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Effect of caffeic acid phenethyl ester in doxorubicin induced descending aorta damage

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Doxorubicin (DOX), a chemotherapeutic agent used in cancer treatment, can cause cardiotoxicity as an adverse effect. In this study, potential protective effect of Caffeic acid phenethyl ester (CAPE), a well-known antioxidant agent, was investigated in doxorubicin induced aortic damage model. Total of 28 adult Wistar albino rats were equally divided into four groups as: Control, DOX, CAPE+DOX, CAPE. Accordingly, 10 µmol/kg CAPE for 10 days and/or 10 mg/kg doxorubicin for 3 days was given intraperitoneally. Control group received saline and ethanol as the vehicles of doxorubicin and CAPE, respectively. GSH, MDA, CuZn-SOD and CAT levels in descending aorta were investigated as the oxidative stress markers and histopathological changes were evaluated. GSH level was significantly higher in CAPE group as compared to the other groups (P <0.05) while there were no significant differences in MDA, CuZn-SOD and CAT levels among the groups (P>0.05). In microscopic view, tunica media of aorta was significantly thinner in DOX group as compared to CAPE group. Tunica media thickness significantly increased in CAPE+DOX group as compared to DOX group. CAPE treatment ameliorates the histopathological changes that are characterized by the reduced wall thickness induced by doxorubicin. However, CAPE treatment did not seem to effect biochemical parameters that are indicative of oxidative stress. The results indicated that CAPE can be protective against doxorubicin induced aortic vessel damage.

Keywords: CAPE, Doxorubicin, Aorta, Antioxidant, Rat

Doxorubicin (DOX), a member of anthracyclines, is a highly effective anti-neoplastic drug used in the treatment of different malignancies such as leukemia, lymphomas, soft tissue sarcomas, solid tumours, and breast, uterus, and lung cancers[1]. However, doxorubicin can also cause cardiotoxicity as an adverse effect. It has been reported that patients receiving doxorubicin and its derivatives may experience heart problems many years even after chemotherapy is ended[2,3]. Doxorubicin has a cytotoxic effect by directly interacting with cellular genetic material and hence may cause DNA damage. It can also impair intracellular molecular homeostasis and cause apoptosis[4]. Most significantly, it can trigger the production of free radical species causing oxidative damage[5]. These effects of doxorubicin may end up with the progression of cardiotoxicity and even fatal congestive heart failure[6]. Cellular vacuolization, sarcomere damage, microtubule damage, myofibril loss, mitochondrial damage and disruption of myocyte structure may occur with doxorubicin toxicity[5,7]. Most of these changes are related to the increased production of free radicals and the development of oxidative damage to cellular structures. Besides in myocytes, excess production of reactive oxygen species (ROS) and impaired cellular homeostasis may also cause endothelial damage and hence vessel dysfunction[8].

Caffeic acid phenethyl ester (CAPE), a phenolic compound obtained by extraction from propolis with strong antimicrobial, anti-inflammatory, antioxidant and anti-neoplastic activities[9,10]. Propolis is a mixture produced by honey bees from substances collected from plants. The content of propolis consists of 10% essential and aromatic oils, 30% wax, 50% resin and herbal balsam, 5% pollen and 5% other substances. CAPE was shown to prevent lipoxygenase activities and lipid peroxidation[11-14]. Therefore, preventive effects of CAPE in cellular damage induced by many toxicants are commonly investigated. CAPE was shown to reduce free radical production and hence cardiotoxicity induced by chemotherapeutic agents[15] and inhibit NF-κβ activation in a dose dependent manner[16]. It has been reported that CAPE can reduce doxorubicin induced myocardial damage[17]. Doxorubicin is known to cause various abnormalities in vessel smooth muscle cells[18]. Hence, possible protective potential of CAPE in preventing vessel damage induced by doxorubicin were aimed to be investigated by examining oxidative stress parameters and tissue histopathology.

