# Effects of caffeic acid phenethyl ester on isoproterenol-induced myocardial infarction in rats

## *Kafeik asit fenetil esterin sıçanlarda isoproterenol indüklü miyokardiyal infarktüs üzerine etkileri*

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## ABSTRACT

**Objective:** Caffeic acid phenethyl ester (CAPE) is a natural product with potent anti-inflammatory, antitumor and antioxidant activities and attenuates inflammation and lipid peroxidation induced by ischemia-reperfusion injury. The purpose of the present study was to investigate the effects of CAPE on isoproterenol (ISO) -induced myocardial infarction.

**Methods:** A randomized controlled experimental design was used in this study. Rats were divided into four groups and treated with saline, CAPE, ISO and ISO+CAPE. Rats were treated with CAPE (10 µmol kg/day i.p.) or saline starting 3 days before injecting ISO (150 mg /kg s.c., 24 hours). Seven days later, rats were sacrificed and the hearts were excised for biochemical analyses and microscopic examination. One-way ANOVA test with post hoc multiple comparisons using LSD method were used for statistical analysis of the data.

**Results:** The administration of ISO alone resulted in higher myeloperoxidase (MPO) activity, lipid peroxidation, superoxide dismutase (SOD) and catalase (CAT) than in the control. The enzyme activities did not change in rat given CAPE alone. CAPE treatment prevented the increase in MPO activity and malondialdehyde, but did not affect the activities SOD and CAT enzymes.

**Conclusion:** In light of these results, we conclude that CAPE prevents MPO-and lipid peroxidation-mediated myocardial injury via inhibition of neutrophil's MPO activity. *(Anadolu Kardiyol Derg 2010; 10: 298-302)*

**Key words:** Caffeic acid phenethyl ester, isoproterenol, myeloperoxidase, myocardial infarction, lipid peroxidation, oxidative stress

## ÖZET

**Amaç:** Kafeik asit fenetil ester (CAPE) güçlü antiinflamatuvar, anti-tümör ve antioksidan özelliklere sahip doğal bir üründür ve iskemi-reperfüzyonun indüklediği inflamasyonu ve lipid peroksidasyonu azaltır. Bu çalışmanın amacı isoproterenol (ISO) indüklü miyokardiyal infarktüs üzerine CAPE tedavisinin etkilerini incelemektir.

**Yöntemler:** Bu çalışmada rasgele deneysel kontrollü araştırma dizaynı seçildi. Sıçanlar dört gruba ayrıldı: Kontrol, CAPE, ISO, ISO+CAPE. CAPE (10 µmol kg/gün i.p.) ve ISO (150 mg /kg s.c.) tek başlarına ratlara 7 gün süreyle verildi. ISO+CAPE grubunda ise ratlara ISO (150 mg /kg s.c.) uygulamasından 3 gün önce CAPE (10 µmol kg/gün i.p.) verilmeye başlandı. Yedi günlük deney periyodunun sonunda ratlar öldürüldü ve biyokimyasal analizler için kan ve doku örnekleri alındı. Verilerin istatistiksel analizi tek-yönlü ANOVA ve post-hoc LSD testi ile yapıldı.

**Bulgular:** Tek başına ISO uygulanması sonucu myeloperoksidaz (MPO) aktivitesi, lipid peroksidasyonu, süperoksit dismutaz (SOD) ve katalaz (CAT) aktiviteleri arttı. Tek başına CAPE uygulaması ise enzim değerlerini kontrole göre değiştirmedi. CAPE tedavisi verilen grupta ise MPO aktivitesi ve malondialdehit seviyesi düşerken, CAT ve SOD aktivitesi değişmedi.

