24 hours



*CCl_4, $^bCCl_4$ + Vitamin E, ^{c}Vitamin E

Figure 5. *Alterations in Serum AST activity of all groups.*
*p<0.001 (Compared to control group)
^{b}p<0.05; ^{c}p<0.001 (Compared to CCl_4 group)

that there were not any differences in GSH-Px activities in the CCl4 + Vitamin E group (p>0.05), while they were higher (p<0.02) in the vitamin E group.

4. Activities of serum ALT and AST (Figure-4-5):

Compared to the control group; serum ALT and AST activities were significantly higher in both the CCl4 and CCl4 + vitamin E groups (p<0.001), while no statistical differences were found in ALT and AST activities in the vitamin E group (p>0.05).

Compared to carbon tetrachloride in the CCl4 + vitamin E group, it was observed that ALT levels were significantly lower (p<0.05), while the changes in AST levels were statistically insignificant (p>0.05). In the Vitamin E group, serum ALT and AST activities were statistically lower (p<0.001).

DISCUSSION

Lipid peroxidation is a process which includes enzymatic and non, enzymatic systems. Lipid peroxidation, stimulated by toxic agents, affects the structural integrity of biologic membranes (2,5,6). It has been reported that CCl4 may cause cellular injury by forming free radicals (14-17). It is known that lipid peroxidation present in liver membrane affects the other phospholipids successively (18). Events resulting from the action of free radicals can mainly be prevented in two ways:

1. Along with catalase and superoxide dismutase, glutathione peroxidase brings about a system aimed at defending the organisms from prooxidant molecules (free radicals and other secondary oxidizer substances).

2. Vitamin E behaves as a chain-broker antioxidant agent by holding chain distributor peroxil radicals. Vitamin E scavenges free radical secondary substances, which are formed both in the process of food storage and in the process of peroxidation of polyunsaturated fatty acids in the gastrointestinal canal (2).

In recent studies, the effect of vitamin E against CCl4 hepatotoxicity has been investigated (2,16). In some studies, it has been determined that vitamin E prevents peroxidative tissue damage by its powerful antioxidant properties (2,4,16). In addition, it has been shown that vitamin E deficiency is an adverse factor in the development of various liver diseases and that administration of vitamin e in these diseases exerts a protective effect against hepatotoxic agent-induced hepatic injury (16,19). However, other studies have not shown an antioxidant protective feature of vitamin E (2,20).

It has been suggested that CCl4 stimulates lipid peroxidation, but this stimulation become clearer

Table 2. Alterations in liver MDA, GSH, GSH-Px levels and serum ALT and AST enzyme activities of all the groups.

Experiment Groups (nmol/g)	MDA (mmol/g) X±SD	Glutathione U/100 mg X±SD	GSH-Px (U/L) X±SD	ALT (U/L) X±SD	AST X±SD
Control	106.5±9.66	5.62±0.35	517.1±6.05	42.44±7.32	71.2±4.96
CCl4 (n=8)	144.17±7.91*	4.36±0.33*	511.2±6.23	86.8±3.63*	113.18±7.95*
CCl4+Vit.E (n=8)	133.62±7.46*a	4.43±0.21*b	515.3±6.50b	75.40±8.31*b	106.66±4.38*b
Vitamin E (n=8)	104.68±3.03c	5.25±0.39c	513.4±4.05a	40.98±3.60c	69.97±5.31c

*P<0.001; (Compared to control group)
aP<0.02; bP>0.05; cP<0.001 (Compared to CCl4 CCl4 group)

in the different fractions of the cell (14-16). The toxic effect of carbon tetrachloride on the liver is via hepatic microsomal cytochrome P-450. Cytochrome P-450 is responsible for CCl4's metabolism to its reactive products. CCl4 is metabolised and trichloromethyl peroxil radicals (CCl3O2) are formed. These substances lead to CCl4 hepatotoxicity by starting lipid peroxidation in the membranes (2,20).

