The effect of caffeic acid phenethyl ester on isoproterenol-induced myocardial injury in hypertensive rats

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Abstract

Objective: The aim of this study is to investigate the effects of caffeic acid phenethyl ester (CAPE) on isoproterenol (ISO)-induced myocardial injury in hypertensive rats.

Methods: Rats were divided into 4 groups (n=29): Control group (n=8), L-NNA (NG-Nitro-L-arginine) group (n=8), L-NNA+ISO (L-NNA+isoproterenol) group (n=7) and L-NNA+ISO+CAPE (L-NNA+ISO + caffeic acid phenethyl ester) group (n=6). ISO (150 mg/kg/day) was given intraperitoneally (i.p.) once a day for 2 consecutive days (at the 12th and 13th days of L-NNA treatment). NG-Nitro-L-arginine (L-NNA) was given orally (25 mg/kg/day) in drinking water for 14 days. CAPE (10 µmol/kg/day) was given (i.p.) for 7 days after the first week. Systolic blood pressure (SBP) was evaluated by the tail-cuff method and biochemical analysis were performed using an autoanalyzer and a spectrophotometer.

Results: SBP in all L-NNA-treated groups was found to be increased at seventh day. AST and LDH levels in LNNA+ISO group were significantly increased compared to control (AST: 125±5 vs. 105±2; LDH: 861±154 vs. 571±46 U/L respectively) ($p<0.05$). Also, ISO caused to extensive necrosis and mononuclear cell infiltration in hypertensive rat myocardium. CAPE application reversed the enhanced AST and LDH levels as well as the extensive necrosis and the mononuclear cell infiltration in LNNA+ISO+CAPE group compared LNNA+ISO.

Conclusion: According to our findings, it might be suggested that CAPE may be a favorable agent to protect the hypertensive myocardium from the injury induced by isoproterenol via mechanisms such as the induction of the antioxidant enzymes and the inhibition of lipid peroxidation.
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Key words: hypertension, isoproterenol, myocardial injury, caffeic acid phenethyl ester, oxidative stress, rat

Introduction

Hypertensive heart disease is a major cause of death associated with high blood pressure and it relates to disorders such as cardiac failure, ischemic heart disease and left ventricular hypertrophy. Myocardial infarction (MI) is an acute condition of myocardial necrosis caused by critical imbalance between the coronary oxygen supply and the demand of the myocardium. There are data arising from experimental and clinical studies concerning the enhanced free radical generation and/or interrupted endogenous antioxidant enzymes production in heart diseases (1). 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 (2).

Nitric oxide (NO) plays an important role in the physiological control of blood pressure (BP) and the alterations in NO synthesis cause vasoconstriction and have been suggested to be involved in the pathogenesis of hypertension (3). Pharmacological inhibition of NO synthesis produces acute and chronic hypertension in many animal species (4).

Caffeic acid phenyl ester (CAPE), a flavonoid-like compound and an active component of propolis from honey bee hives (5). CAPE has strong antimicrobial, antiviral, antiinflammatory, antineoplastic, antiarrhythmic, cardioprotective and antioxidant properties (6-11). CAPE can completely block the production of reactive oxygen species (ROS) in human neutrophils and in the xanthine/xanthine oxidase (XO) system at a concentration of 10 µmol (12). In the previous studies it has been shown that CAPE preserves heart tissue from isoproterenol-induced cardiac dam-

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age and restores the impaired antioxidant enzyme activity in the rat kidney and heart (13-17). Furthermore, it was reported that CAPE (10 μmol/kg) application significantly reversed the increased MDA, decreased NO levels and the increased diameters of myocardial myofibrils in cadmium-induced hypertensive rats (11).

A toxic dosage of isoproterenol causes characteristic myocardial damage and subsequently results in mild heart failure in experimental conditions (18). To the best of our knowledge, the effects of CAPE on ISO-induced myocardial damage in hypertensive rats have not yet been known. Therefore, the aim of the present study was to investigate the effects of CAPE against ISO-induced myocardial injury in an experimental hypertensive rat model. In this study, We used NG-Nitro-L-arginine (L-NNA), an inhibitor of nitric oxide synthase (NOS) enzyme, to produce hypertension and isoproterenol (ISO) to induce myocardial infarction.

