Hepatoprotective Effect of Caffeic Acid Phenethyl Ester (CAPE) on Carbon Tetrachloride (CCl4) Induced Acute Hepatotoxicity in Rats

Karbontetrakloridle Akut Hepatotoksite Oluşturulan Ratlarda Caffeic Acid Phenethyl Ester (CAPE)'in Karaciğeri Koruyucu Etkisi

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ABSTRACT

In this study, hepatoprotective activity of CAPE, which has antiinflammatory, anticancer, antiviral, and immunomodulatory effects, was investigated in the CCl4 induced liver injury model in rats. Twenty-one Sprague Dawley rats were divided into the three groups: Control (n=7), CCl4 (n=7), CCl4 + CAPE (n=7). The serum levels of AST, ALT, LDH, and bilirubin were measured on the first and last day (10 th day) in all experimental animals. Histopathological examinations were carried out in the rats sacrificed on the tenth day. For histopathological evaluation, livers of all rats were removed and processed for light microscopy. The levels of unconjugated bilirubin, AST, ALT, ALP in CCl4 group were markedly higher than those in the control group ($p<0.01$, $p<0.01$, $p<0.001$ and $p<0.05$ respectively). The values of these parameters in CAPE+CCl4 group were lower than those in CCl4 ($p<0.01$, $p<0.05$, $p<0.05$, $p<0.001$ respectively). Histopathological findings also corfirmed that CCl4 induced hepadic damage and support the wiev that CAPE has a hepatoprotective activity. The results of this study indicate that CAPE has a hepatoprotective action in acute liver injury.

Key words: *Carbon tetrachloride(ccı4), acute hepatotoxicity, caffeic acid phenethyl ester(cape), rat.*

ÖZET

Bu çalışmanın amacı antiinflamatuar, antikanser, antivitral ve immünomodulatuar etkileri olan CAPE'nin karaciğeri koruyucu aktivitesini CCl4 ile oluşturulan karaciğer hasar modelinde araştırmaktır. Yirmibir Sprague Dawley ratlar üç gruba ayrıldı: Kotrol (n:7), CCl4 (n:7), CCl4 + CAPE (n:7). Bütün hayvanlarda birinci ve son günde (onuncu gün) AST, ALT, LDH ve bilirubin serum düzeyleri ölçüldü. Histopatolojik incelemeler onuncu günde sakrifiye edilen ratlarda yapıldı. Histopatolojik değerlendirme için ratların karaciğerleri çıkarıldı ve ışık mikroskopu için işlemden geçirildi. Unconjugated bilirubin, AST, ALT, ALP düzeyleri CCl4 grupta kontrol grubundakilerden belirgin olarak daha yüksekti (sırasıyla p<0.01, p<0.01, p<0.001 ve p<0.05). Bunların değerleri CAPE+CCl4 grupta CCl4 grubundakilerden daha düşüktü (sırasıyla p<0.01, p<0.05, p<0.05, p<0.001). Histopatolojik bulgular aynı zamanda CCl4 ile oluşturulan hepatic zarara sebep olduğunu ve CAPE'nin karaciğeri koruyucu aktiviteye sahip olduğunu gösterdi. Bu çalışmanın sonuçları akut karaciğer hasarında CAPE'nin karaciğeri koruyucu etkiye sahip olduğuna işaret eder.

Anahtar kelimeler: *Karbon tetrachlorid, akut hepatotoksisite, caffeic acid phenethyl ester, rat.*

INTRODUCTION

Numerous substance are known to cause liver damage. One of these chemicals is carbon tetrachloride (CCl4) which is a xenobiotic that induces hepatotoxicity in humans as well as in animals (1,2). Carbon tetrachloride (CCl4), awell-known model compound for producing chemical hepatic injury, is biotransformed by hepatic microsomal cytochrome P450 to trichloromethyl-free radicals (CCl3) (3-5) In addition, CCl4 induces elevated levels of hepatic enzymes in serum that are markers of liver cell damage (6). Furthermore, histopathological changes occur in the liver after CCl4 administration (6,7-10).

