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RESEARCH ARTICLE

Protective Effect of Caffeic Acid Phenethyl Ester Against Lead Acetate-induced Hepatotoxicity in Mice

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Summary

The toxicity of lead in the liver seems to relate to the generation of reactive oxygen species (ROS). Caffeic acid phenethyl ester (CAPE), a flavonoid like compound, is one of the major components of honeybee propolis which has been demonstrated to have antioxidant, free radical scavenger. This study was designed to investigate the protective effects of the CAPE against lead acetate-induced hepatotoxicities in mice. A total of 25 mice were equally divided into 5 groups which were: 1st control, 2nd injected with intraperitoneally (IP) %1 ethanol. 3rd injected with IP CAPE, 4th injected with IP lead-acetate, 5th injected with IP CAPE + lead acetate. Experimental animals were decapitated at the end of the study. Following systemic necropsies of the animals, tissue paraffin blocks were prepared and cut into 5 µm thick sections for hematoxylen-eosin staining. The samples were later monitored under a light microscope for histopathological changes. In conclusion, livers which were treated with 1% ethanol and CAPE were similar to control group. In the group which was treated with lead some degenerations and necrosis were observed. In the group which was treated with CAPE and lead, levels of necrosis and degenerations was observed in less degree.

Keywords: *Lead, CAPE, Histopathology, Liver*

Farede Kurşun Asetatla Oluşturulan Hepatotoksisiteye Karşı Kafeik Asit Fenetil Esterin Koruyucu Etkisi

Özet

Karaciğer'de kurşun toksisitesinin reaktif oksijen türlerinin (ROS) üretimiyle ortaya çıktığı düşünülmektedir. Yapıca flavanoidlere benzeyen, bal arısı propolisinin aktif bir bileşeni olan kafeik asit fenetil ester (CAPE)'in antioksidan ve serbest radikalleri yok edici özellikleri gösterilmiştir. Bu çalışma, farede kurşun asetatla oluşturulan hepatotoksisiteye karşı CAPE'nin koruyucu etkisini araştırmak için dizayn edilmiştir. 1. grup kontrol, 2. grup intraperitonal (IP) yolla %1 etanol uygulanan grup, 3. grup IP olarak CAPE uygulanan grup, 4. grup IP olarak kurşun asetat uygulanan grup, 5. grup ise IP olarak CAPE + kurşun asetat uygulanan grup olmak üzere toplam 25 fare 5 gruba ayrılmıştır. Çalışma sonunda hayvanlar dekapite edildi. Hayvanların sistemik nekropsilerinin ardından elde edilen dokular parafin bloklara alınıp, hematoksilen-eozinle boyanmak üzere 5 µm lik kesitler alınmıştır. Örnekler histopatolojik değişiklikleri gözlemlemek için ışık mikroskobunda incelenmiştir. Sonuç olarak etanol ve CAPE uygulanan gruptaki hayvanlara ait karaciğer, kontrol grubu hayvanlarının karaciğer dokusuyla benzerdir. Kurşun uygulanan gruptaki hayvanların karaciğerlerinde bazı dejenerasyon ve nekrozlar gözlemlenmiştir. CAPE ve kurşunun birlikte uygulandığı grupta nekroz ve dejenerasyonların şiddetinin azaldığı görülmüştür.

Anahtar sözcükler: *Kurşun, CAPE, Histopatoloji, Karaciğer*

INTRODUCTION

Lead is a toxic metal with no function in the body. As mines were taken out and started to be used on the earth, it spread into the biosphere and its amount in humans' body significantly increased in parallel with industrialization [1]. Upon widespread use of lead, both ecological balance has been unsettled and it has entered

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into the food chain resulting in vital health problems for humans [2]. The most important lead contamination sources: lead added to petrol, lead-based paints, lead soldered conserved food cans, ceramic glasses and industrial pollutions [3].

Lead may cause physiological, biochemical and behavioral malfunctions in the organism depending on amount [4]. Main targets of the lead are hematopoetic system, neural system and renal system. Besides, reproduction system and immune system were recorded to have been damaged by metal [5]. Also it is known to have undesired effects related with respiration and sight as well as hepatic system and reproduction [6].