Methods

Animals

The study was checked for compliance with ARRIVE guidelines for presentation of experimental animal studies (19). The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals [DHEW Publication (NIH) 8523, 1985] and approved by the Fırat University Animal Experimentation Ethics Committee (FUAEEC). Twenty-nine Sprague-Dawley male rats, 200-250 g, were used in experiments. The animals were housed in quiet rooms with: 12 hours light/dark cycle (7 a.m. to 7 p.m.) and allowed a commercial standard rat diet and water ad libitum.

Experimental protocols

Rats were divided into 4 groups (n=29): Control group (n=8), L-NNA (NG-Nitro-L-arginine) group (n=8), L-NNA+ISO (L-NNA+isoproterenol) group (n=7) and L-NNA+ISO+CAPE (L-NNA+ISO + caffeic acid phenethyl ester) group (n=6). Systolic blood pressure (SBP) was measured by tail-cuff method (MAY BPHR 9610-PC, Commat Ltd., Ankara, Turkey) for 3 consecutive days before the starting the protocol and then at 3- to 4-day intervals during the 2-week study period. L-NNA was given orally (25 mg/kg/day) in drinking water to rats during 14 days to produce hypertension. To induce myocardial infarction, ISO was given intraperitoneally to rats (150 mg/kg/day) once a day for 2 consecutive days (20). Control group: untreated, LNNA group: rats were treated with L-NNA (25 mg/kg/day), LNNA+ISOgroup: L-NNA treated rats were given ISO (150 mg/kg) for 2 consecutive days in the 12^{th} and 13^{th} days of L-NNA treatment, LNNA+ISO+CAPE group: L-NNA treated rats were also given CAPE (10 μmol/kg/day) during 7 days after the first week and treated with ISO (150 mg/kg) for 2 consecutive days at the 12^{th}-13^{th} days of L-NNA treatment.

Animals were sacrificed at 48^{th} hour after second isoproterenol administration. All rats fasted about 12 hours, but had free access to water at the last administration of the drug. Then, the rats were anesthetized with ketamine (60 mg/kg) and Xylazine (5 mg/kg, i.p.) at the end of the experiments, respectively. Blood was collected, serum was separated and used for various biochemical analyses. The heart tissue was excised immediately from the rats, washed with prechilled physical saline and used for further biochemical estimations. The tissues homogenized with prechilled physical saline in tissue homogenizer, then centrifuged at 3000 g for 10 min at 4°C, and the supernatant was used for the estimation of various biochemical parameters.

Biochemical analysis

Serum aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase MB isoenzyme (CK-MB) enzyme activities were measured with a Beckman Coulter LH 750 (Fullerton, CA, USA) autoanalyzer. The protein content in the heart were analysed in homogenate, supernatant and extracted samples according to the method of Lowry et al. (21). Malondialdehyde (MDA) levels in heart homogenate were measured by the thiobarbituric acid reaction by the method of Esterbauer et al. (22). The values of MDA were expressed as nmol/g protein. Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia et al. (23). The enzymatic reaction in the tube containing NADPH, reduced glutathione, sodium azide and glutathione reductase was initiated by addition of H2O2, and the change in absorbance at 340 nm was monitored by a spectrophotometer. Total superoxide dismutase (SOD) activity was determined according to the method of Sun et al. (24). The SOD activity was expressed as U/mg protein. Catalase (CAT) activity was determined according to Aebi's method (25). Since NO measurement is very difficult in biological specimens, tissue nitrite (NO2-) and nitrate (NO3-) were estimated as an index of NO production, and the colorimetric assay based on the Griess reaction for assessment of NO activity was used (26).