Caffeic acid phenethyl ester (CAPE) is an active component of honeybee propolis extracts and has been used for many years as a folk medicine (11) CAPE is a small, lipid-soluble flavonoid-like compound that has anti-inflammatory (12) antiviral, immunomodulatory (13) antitumoral (14) neuroprotective (15) and anti-atherosclerotic (16) and antioxidant activities (17). It has been found that CAPE has protective effects against carbon tetrachloride- induced liver in the crhonic period (18) and kidney injuries in rats (19,20) and mice (21) and against cisplatin- induced hepatic oxidative damage (22). In vitro studies showed that CAPE is effective against experimentally produced liver toxicity (21). It has antiproliferative and antioxidant properties and has been shown to inhibit lipooxygenase activities as well as suppress lipid peroxidation (23-27). It was shown in a previous study that CAPE preserved heart tissue from doxorubicin-induced oxidant injury (28).

The aim of the present study was to investigate whether treatment of rats with CAPE prior to CCl4 administration prevents CCl4-induced acute hepatotoxicity. For this purpose, we designed to determine the histopathological effects of CCl4 and the possible protective effect of CAPE on acute phase tissue damage of rat liver.

MATERIALS AND METHODS

Animals and treatment

Twenty-one Sprague Dawley rats, initially weighing 150-170 gr. at 14-16 weeks old, were used in this study. The animals were fed with a Standard diet, kept on a physiological day/night rhythm and maintained in an ambient temperature of 22 °C during the experimental procedures. The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals.29 Rats were randomly divided into three groups: rats given CCl4 (0.8 ml/kg body weight per 1ml olive oil, Merk KgaA, 64271 Darmstad, Germany) as a CCl4-induced acute hepatotoxicity model (n = 7); rats given CCl4 plus CAPE (20 µgr/kg body weight per 1ml olive oil) (n = 7); and rats given isotonic saline solution (0.2 ml/kg body weight) alone as a control group (n = 7). Application of CAPE in CCl4 induced acute hepatotoxicity

The CAPE was synthesized by the standard method of Grunberger11 and administered intraperitoneally once a day at a dose of 20 µgr/kg body weight. The first dose of CAPE was given 3 day prior to CCl4 injection and continued until sacrifice.

Anesthesia

Rats were anesthetized with a cocktail of ketamine hydrochloride (50mg/kg) and xylazine (5 mg/kg) which were administered intraperitoneally (i.p.) before the animals were sacrificed.

Serum biochemistry

Table 1. *Biochemical parametes in serum and statistical comparisons of the groups.*

Parameters	*Control*		*CCl4*		*CCl4+CAPE*	
	n	*Mean ±SEM*	*n*	*Mean ±SEM*	*n*	*Mean ±SEM*
AST	*7*	*193.40±11.88*	*7*	*2192.16±592.99b*	*7*	*1008.00±162.79d*
ALT	*7*	*63.80±2.96*	*7*	*1363.83±348.62c*	*7*	*685.66±136.48 d*
ALP	*7*	*354.25±19.36*	*7*	*487.50±48.59a*	*7*	*567.83±22.41c*
Unconjugated bilirubin	*7*	*0.112±0.007*	*7*	*0.495±0.114 b*	*7*	*0.198±0.032e*
Conjugated bilirubin	*7*	*0.050±0.004*	*7*	*0.213±0.099 b*	*7*	*0.041±0.005 e*

Results os post hoc LSD (least-squares difference test), a:Statistical difference with control group at p< 0. 05. b:Statistical difference with control group at p< 0.01. c:Statistical difference with control group at p< 0.001. d: Statistical difference with CCl4 group at p <0.05. e: Statistical difference with CCl4 group at p< 0.01.

ALT, AST and ALP serum activities were measured on the first and last day (10 th day) in all experimental animals to assess hepatotoxicity. ALT, AST, ALP, conjugated and unconjugated bilirubin activities in blood serum were evaluated by an autoanalyzer using spectrophotometric diagnostic kits.