Caffeic acid phenethyl ester (CAPE), a flavonoid like compound, and is an active component of honey bee propolis [7]. It has been used for alternative medicine in Middle Eastern countries for long years [8]. It has been shown in many studies that CAPE has antioxidant [9], anti-inflammatory [10], anti carcinogenic [11], antiviral [12], immuno-modulator [13] anti-hepatoxic [14], neuroprotective [15] and anti-atherosclerotic [16] effects. No hazardous effect of CAPE on normal cells has been reported so far [15].

In a study comparing antioxidant effects of galangin, one of the propolis components, with CAPE, it was observed that both components clear reactive oxygen species. Moreover; CAPE was shown to clear oxygen radical and reactive oxygen species generated by xanthine oxidase system more effectively than galangin and decrease the malondialdehyde (MDA) level far better than galangin [17].

In present study, lead acetate was given to mice to create hepatotoxicity and livers of the mice were investigated to observe protective effects by histopathological methods. As a consequence, the debate over whether or not CAPE will constitute a type of treatment for preventing potential damage to arise in case of exposure to lead will be clarified.

MATERIAL and METHODS

Animals and Treatment

Twentyfive adult mice which are 10-12 weeks old and weigh 30-35 gram were used in this research. Mice were acclimated to laboratory conditions with regular temperature control ranging from 23±2°C and with balanced diet and water *ad libitum*. Mice were divided into the 5 groups and each group had 5 mice. Group 1 was injected with intraperitoneally (IP) 1 ml serum physiologic (0.9% NaCl), group 2 was injected with IP 1% ethanol (Carlo Erba,USA), group 3 was injected with IP 8.5 mg/kg CAPE (≥97% pure, Sigma Aldrich, USA), and group 4 was injected with 50 mg/kg lead in the form of lead acetate (Sigma Aldrich, USA) via IP means for 5 days [18-20] and lead acetate was solved in 1 ml serum physiologic on a daily basis always before administration. Group 5 was injected with IP 8.5 mg/kg CAPE starting 3 days before the experiment, and injection of lead in the form of lead acetate with CAPE follow on for 5 days. CAPE solution was solved in 1% ethanol on a daily basis always before administration.

Histopathologic Examinations

After the last injection, liver tissues were dissected and received liver tissue was fixed in 10% formaldehyde solution for 48 h, and then paraffin blocks were prepared by means of routine histological methods. After that, cross sections with 5 μm. thickness were taken from those blocks and stained by means of hematoxylen-eosin staining method, and finally histopathological changes were monitored under the light microscope.

RESULTS

Morphological Findings

Macroscopic examination showed that bud-shaped oedema glands emerged in liver tissues of the animals given lead; liver was looked like faded. On the other hand, in the group administered CAPE together with lead, these formations were occured less and view of liver tissue was similar to the control group *(Fig. 1)*.

Microscopic Findings

Light microscopic examinations demonstrated that liver tissues of the animals administered CAPE had a view similar to normal *(Fig. 2)* while liver tissues of those administered ethanol of 1% noted slight degeneration and necrosis causing from administering alcohol *(Fig. 3)*. Severe degeneration, widespread necrosis areas and hydrophic and vacuolar degeneration were observed in the animals administered lead only *(Fig. 4)*. Whereas these formations were occurred less in the animals administered CAPE together with lead *(Fig. 5)*.

DISCUSSION

It was observed that severity of the degenerations was decreased in the animals administered lead together with CAPE in our study. Lead toxicity was studied by many scientist and its effects on various tissues and organs were determined [3,4,6,21-23]. Slight oxidative stress on hepatocytes was seen in the liver of the animals administered lead [21]. Daggett et al.[22] reported that lead application did not have effects on oxidative stress on rats' kidney structure whereas it decreased glutathione (GSH) levels and increased malondialdehyde MDA levels in the liver tissue. Nakagawa [23] determined that GSH levels