Histological examination

For light microscopic examinations, cardiac samples were fixed at 10% neutral buffered formalin. Tissues were embedded in paraffin following dehydration with graded alcohol series. Several 5 μm thick transverse sections were obtained from the tissue blocks and stained with hemotoxylin and eosin for histological evaluation. Sections were examined and photographed with Olympus DP20 camera attached-Olympus CX41 photomicroscope for characteristic histological changes.

Chemical reagents

NG-nitro-L-arginine, superoxide dismutase, malondialdehyde, myeloperoxidase, xanthine oxidase diagnostic agents and caffeic acid phenyl ester and isoproterenol were bought from Sigma Chemical Co (St Louis, USA).

Statistical analysis

The distribution of the groups was analyzed with one sample Kolmogrov-Smirnov test. Experimental groups showed normal

distribution for blood pressure and antioxidant parameters. One-way ANOVA test was performed and posthoc multiple comparisons were made using least-squares differences to analyze antioxidant parameters and blood pressures at the end of study. ANOVA for repeated measures was used and posthoc multiple comparisons performed Dunnett test to analyze repeated blood pressure measurements. Groups have abnormal distribution for AST, LDH and CK-MB, so that Kruskal-Wallis test was performed to analyze the data and nonparametric Tukey HSD test were used to posthoc analyze. Results are presented as mean±SEM; $p<0.05$ was regarded as statistically significant.

Results

Blood pressure

SBP values were shown in Table 1. The average SBP in L-NNA treated groups increased throughout the study. At the end of the study, the average SBP in isoproterenol treated rats were reversed initial levels compared on day 7th of the same groups. There were no any differences between LNNA and LNNA+ISO groups for L-NNA consume (data not shown).

Biochemical results

The serum levels of AST, LDH and CK-MB in all groups were summarized in Table 2. The levels of AST and LDH in LNNA+ISO group were increased compared to control but not significant. CAPE treatment reversed the enhanced levels of AST and LDH in LNNA+ISO+CAPE group. There were no statistically significant difference in the levels of CK-MB among all groups. The serum levels of AST, LDH and CK-MB increased in LNNA group, but it was not significant in statistic.

Table 3 summarizes the activities of heart SOD, GSH-Px and CAT enzymes and MDA levels in all groups. SOD enzyme activity in LNNA and LNNA+ISO decreased compared to control group. The activity of the CAT enzyme in LNNA+ISO group was significantly increased compared to control group. CAPE treatment enhanced activities of SOD and CAT enzymes in LNNA+ISO+CAPE group but not significant for SOD. The levels of MDA in LNNA+ISO group were significantly increased compared to control group and CAPE treatment reversed the enhanced levels of MDA. There were no differences in NO levels among all groups. Really, L-NNA treatment decreased NO levels but not significant statistically.

Histological results

In histological evaluation, control tissues showed normal cardiac histology (Fig. 1). LNNA caused diffuse edema, myocytolysis and fiber disorganization (Fig. 2). In LNNA+ISO group, marked necrosis, hemorrhage and mononuclear cell infiltration was observed (Fig. 3). CAPE administration decreased degeneration and improved cardiac histology (Fig. 4). However signs of degeneration like increased eosinophily persisted in some areas.

Discussion

The present study has targeted the hypothesis that CAPE may prevent the myocardial damage caused by ISO in L-NNA-

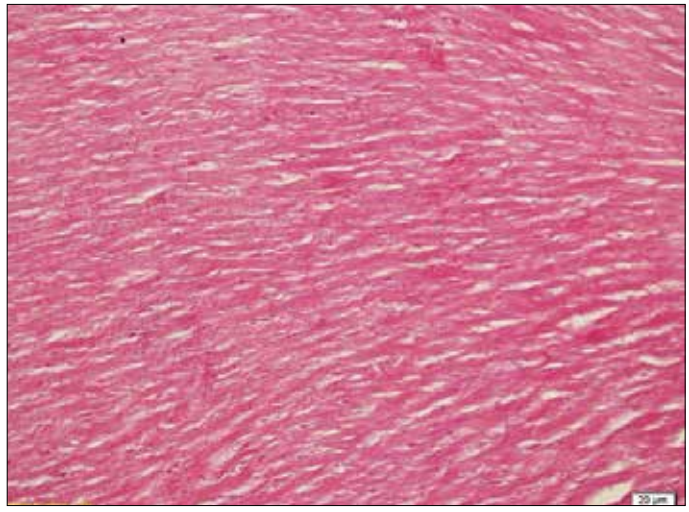

Figure 1. Control; Normal cardiac histology (HE x200)