Histopathological analysis of liver

Liver tissue specimens were fixed in neutral formalin solution (10%). Tissue specimens were embedded in paraffin wax and sectioned (thickness, 3 μm). For histopathological evaluation, sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome, and examined with a BX50-3 Olympus light microscope (Olympus Optical, Tokyo, Japan). Number of mitotic and apoptotic cells, and ballon degeneration was calculated per 10 high-power fields.

Statistical analysis

Data are expressed as means±standard deviation. The one-way analysis of variance (ANOVA) and post hoc multiple comparison tests (least-squares difference, Tukey's HSD) were performed on the data of biochemical and histopathological variables to examine the differences among groups. All analyses were made using the SPSS statistical software package; $p<0.05$ was considered statistically significant.

RESULTS

The effects of CAPE pretreatment on the CCl4-induced elevation of serum levels ALT, AST, ALP, unconjugated and conjugated bilirubin are shown in Table 1. CCl4 caused hepatotoxicity in rats, as indicated by the increases in ALT, AST and ALP serum levels. However, CAPE pretreatment reduced the CCl4-induced elevations in

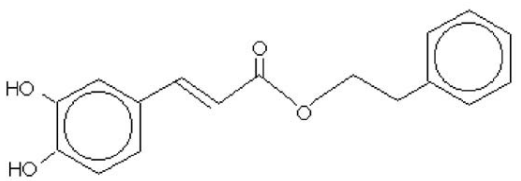

Figure 1*.The structure of caffeic acid phenethyl ester. Note that the two hydroxyl groups located in the first ring show antioxidant activity. The antioxidant activity of the compound depends not only on the hydroxyl groups or catechol rings but also on the partition coefficient or hydrophobicity of CAPE.*

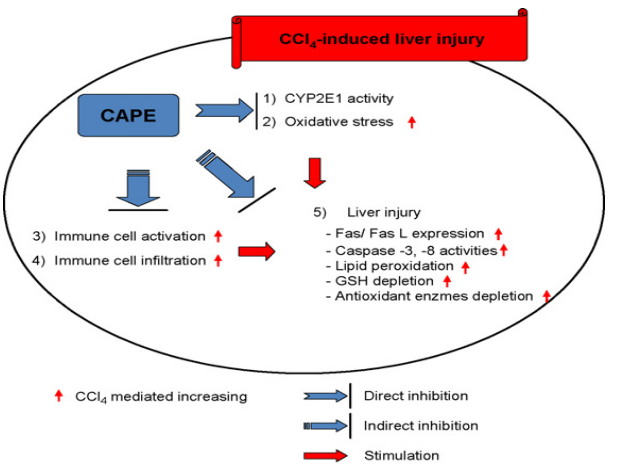

Figure 2*.Diagram shows that protective mechanisms of CAPE against CCl4-induced liver damage.*

Table 2. *Histopathological parameters and statistical comparisons of the groups.*

Parameters	Control		CCl4		CCl4+CAPE	
	n	Mean ±SEM	n	Mean ±SEM	n	Mean ±SEM
Balloon degeneration	7	0.12±0.10	7	31.50±4.00b	7	3.10±0.23d
Apoptosis	7	0.10±0.10	7	1.4±0.30a	7	0.50±0.22c
Mitoses	7	0.090±0.10	7	0.40±0.22e	7	0.20±0.13

Results os post hoc Tukey's HSD Test, a: Statistical difference with control group at p< 0.01. b: Statistical difference with control group at p <0.001. c: Statistical difference with CCl4 group at p< 0.05. d: Statistical difference with CCl4 group at p <0.001. e: Statistical difference with control group at p< 0.05.

ALT, AST, unconjugated and conjugated bilirubin serum levels (Table 1).

Livers of rats in the control group showed a normal histological appearance. Histopathological analysis showed classic histopathology in livers of rats treated with CCl4. Hepatic ballon degeneration, focal necrosis, and apoptotic cells were observed in rat livers. However, the severe hepatic lesions (necrosis, ballon degeneration,) induced by CCl4 were remarkably ameliorated by CAPE. Consistent with this histopathological data, apoptosis was also either markedly prevented or minimized in CAPE given group (Table 2). According to our biochemical and histopathological findings, it is concluded that CAPE treatment prevents CCl4-induced liver damage in rats.