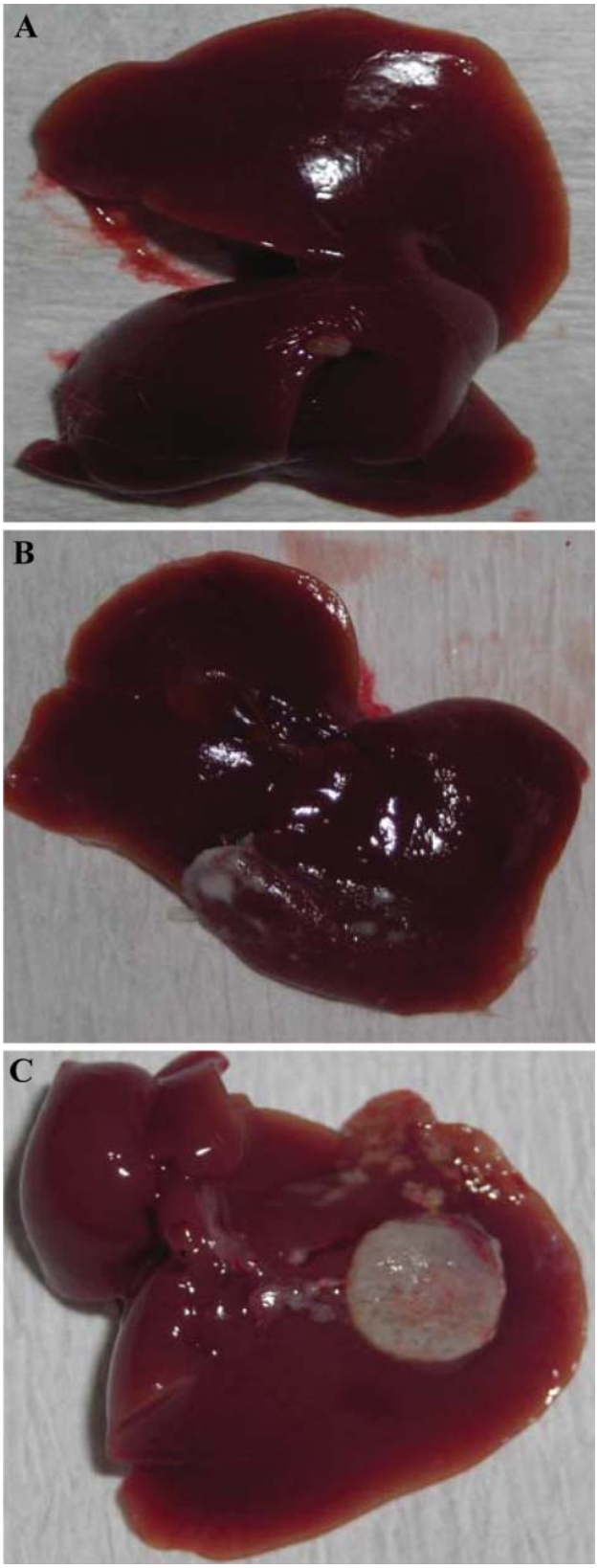

Fig 1. Macroscopic view of the liver tissues obtained from the control and experiment groups. A- Control group, B- Lead + CAPE group, C- Lead group

Şekil 1. Kontrol ve deneme gruplarından elde edilen karaciğer dokularının makroskobik görünüşü. A- Kontrol grubu, B- Kurşun + CAPE grubu, C- Kurşun grubu

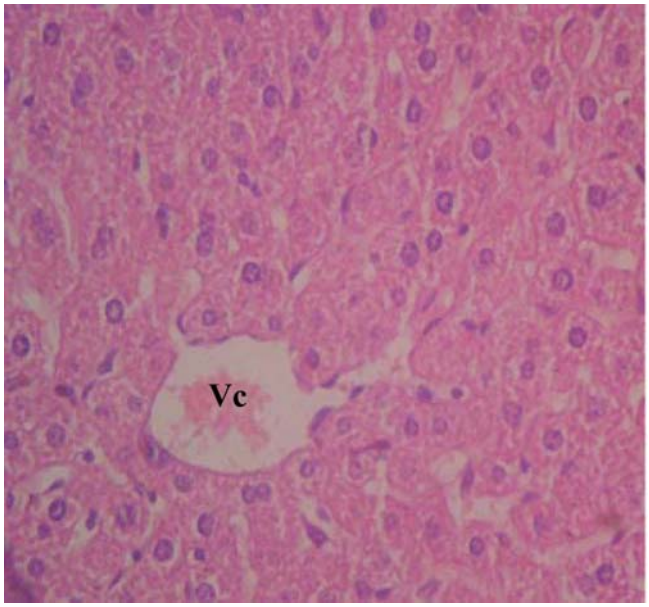

Fig 2. Microscopic view of the hepatocytes and sinusoidal structures in the liver tissue of the animals administered CAPE (Vc: Vena centralis), H-E x40

Şekil 2. CAPE uygulanan hayvanların karaciğer dokusunda hepatosit ve sinusoidal yapıların mikroskobik görünümü (Vc: Vena sentralis), H-E x40