**Sonuç:** Mevcut bulgular CAPE tedavisinin nötrofil MPO aktivitesini inhibe ederek MPO ve lipid peroksidasyon aracılı miyokardiyal hasarı önlediğine işaret etmektedir. *(Anadolu Kardiyol Derg 2010; 10: 298-302)*

**Anahtar kelimeler:** Kafeik asit fenetil ester, isoproterenol, lipid peroksidasyonu, miyokardiyal infarktüs, oksidatif stres, miyeloperoksidaz

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## Introduction

Myocardial infarction (MI) commonly known as heart attack, is the leading cause of death in both men and women in developed and developing countries. Despite all basic and clinical improvements, MI is still one of the most common and severe health problems in a modern world (1). MI is the acute condition of myocardial necrosis caused by critical imbalance between the coronary oxygen supply and the demand of the myocardium. Experimental and clinical studies have shown that there is enhancement of free radical generation and/or interrupted production of endogenous antioxidant enzymes in heart diseases (2). Increased levels of reactive oxygen species and increased migration of neutrophils to the ischemic tissue play an important role in the pathophysiology of ischemic myocardial injury (3).

Isoproterenol (ISO), a synthetic catecholamine and β- adrenergic receptor agonist has been found to cause MI as a result of development of myocardial necrosis in experimental animals (4). One of the prominent possible mechanisms of ISO-induced cardiac injury is high production of cytotoxic free radicals and lipid peroxides by the way of auto-oxidation of catecholamines (5, 6).

Caffeic acid phenethyl ester (CAPE), a flavonoid-like compound and an active component of propolis from honeybee hives, has been used in traditional medicine for decades (7). It has strong antimicrobial, antiviral, anti-inflammatory and antineoplastic properties and has also been shown to decrease the oxidative damage (8). It has been reported that CAPE completely blocks the production of reactive oxygen species (ROS) in human neutrophils and in the xanthine/xanthine oxidase (XO) system at a concentration of 10μmol (9). Parlakpınar et al. (10) reported that CAPE acts in the heart as a potent scavenger of free radicals to prevent the apoptotic effect of myocardial ischemia/reperfusion injury. The authors showed that CAPE inhibits lipid peroxidation and suppresses oxidative stress (9, 11). It was shown in the previous studies that CAPE preserved heart tissue from doxorubicin-induced cardiac damage (11).

However, the possible protective effects of CAPE on ISO-induced MI have not yet been investigated.

Therefore, the aim of the present study was to investigate the protective effects of CAPE against ISO-induced myocardial infarction through oxidant injury and myocardial damage in a rat model.

## Methods

### Animals

A randomized controlled experimental design was used in this study. Wistar-albino female rats, 250-300 g, were used in experiments. All experiments were conducted at Fırat University. Animals were kept in a room maintained at an ambient temperature and humidity (25±5°C, 55±5%) under a day/night regime (day 7:00-19:00 and night 19:00-7:00) and allowed a commercial standard rat diet and water *ad libitum.* The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (DHEW Publication (NIH) 8523, 1985). The study protocol was approved by the Animal Ethical Committee of Kahramanmaraş Sütçü İmam University (Turkey).

### Experimental protocols

To induce experimental myocardial infarction, ISO was given subcutaneously (s.c.) to rats (150mg/kg) dissolved in 1 ml of saline once a day for 2 consecutive days (12). CAPE was dissolved in 95% ethanol and further diluted in saline. The animals were divided into four groups. Group control (n=7)-rats were given 1 ml of saline i.p. daily for 7 days; Group CAPE (n=8) - rats were treated with CAPE (10 μmol /kg/day) in 1 ml of saline i.p. daily for 7 days; Group ISO (n=7)-rats were s.c. injected with ISO in 0.5 ml of saline (150 mg/kg) once a day for 2 consecutive days; Group ISO+CAPE (n=6)-rats were treated with CAPE (10 μmol / kg/day) in 0.5 ml of saline i.p. daily for 7 days and rats were s.c. injected with ISO in 0.5 ml of saline (150 mg/kg) once a day for 2 consecutive days on the $4^{th}$-$5^{th}$ day of CAPE treatment.

All rats were fasted for 12 hours after the last drug administration, but had free access to water. Then, the rats were anaesthetized with (ketamine+xylazine (60 mg/kg+5 mg/kg, i.p.) at the end of the experiment. Blood was collected, serum were separated and used for various biochemical estimations. The heart tissue was excised immediately from the rats, washed with pre-chilled physical saline, and used for further biochemical estimations. The tissues homogenized with pre-chilled physical saline in tissue homogenizer, were then centrifuged at 3.000 x g for 10 min at 4°C, and the supernatant was used for the estimation of various biochemical parameters.