Table 1. Systolic blood pressure at days initial, 7th and 14th of L-NNA treatment

		SBP (mmHg)			F values (ANOVA for repeated measurements)
Group		Initial	Day 7th	Day 14th	
Control	(n=8)	107±1	106±1	107±1	0.273
L-NNA	(n=8)	110±1	134±1^{ax}	145±2^{abx}	69.656
L-NNA+ISO	(n=7)	108±1	133±2^{ax}	107±2^{by}	58.595
L-NNA+ISO+CAPE	(n=6)	108±1	136±2^{ax}	102±1^{byz}	90.111
F values (one-way ANOVA)		0.671	55.750	87.257	
P values (Between Groups)		0.577	<0.001	<0.001	

$^{a}P<0.001$, vs initially; $^{b}P<0.001$, vs day 7th; $^{x}P<0.001$, vs Control; $^{y}P<0.001$, vs L-NNA ; $^{z}P<0.05$, vs L-NNA+ISO. Results are represented as mean±SEM. ANOVA for repeated measures was used and post hoc multiple comparisons performed Dunnett test. Control: untreated, LNNA: rats were treated with L-NNA (25 mg /kg/day) for weeks, LNNA+ISO: L-NNA treated rats were given isoproterenol (150 mg/kg/day) for 2 consecutive days in the 12th and 13th days of L-NNA treatment, LNNA+ISO+CAPE: L-NNA treated rats were given CAPE (10 µmol / kg/day) for 7 days after the first week and treated with isoproterenol (150 mg/kg/day) for 2 consecutive days at the 12th-13th days of L-NNA treatment

induced hypertensive rats. CAPE prevented the inceased MDA, AST and LDH levels induced by ISO as well as the extensive necrosis and the mononuclear cell infiltration in hypertensive heart tissue. According to present results, this study has demonstrated that CAPE might protect the hypertensive myocardium against to the injury induced by ISO application via antioxidant effects.

Table 2. Biochemical parameters of serum in all groups

Group		AST (U/l)	LDH (U/l)	CK-MB (U/l)
Control	(n=8)	105±2	571±46	179±19
LNNA	(n=8)	121±8	778±151	312±110
LNNA+ISO	(n=7)	125±5[a]	861±154[b]	458±257
LNNA+ISO+CAPE	(n=6)	106±9	372±46[c]	321±194

[a]*P*<0.01 compared with control group; [b]*P*<0.05 compared with control group; [c]*P*<0.001 compared with LNNA+ISO group; Results are represented as mean±SEM. Kruskal-Wallis test was performed to analyze the data and nonparametric Tukey HSD test were used to posthoc analyze. Control-untreated, LNNA-rats were treated with L-NNA (25 mg/kg/day) for weeks, LNNA+ISO- L-NNA treated rats were given isoproterenol (150 mg/kg/day) for 2 consecutive days in the 12th and 13th days of L-NNA treatment, LNNA+ISO+CAPE- L-NNA treated rats were given CAPE (10 µmol/kg/day) for 7 days after the first week and treated with isoproterenol (150 mg/kg/day) for 2 consecutive days at the 12th-13th days of L-NNA treatment