Even though some reactions between the non-ionized CCl3O2 and vitamin E have been reported, the mechanism of protective effect against CCl4 hepatotoxicity has not been clarified entirely (2). However, it has been reported that this effect may probably depend on the dose of CCl4, and concentrations of antioxidants and vitamin E in the liver (20). Angelow et al. (19) explained the causes of selective damage that CCl4 brings about over cytochrome P-450, as the presence of many local factors altogether. It has been suggested in the metabolisation of CCl4 to CCl3, factors such as lipid peroxidation, micro structure of phospholipid, oxygen pressure, concentrations of antioxidant and "Hem" reducing enzyme activity may be effective.

In our study, we determined that lipid peroxidation increased significantly in the CCl4 and CCl4 + vitamin E groups, relative to those of the control group, that vitamin E administration did not significantly affect the enhancement of lipid peroxidation compared to CCl4 group, ant that a significant lipid peroxidation decrease was found in CCl4 + vitamin E and vitamin E groups. Our results are consistent with other studies showing that vitamin E has no effect against CCl4 hepatotoxicity (2,20).

Some investigators have demonstrated that glutathione has important effects on CCl4 hepatotoxicity (6,21-23). Lipid peroxidation isei controlled by glutathione in hepatocytes. The most important mechanism for detoxification of oxidants is the GSH redox cycle. This cycle is related with the hexose monophosphate shunt (5). Metallic ions such as cadmium, mercury, lead, CCl4 and antibiotics inactivate this system by their binding cepability to glutathione sulphydrile groups. As a result, efficiency of glutathione on defence mechanisms decreases (5). It has been suggested that liver glutathione levels are decreased by the effect of xenobiotics such as bromobenzen paracetamol, besides CCl4, and this decrease raises the toxic effects of xenobiotics. Also, it has been shown that the presence of liver GSH levels in normal ranges in an important factor to prevent xenobiotic toxicity (21,22,24). In a study, in which the relation between CCl4 hepatotoxicity and GSH content of liver was investigated, it was reported that there is no relation between CCl4- induced hepatic injury and liver GSH content (14). However, most researchers have found a decrease in GSH levels resulting from CCl4 hepatotoxicity (17,21,24,25).

We detected significantly decreased GSH levels in the CCl4 and CCl4 + vitamin E groups, when compared to control group. Compared to the CCl4 group, we found that there were no significant alterations in the CCl4 + vitamin E group, while glutathione levels in vitamin E group increased. Our results, which are consistent with the literature have shown that CCl4 hepatotoxicity increases GSH levels.

Hepatotoxic agents lead to depression of the activities of antioxidants (such as GSH-Px) (21,22,25). GSH-Px defends the cells from endogenous and exogenous oxidizing agents. It creates a defense system against the organic hyperperoxide or H2O2 by reducing oxidizing glutathione (GGGS), and prevents lipid peroxidation that is formed by oxygen and free radicals (5,21,24). It has been shown that antioxidants exhibit a protective effect, depending on the dose, against the CCl4 hepatotoxicity (21).

In our study, it was not found that liver GSH-Px levels attered significantly in the CCl4, CCl4 + vitamin E and vitamin E groups, relative to those of control group. When compared to the CCl4 group, an important difference was not found in the CCl4 + vitamin E group, while an increase was observed in the vitamin E group. Our results suggest that CCl4 administration does not effect liver GSH-Px levels in rats, and this demonstrates consistency with the, literature (25).

It has been demonstrated that CCl4 increases serum enzyme activities, while vitamin E perevents this increase (2,15,25). Compared to the control group; we determined that serum ALT and AST activities of CCl4 and CCl4 + vitamin E groups increased significantly, depending on CCl4 hepatotoxicity. Compared to the CCl4 group, it was found that there was no change in serum transaminase activity in the CCl4 + vitamin E group, while a significant decrease was observed in the vitamin E group. Our study has demonstrated that vitamin E has no effects on increased transaminase levels resulting from CCl4 hepatotoxicity. This re-

sult is also consistent with the literature (2,20).

Some investigators suggest that antioxidants and their combinations (such as vitamin E, sodium selenite, ubiquinone-9) block lipid peroxidation, have a protective effect on CCl4 - induced rat liver injury, and decrease the ALT and AST activities in blood serum significantly (14,15). However, we are of the opinion that vitamin E does not significantly affect the known hepatotoxic effect of CCl4, that protective effect of vitamin E is still contraversial, and that further studies are necessary on this subject.

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