### Biochemical estimations

Serum aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK) enzyme activities were measured with an Olympus AU600 (Japan) autoanalyzer. The protein content of the heart were analyzed in homogenate, supernatant and extracted samples according to the method of Lowry et al. (12). Lipid peroxidation (as malondialdehyde) levels in heart homogenate were measured with the thiobarbituric acid reaction by the method of Esterbauer and Cheeseman (13). The values of malondialdehyde (MDA) were expressed as nmol $g^{-1}$ protein. Myeloperoxidase (MPO) activity was determined using a 4-aminoantipyrine/phenol solution as the substrate for MPO-mediated oxidation by $H_2O_2$, and changes in absorbance at 510 nm were recorded (14). One unit of MPO activity was defined as the amount of protein that degrades 1 mol $H_2O_2$ $min^{-1}$ at 25°C. Data were presented as mU $g^{-1}$ protein. Total superoxide dismutase (SOD) activity was determined according to the method of Sun et al. (15). The principle of the method is based on inhibition of nitro blue tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lysate after 1.0 ml of ethanol chloroform mixture (5: 3, v/v) was added to the same volume of sample and centrifuged. One unit of superoxide dis-

mutase (SOD) was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as U $mg^{-1}$ protein. Catalase (CAT) activity was determined according to Aebi's method (16).

### Chemical reagents

Superoxide dismutase, malondialdehyde, myeloperoxidase, xanthine oxidase diagnostic agents and caffeic acid phenyl ester and isoproterenol were bought from Sigma Chemical Co. (St. Louis, MO, USA).

### Statistical analysis

Data were analyzed by using a commercially available statistical software package (SPSS for Windows v. 12.0, Chicago, IL, USA). Distribution of the variables was analyzed with one sample Kolmogorov-Smirnov test. All groups showed normal distribution, so that parametric statistical methods were used to analyze the data. One-way ANOVA test was performed and post hoc multiple comparisons were made using least-squares differences test. Results are presented as mean±SEM; $p<0.05$ was regarded as statistically significant.

## Results

The serum levels of AST, LDH and CK in all groups are summarized in Table 1. The levels of AST and CK in ISO group were significantly increased compared to control ($p<0.01$). Serum AST levels were lower in ISO+CAPE group compared with ISO group ($p<0.05$). There were no statistically significant differences in the levels of CK between ISO+CAPE and ISO group. The serum levels of AST and CK were not different between CAPE and control groups. There were no statistically significant differences in the levels of LDH among all groups.

Table 2 summarizes the activities of heart MPO, SOD and CAT enzymes and MDA levels in all groups. These enzyme activities in heart were not different between CAPE and control groups. The activities of SOD and CAT enzymes in ISO group were significantly decreased compared to control group. The levels of MDA and MPO activity in ISO group were significantly increased compared to control group ($p<0.01$). The activities of MPO and the levels of MDA were lower in ISO+CAPE group compared with ISO group ($p<0.01$). The activities of SOD and CAT enzymes in ISO+CAPE group were not different from ISO group.

**Table 1. The levels of cardiac enzymes in serum of control, CAPE, ISO and ISO+CAPE groups**

| Groups | AST, U/l | LDH, U/l | CK, U/l |
|---|---|---|---|
| Control, (n=7) | 111±8 | 538±41 | 196±34 |
| CAPE, (n=8) | 139±21 | 663±54 | 228±47 |
| ISO, (n=7) | 227±58[a] | 649±92 | 435±61[a] |
| ISO+CAPE, (n=6) | 135±12[b] | 656±78 | 549±61[a] |
| *F | 2.52 | 0.71 | 10.33 |
| *p | 0.04 | 0.54 | 0.001 |