L-NNA application caused high blood pressure throughout 14 days and ISO returned SBP to normal values. The reason of this situation may be to development of heart failure as a result of mild myocardial infarction (18). Data regarding the effects of CAPE on blood pressure are controversial: CAPE administration does not affect the hemodynamic parameters or it may exert a hypotensive effect via a central parasympathetic control mechanism on heart rate (16, 27). We have not given CAPE alone to animals, but it slightly reduced the blood pressure in LNNA+ISO group. Additionally, we have given CAPE to animals for a subacute period (seven days), but they gave it acutely. Probably, the slight hypotensive effect of the agent will be balanced in this subacute period. On the other hand, it has been reported that CAPE has a vasorelaxant effect on porcine coronary artery ring segments via the induction of NO and adrenergic beta-receptors (28). Indeed, CAPE treatment improved the levels of NO decreased by ISO administration in the heart (0.010±0.002 vs. 0.020±0.004, µmol/g wet tissue). As a result, CAPE can act as positive inotropic agent via the induction of beta-adrenoceptors and dilate coronary arteries inducing NO without affecting blood pressure. We suggest that CAPE might be useful in both hypertensive or normotensive patients with a heart attack.

Table 3. The levels of MDA and NO, the activities of SOD, GSH-Px and CAT enzymes in all groups

Group		MDA, nmol/g protein	SOD, U/mg protein	GSH-Px, U/g protein	CAT, ku/mg protein	NOx, µmol/g wet tissue
Control	(n=8)	2.50±0.22	0.890±0.095	3.53±0.15	0.196±0.012	0.023±0.009
LNNA	(n=8)	2.37±0.16	0.646±0.044[a]	3.45±0.11	0.236±0.023	0.017±0.001
LNNA+ISO	(n=7)	3.22±0.25[a]	0.591±0.040[a]	3.31±0.17	0.263±0.011[c]	0.010±0.002
LNNA+ISO+CAPE	(n=6)	2.55±0.19[b]	0.737±0.116	3.63±0.24	0.312±0.013[b]	0.020±0.004
F values (one-way ANOVA)		3.176	2.975	0.603	10.339	0.890
P values (Between groups)		0.042	0.051	0.619	<0.001	0.460

[a]*P*<0.05 compared with control group; [b]*P*<0.05 compared with LNNA+ISO group; [c]*P*<0.01 compared with control group. Results are represented as mean±SEM. One-way ANOVA test was performed and posthoc multiple comparisons were made using LSD test. Control- untreated, LNNA-rats were treated with L-NNA (25 mg/kg/day) for weeks, LNNA+ISO-L-NNA treated rats were given isoproterenol (150 mg/kg/day) for 2 consecutive days in the 12th and 13th days of L-NNA treatment, LNNA+ISO+CAPE-L-NNA treated rats were given CAPE (10 µmol/kg/day) for 7 days after the first week and treated with isoproterenol (150 mg/kg/day) for 2 consecutive days at the 12th-13th days of L-NNA treatment;
CAT - catalase; GSH-Px - glutathione peroxidase; MDA - malondialdehyde; Nox - nitrite (NO^{2-}) and nitrate (NO^{3-}), SOD - superoxide dismutase

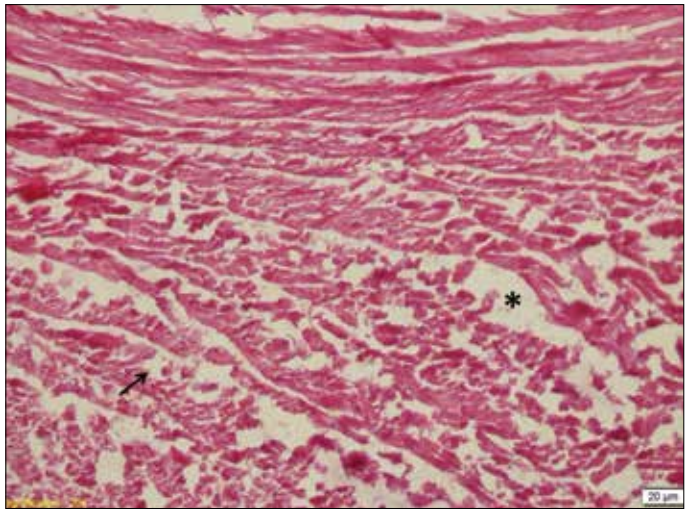

Figure 2. LNNA; Degeneration, edema (*) and myocytolysis (→) (HE x200)