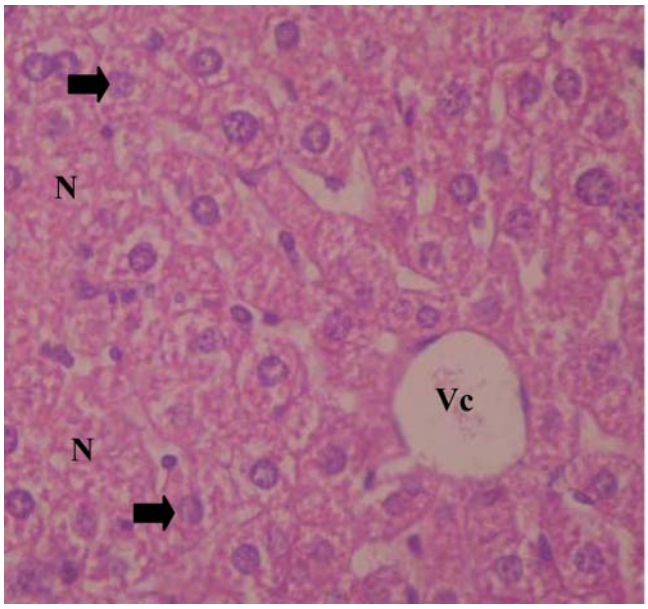

Fig 3. Focal necrosis areas (N) and hydropic degeneration (arrows) in the liver tissue of the animals administered ethanol (Vc: Vena centralis), H-E x40

Şekil 3. Etanol uygulanan hayvanların karaciğer dokusunda hidropik dejenerasyon (oklar) ve fokal nekrozis bölgeleri (N) (Vc: Vena sentralis), H-E x40

decreased depending on lead. In another study, Wang et al.[24] stated that administration to subcronical lead caused with decreases in hemoglobin, glutathione peroxidase (GSH-Px), superoxide dimutase (SOD) levels in the blood and GSH levels in the hepatocytes. It has been noted that

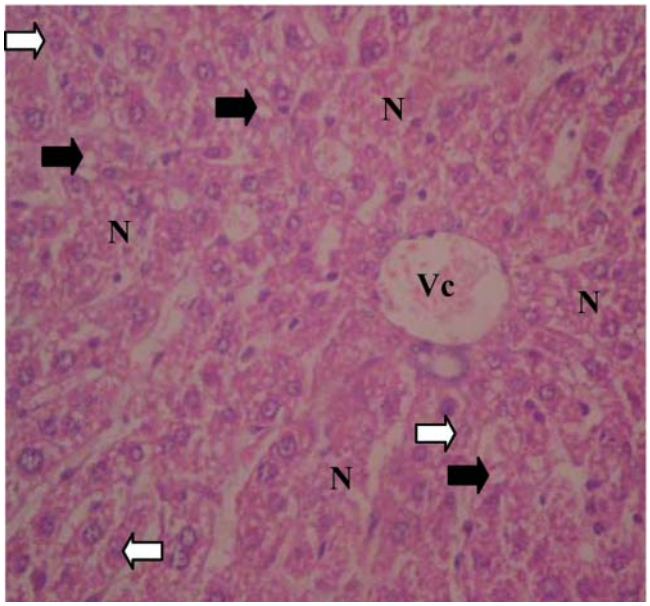

Fig 4. Microscopic view of the liver tissue of the animals administered lead. Widespread necrosis areas (N), hydropic (white arrows) and vacuolar degeneration (black arrows) areas (Vc: Vena centralis), H-E x40

Şekil 4. Kurşun uygulanan hayvanların karaciğer dokusunun mikroskobik görünümü. Yaygın nekrozis bölgeleri (N), hidropik (beyaz oklar) ve vakuolar dejenerasyon (siyah oklar) bölgeleri (Vc: Vena sentralis), H-E x40