Data are presented as mean+/-SEM.
*One-way ANOVA test and posthoc LSD test:
[a]$p<0.01$ compared with control group.
[b]$p<0.05$ compared with ISO group.
Control: untreated
CAPE: rats were treated with CAPE (10 µmol/kg/days) for 7 days
ISO: rats were treated with isoproterenol (150 mg/kg) for 2 consecutive days
ISO+CAPE: rats were treated with CAPE (10 µmol/kg/days) for 7 days and treated with isoproterenol (150 mg/kg) for 2 consecutive days at the 4th-5th days of CAPE treatment
AST - aspartate aminotransferase, CAPE - caffeic acid phenethyl ester, CK - creatine kinase, ISO - isoproterenol, LDH - lactate dehydrogenase

**Table 2. The cardiac antioxidant enzyme levels in rats of control, CAPE, ISO and ISO+CAPE groups**

| Groups | MDA, nmol/g protein | MPO, U/g protein | SOD, U/mg protein | CAT, k/mg protein |
|---|---|---|---|---|
| Control (n=7) | 8.43±0.46 | 1.51±0.14 | 0.187±0.011 | 0.153±0.008 |
| CAPE ( n=8) | 7.93±0.41 | 1.77±0.11 | 0.168±0.004 | 0.134±0.012 |
| ISO (n=7) | 10.14±0.31[a] | 1.97±0.14[b] | 0.153±0.008[b] | 0.122±0.011[a] |
| ISO+CAPE (n=6) | 7.47±0.66[c] | 1.43±0.08[c] | 0.154±0.007[b] | 0.098±0.006[b] |
| *F | 6.23 | 3.81 | 4.41 | 4.23 |
| *p | 0.003 | 0.02 | 0.01 | 0.01 |

Data are presented as mean ± SEM
*One-way ANOVA test and posthoc LSD test:
[a]$p<0.05$ compared with control group
[b]$p<0.01$ compared with control group
[c]$p<0.01$ compared with ISO group
Control: untreated
CAPE: rats were treated with CAPE (10 µmol/kg/days) for 7 days
ISO: rats were treated with isoproterenol (150 mg/kg) for 2 consecutive days
ISO+CAPE: rats were treated with CAPE (10 µmol/kg/days) for 7 days and treated with isoproterenol (150 mg/kg) for 2 consecutive days at the 4th-5th days of CAPE treatment
CAPE - caffeic acid phenethyl ester, CAT - catalase, ISO - isoproterenol, MDA - malondialdehyde, MPO - myeloperoxidase, SOD - superoxide dismutase

## Discussion

In the present study, the effect of CAPE was determined in ISO-induced myocardial infarction in rats. We have shown that CAPE treatment successfully improved ISO-induced myocardial injury. Isoproterenol administration caused myocardial damage, which was reflected by a significant increase in serum AST, CK levels.

Increases in these serum enzymes in rats given ISO have been shown in previous studies (17, 18). Serum LDH level was not changed by ISO treatment. A possible reason for this is that LDH begins to rise in 24-48 hours following MI and peaks in 3-5 days (19). Rats were sacrificed 48 hours after second ISO administration in this study. Levels of AST, LDH and CK enzymes did not change in rats given CAPE alone. CAPE treatment prevented the increase in AST level, but did not affect the increase in CK level in ISO-induced myocardial infarction. Despite the increased ISO-induced oxidative stress parameters in heart, cardiac marker enzymes did not correlate with oxidative stress level in CAPE treated rats. It has been reported that the oxidant/antioxidant ratio is low in vitamin E (an antioxidant) treated

humans in comparison with placebo, but vitamin E treatment did not affect on the CK activity in humans (20). The researchers reported that there is no correlation between serum aminotransferase levels and the activities of antioxidant enzymes (21).

Isoproterenol-induced MI serves as a standardized model to investigate cardiac function and the beneficial effects of cardioprotective drugs. ISO, in large dose produces structural damages and functional impairments in the heart caused by myocardial necrosis. In this model, cardiotoxicity does not only occur via adrenoreceptor activation (22), but also ISO induces excessive production of free radicals resulting from oxidative metabolism of catecholamines and thus, the free radicals cause cell necrosis and contractile failure in the heart (23). In accordance with previous studies, it was observed that 150 mg/kg dose of ISO induced lesions in the myocardium and significantly altered biochemical parameters and antioxidant enzyme activities in the present study (24).