DISCUSSION

CCl4, which is an intrinsic hepatotoxin, was used to induce hepatic damage in this study since it has previously been shown to exert its toxic effects on the liver (1,2). Administration of CCl4 to rats causes severe liver injury which is recognized histopathologically together with an increase in serum levels of the hepatic enzymes AST and ALT, which are indices of liver cell damage (6). Liver injury induced by CCl4 is a common model for screening the hepatoprotective activity of drugs because this chemical is a potent hepatotoxin and a single exposure can rapidly lead to severe hepatic necrosis and steatosis (30-32). Increases in serum AST, ALT and ALP levels by CCl4 have been attributed to hepatic structural damage because these enzymes are normally localized to the cytoplasm and are released into the circulation after cellular damage has occurred (33).

Some studies have shown that CCl4 administration causes increased serum levels of AST, ALT and ALP (6,7,9,34,35). The hepatotoxicity of CCl4 was confirmed in our study by a significant elevation of serum levels of AST, ALT, ALP, conjugated and unconjugated biluribin. Additionally, it has been reported that CCl4 causes necrosis (7,9,35-37) steatosis and ballon degeneration of hepatocytes, increase in mitotic activity (6) in liver. It has also been reported that CCl4 causes apoptosis in liver (9,10,38,39). We also observed all of those findings in CCl4-induced acute hepatotoxicity in rats.

Previous experimental studies have shown that lipid peroxidation can be prevented by CAPE in spinal cord and kidney after ischemia reperfusion (25,40). We have previously shown that CAPE administration prevents cisplatin-induced nephrotoxicity in rats (20). Similarly, Fadillioglu et al. (28) have reported that CAPE inhibits cardiotoxicity induced by doxorubicin in rat. Additionally, CAPE scavenges reactive oxygen species (ROS) and guards against GSH depletion in mice brain tissues (15). CAPE has these protective effects on the basis of antioxidant actions, but the exact mechanisms of anti-oxidant properties of CAPE are not known yet. However, it has been speculated that CAPE may affect transcription and/or translation of genes and gene products of anti-oxidant enzymes (20). It can be attributed to the presence of the two phenolic groups in its catechol ring (40). Like some other antioxidant compounds (i.e. vitamins C and E), CAPE can show antioxidant activity with its two hydroxyl groups located in one of the ring structures (Figure 1) (20).

In the present study, CAPE treatment significantly reduced elevated serum levels of AST, ALT, ALP, conjugated and unconjugated biluribin. Our results are supported by several reports that CAPE has protective effects against oxidative damage in various tissues (18,41-43). On the other hand, some studies has revealed that CAPE has inhibitory effect on neutrophil sequestration into the tissue protecting the tissue from ROS produced in huge amount in relevant tissues by neutrophils (44). Generally, neutrophils accumulate in liver damaged by toxicants, such as diethylnitrosamine and CCl4, and

then release inflammatory cytokines, like tumor necrosis factor, interleukin (IL)-1 and IL-6, which also have toxic effects in CCl4-induced liver injury (45). In previous studies, CAPE exhibited anti-atherosclerotic and anti-inflammatory activities via inhibition of NF-_B activity (46) and protected against Fas-mediated cell apoptosis (47). Consistent with these data, our results showed that CAPE dramatically prevented CCl4-induced liver injury by depleting balloon degeneration and preventing hepatic apoptosis.

In the recent study, Lee and colleagues (21) demonstrated that CAPE has potent hepatoprotective effects against CCl4-induced hepatic damage in mice. In this study, the hepatoprotective effects of CAPE may be caused by its ability to block the bioactivation of CCl4 by inhibiting CYP2E1 activity, in combination with its ability to scavenge free radicals. In addition, CAPE may be able to block infiltration and activation of neutrophils through its anti-inflammatory and anti-caspases activities by inhibiting Fas/FasL protein expression in CCl4-induced hepatic damage (Figure 2) (21).

In conclusion, We showed the protective effect of CAPE both histologically and biochemically in CCl4-induced hepatic damage. The results of this study indicate that CAPE has a hepatoprotective action in CCl4-induced acute liver injury.

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