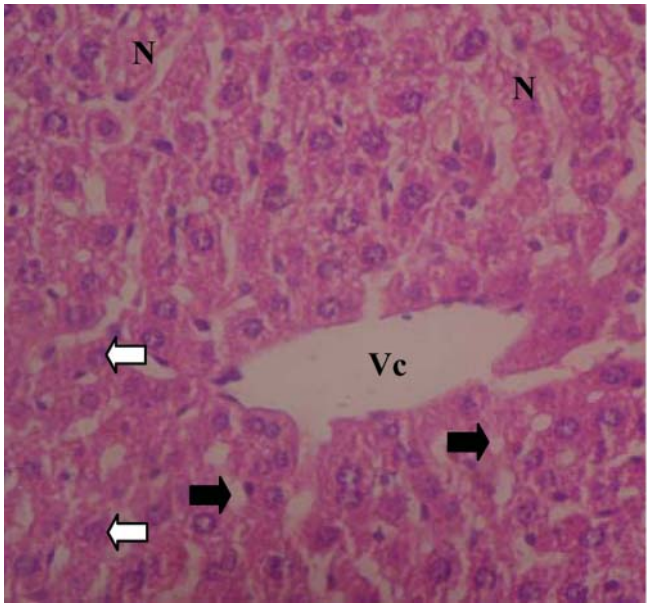

Fig 5. Microscopic view of the liver tissue of the animals administered lead together with CAPE. Focal necrosis areas (N), hydropic (white arrows) and vacuolar degeneration (black arrows) areas (Vc: Vena centralis), H-E x40

Şekil 5. CAPE ile birlikte kurşun uygulanan hayvanların karaciğer dokusunun mikroskobik görünümü. Fokal nekrosis (N) bölgeleri, hidropik (beyaz oklar) ve vakuolar dejenerasyon (siyah oklar) bölgeleri (Vc: Vena sentralis), H-E x40

nitric oxide (NO) level decreased due to the lead application [25].

Similarly, the effects of the lead were determined histopathologically in animal models administered lead. Shalan et al.[6], reported that the expansion of sinusoidal structures, mononuclear cell infiltration and focal necrosis areas were occured depending on exposure to lead. Furthermore, El-Sokkary et al.[26], noted that application of 100 mg/kg lead caused necrosis and cytoplasmic vacuolization in the liver and degeneration and necrosis were seen in the kidney of the rats. Carmignani et al.[25], found out that intoxication rising upon administration of 15-60 ppm lead in drinking water caused increases in hepatocytes' activities in the liver. In our study, degeneration and necrosis areas in the liver were similar to abovementioned literature in the animals administered lead [6,25,26].

Various mechanisms are involved in the process of ethanol-induced tissue impairment. Oxidative stress and its effects are among the most important. Most of the studies showed protective effects of the antioxidant agents in various models of ethanol induced injury [27].

Protective function of CAPE on antioxidant system and other tissues was demonstrated in many studies. Cüre [7], studied on protective role of CAPE against oxidative stress rising upon excessive iron administration in rats, and reported that the increase in the level of MDA, by the application of iron decreased due to administration of CAPE. Lee et al.[14], revealed the increase of the MDA level and decrease of the GSH level in the oxidative stress occurred by carbon tetrachloride whereas CAPE application relieved the effect of such oxidative damage, and as a consequence of histopathological examinations, degenerations together with focal necrosis areas emerged in hepatocytes and hepatic cordones in the liver tissues, and CAPE application helped to relieve severity of these effects on the liver. In another study, vacuolization in molecular layer, neuroglial cell infiltration and picnotic cells were determined in prefrontal cortex sections obtained from rats exposed to cigarette smoke. On the other hand, structural changes caused by exposure to smoke were significantly decreased rats injected CAPE together with exposure to cigarette [28]. Mollaoğlu et al.[29], identified the increase of the MDA level due to the cadmium effect and the decrease of NO level, but application of CAPE together with cadmium, NO level increased while MDA level decreased. In a similar study, Parlakpınar et al.[30], determined the increase of the MDA and NO levels while decreasing of SOD, CAT and GSH levels by the application of gentamisin (GEN), decreased MDA and NO levels while increased SOD, catalase (CAT) and GSH levels by the application of CAPE together with GEN. As a consequence of histopathological examination of kidneys revealed that tubular necrosis emerged apparently, but by the application of CAPE together with GEN severity of such necrosis decreased. In our study, it was found out that severity of degeneration decreased in liver tissues of the animals administered CAPE together

with lead. This protective effect may possibly thought to take place by means of CAPE's inducing antioxidant system and thus decreasing oxidative damage.

As a conclusion; lead which is widespread in the environment was toxic at 50 mg/kg dose, whereas CAPE had a preventive effect at the same dose of lead. In view of this data it may possibly was though that CAPE can be used for treatment purpose against potential hazardous effects that might rise in humans and other living creatures due to lead toxication.

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