Myeloperoxidase is a neutrophil and monocyte enzyme that amplifies the reactivity of hydrogen peroxide through generation of hypochlorous acid, free radicals and reactive nitrogen species (25). MPO and its oxidative products play a key role for the enzyme in promotion of lipid peroxidation, protein nitration and other oxidative modifications in acute myocardial infarction (26). Similar to previous studies, MPO activity increased significantly in animals given ISO in the present study (20). Caffeic acid phenethyl ester treatment prevented the increase in MPO activity; accordingly, CAPE suppresses neutrophils infiltration into the injured myocardium. As a result, less oxygen free radicals generated and thus, CAPE would protect the heart from a serious oxidative damage. In addition, the protective effects of CAPE against myocardial and hepatic injuries have been shown, which are partly mediated by the inhibition of inflammatory responses (27, 28). The data obtained from recent studies showed that the leukocyte and neutrophil counts directly reflect the extent of myocardial damage in humans (29). In a similar way, researchers have demonstrated that neutrophils may contribute to tissue injury by the release of leukotrienes, free oxygen radicals and hydrolytic enzymes in ISO induced MI (11, 30). In recent years, important findings on the relationship between MPO and coronary artery disease were obtained. The level of plasma MPO enzyme alone predicts independently the early risk of MI (31). Increase in leukocyte and MPO levels are associated with coronary artery disease (32). These results allow suggesting that both neutrophil and MPO enzyme play a key role in the progress of MI.

Lipid peroxidation is an important index of oxidant injury in ISO induced necrotic damage of the heart. Isoproterenol-induced MI may develop through the induction of free radical-mediated lipid peroxidation, as a result of stressed condition (5). Malondialdehyde is a major lipid peroxidation end-product and our present observations are consistent with the previous findings indicating the increases of lipid peroxidation (20, 33). CAPE treatment significantly decreased the MDA levels by preventing formation of lipid peroxides from fatty acids. The neutrophil accumulation and the activation of MPO enzyme in MI are probably indirectly responsible for lipid oxidation. Really, MPO and its oxidative products play a key role in the lipid peroxidation in MI (26). Moreover, the correlation between neutrophils and MDA levels has been shown (34). The data obtained suggest that the accumulation of neutrophils and the activation of MPO by neutrophils lead to lipid peroxidation.

In the present study, SOD and CAT activities decreased significantly in the ISO given rats. The decline in SOD enzyme level may lead to excessive formation of superoxide anions, may give serious damage to the myocardium (35). Hydrogen peroxide scavenging enzyme CAT also decreased significantly after ISO administration. The decline in these enzyme levels may be explained by the fact that excessive superoxide anions may inactivate SOD, thus, resulting in an inactivation of the $H_2O_2$ scavenging enzymes (33). CAPE treatment did not affect the activities SOD and CAT enzymes. Probably, CAPE does not indicate a direct effect on these antioxidant enzymes but it may have an indirect effect on these enzymes. As a strong possibility, the essential effect of CAPE on myocardial infarction is to prevent neutrophils infiltration into the affected myocardial areas (27). CAPE should not prevent, even indirectly, the increases in the activities of SOD and CAT enzymes for rats sacrificed early (48 hours after second ISO administration).

### Study limitations

We should have measured the serum CK-MB levels in groups, because CK-MB isoenzyme is more specific than total CK enzyme in detecting myocardial injury. Second limitation, the protective effect of CAPE on myocardial injury was not confirmed by the histopathological findings.

## Conclusion

We demonstrated an increase in lipid peroxidation and MPO activity and a decrease in SOD and CAT activity in heart tissue of rats given ISO, and MPO- and lipid peroxidation-mediated myocardial injury was prevented by CAPE treatment. Our results collectively suggest that CAPE may be an available agent to protect the myocardium from infarction via inhibition of neutrophil MPO activity. Further studies are needed to elucidate the protective effects of CAPE against myocardial infarction in a clinical study designed in humans.

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**Conflict of interest:** None declared.

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