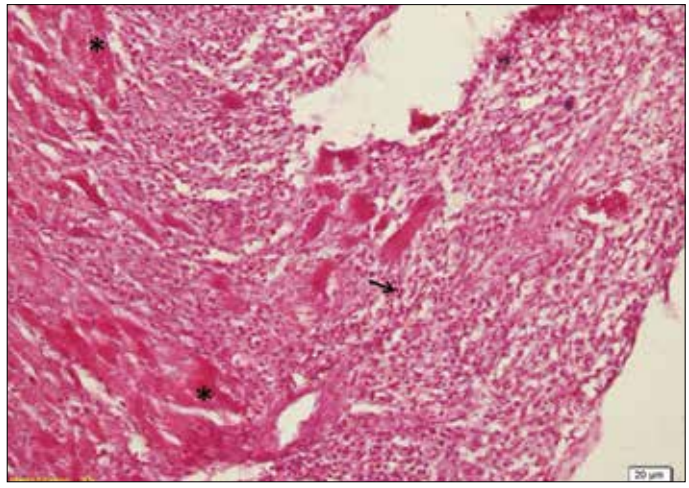

Figure 3. LNNA+ISO; Extensive necrosis (*) and mononuclear cell infiltration (→) (HE x200)

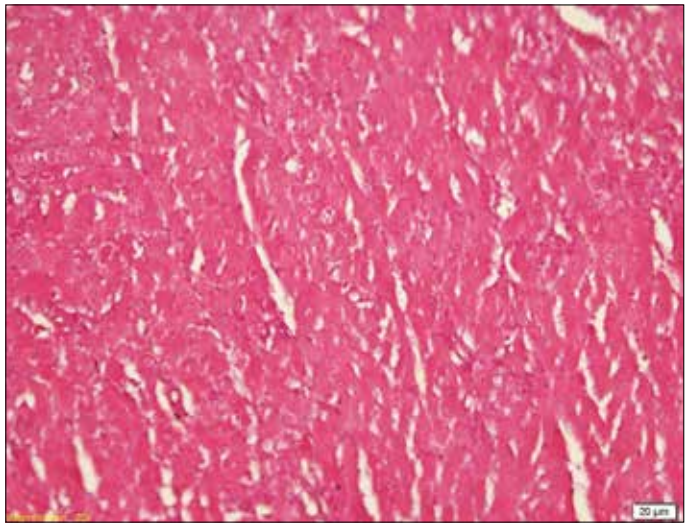

Figure 4. LNNA+ISO+CAPE; Improved cardiac histology (HE x200)

L-NNA alone caused to decrease in SOD activity compared to control. In the previous studies reported that L-NNA decreases the activities of antioxidant enzymes and enhances lipid peroxidation (29). In accordance with previous studies, it was observed that 150 mg/kg dose of ISO induced a myocardial damage and significantly altered biochemical parameters and antioxidant enzyme activities in the present study (13). In the present study ISO administration caused myocardial damage which was reflected by a significant increase in serum AST and LDH levels. Serum CK-MB level was not changed by isoproterenol treatment probably as a result of the killing of rats at 48th hour after second isoproterenol administration in this study. Indeed, CK-MB begins to elevate at 4-6 hours after the start of myocardial infarction and reaches a peak at 12-24 hours and returns to normal in 36-72 hours (30). CAPE treatment prevented the increase in LDH and AST level in ISO-induced myocardial infarction. ISO increased oxidative stress parameters and deteriorated antioxidant enzymes in heart tissue. Despite the increased oxidative stress parameters in heart, cardiac marker enzymes may not correlate with the activity of antioxidant enzymes (31).

Malondialdehyde is a major lipid peroxidant end product and our present results were consistent with the previous findings indicating the increases of lipid peroxidation (32). CAPE treatment significantly decreased the MDA levels by probably preventing the formation of lipid peroxides. Myeloperoxidase (MPO) is a neutrophil and monocyte enzyme that amplifies the reactivity of hydrogen peroxide (33). We did not measure MPO activity, but we previously showed that CAPE decreases the rised MPO activity in heart (13). MPO and its oxidation products can play a key role for the enzyme in promoting of lipid peroxidation and other oxidative modifications in acute myocardial infarction (34). The activation of MPO enzyme in myocardial infarction is associated and positively correlated with lipid oxidation.

Superoxide dismutase, CAT and GSH-Px are the main antioxidant enzymes in the body. First, the function of SOD is to convert superoxide anion free radicals (O_2-) to H_2O_2 and to molecular oxygen and therefore, the decline in SOD enzyme level may lead to excessive formation of superoxide anions and might induce a serious damage of myocardium (35). In the present study, SOD activity decreased and CAT activity increased significantly in the ISO treated rats. The decline in SOD activiy may be explained by the fact that excessive superoxide anions may inactivate SOD enzyme (32). CAPE treatment improved unsignificantly SOD activity. The increase in the enzyme activity was 24% and this rise is really significant because it is greater than 20% (36). Second, H_2O_2 is converted to O_2 and H_2O using catalase. H2O2 scavenging enzyme CAT increased significantly after ISO administration and CAPE enhanced CAT activity much more. We cannot show that how CAPE alone affects the activities of antioxidant enzymes because it was not studied. On the other hand, we have shown previously that CAPE alone may directly increase CAT activity (37). Generally, CAPE does not affect GSH-Px activitiy in normal or injured animals. It is likely that the current increase in CAT enzyme is sufficient to break raised the amount of H_2O_2. Probably, CAPE indicates a direct effect on CAT and may exist an indirect effect on other antioxidant enzymes.

As a result, we demonstrated an increase in lipid peroxidation and a decrease in SOD and CAT activity in heart tissue of hypertensive rats given ISO and oxidative stress-mediated possible myocardial injury was prevented by CAPE treatment.

In the present study CAPE administration improved cardiac tissue structure histologically. The protective effects of CAPE on cardiac tissue in myocardial ischemia reperfusion injuries were previously reported (9, 38, 39). Çağlı et al. (39) and Parlakpınar et al. (16) showed decreased apoptosis in ischemia reperfusion-induced cardiac injury. However this is the first study in the literature evaluating the effects of both LNNA and CAPE on cardiac tissue from a histological point of view.

Study limitations

Our study has some limitations. First, in our study, biomarkers such as troponins (T and I) and total CK that are important parameters for the diagnosis of myocardial infarct were not performed. Secondly, after ISO application, EKG recording was not made. But, on the other hand, the light microscopic examination of heart tissues for all groups which shows the injury directly was performed to the evaluation of the ISO-induced myocardial injury.

Conclusion

The current study suggests that CAPE may help to protect against to myocardial injury induced by isoproterenol via inhibition of lipid peroxidation and induction of antioxidant enzymes in hypertensive rats. Further experimental and clinic studies are needed to elucidate the preventive effects of CAPE against myocardial damage.

Conflict of interest: None declared.

Peer-review: Partially external peer-reviewed.

Authorship contributions: Concept - S.İ., N.Y., S.M., S.O., E.Ş.; Design - S.İ., S.O., N.Y., E.N., S.M.; Supervision - S.İ., N.Y., S.M., S.O.; Resource - S.İ., E.N., E.Ş., N.Y., S.M.; Materials - S.İ., S.O., E.N., N.Y., S.M.; Data collection&/or Processing - S.İ., S.O., E.Ş.; Analysis &/or interpretation - S.İ., S.M., S.O., E.Ş.; Literature search - S.İ., N.Y., S.O., E.Ş.; Writing - S.İ., N.Y., E.N., S.M., S.O., E.Ş.; Critical review - S.İ., N.Y., E.N., S.M., S.O., E.Ş.

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