Materials and Methods

Experimental procedure

Ethical approval of the study was obtained from the Animal Ethics Committee of İnönü University

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(Protocol No: 06.01.2022, 2022/1-2). A total of 28 adult male Wistar albino rats were caged in an environment with a regulated temperature of 21°C, a humidity level of 55-60%, and a 12 h light and 12 h dark cycle throughout the experimental period. Regular rat food and water were provided *ad libitum*. Rats were randomly divided into four groups (n=7) as Control, CAPE, DOX, and CAPE+DOX. Control group was given a mixture 0.5 mL of saline and 2.5% ethanol as the vehicles of doxorubicin and CAPE, respectively. While CAPE group was given 10 µmol/kg body weight (bw) CAPE per day (Lot. No: 1016880, Bachem-Switzerland), intraperitoneally (ip) for a period of 10 days, DOX group received 10 mg/kg bw doxorubicin (Adrimycin-Deva Holding A.Ş.-Türkiye) for a period of 3 days (total of 30 mg/kg). CAPE+DOX group was given 10 µmol/kg bw CAPE ip for 10 days and 10 mg/kg bw doxorubicin ip for the last 3 days. At the end of the experimental period, all rats were sacrificed under xylazine/ketamine anaesthesia, the descending aorta were collected for biochemical and histopathological investigations.

Biochemical analysis

Tissue homogenates were prepared from aorta samples and the homogenates were later used to determine the levels of reduced glutathione (GSH), malondialdehyde (MDA), catalase (CAT) and copper-zinc superoxide dismutase (CuZn-SOD). GSH levels were measured as described by Ellman[19]. The method is based on the reaction of reduced GSH with 5,5-dithiobis-2-nitrobenzoic acid and the results are read spectrophotometrically at 410 nm. Results were expressed as nmol/g wet tissue (gwt). Mihara & Uchiyama's[20] method was used to measure tissue MDA levels spectrophotometrically at 535 nm and 520 nm. Results were given as nmol/gwt. CAT activity was determined according to method of Lück[21]. CAT activity in aorta tissue samples was determined by spectrophotometric measurement of the degradation of 30 mmol/L H_2O_2 in 50 mmol/L potassium phosphate buffer (pH 7.0) at 240 nm at 15 second intervals over a 150 second period. Results were expressed as K/g protein. CuZn-SOD activity was performed according to the method of Sun *et al*.[22], in which nitroblue tetrazolium was reduced by xanthine-xanthine oxidase, which acts as a superoxide generator, and the results were read spectrophotometrically at 560 nm. Results were given as U/g protein.

Histopathology

Samples of descending aorta were fixed in 10% formalin solution and then routinely processed for preparation of paraffin blocks. Tissue samples were prepared by cutting 4 µm thick sections and then staining with hematoxylin-eosin (H&E). Overall tissue structure was evaluated and the thickness of tunica media was measured. Histopathological evaluations and measurements were performed using a Leica DFC280 light microscope and Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

Statistical analysis

The minimum sample size required to find a significant difference were calculated as 7 for each group, as the amount of Type I error (alpha) was 0.05, the power of the test (1-beta) was 0.8, and the predicted effect size for GSH (nmol) was 1.80. Statistical analysis of quantitative data were performed using PAST 4.10 and IBM SPSS Statistics 28.0 for Windows (New York; USA). Normal distribution of data was evaluated by the Shapiro–Wilk test ($P>0.05$). Since the data did not show a normal distribution, they were given as median and interquartile range. Kruskal-Wallis H test was used for intergroup comparison and Conover test was used for post-hoc analysis. $P<0.05$ was considered statistically significant. American Psychological Association (APA) 6.0 style was used to report statistical differences.

Results and Discussion

Biochemical analysis

The results of the biochemical analysis were summarised in Table 1. The most noteworthy changes were observed in the tissue levels of GSH. Although there was no significant difference between the control and DOX group ($P>0.05$) in terms of GSH level, it was significantly higher in CAPE group as compared to CAPE+DOX and DOX groups ($P<0.05$). However, GSH level in CAPE+DOX group was significantly lower than that of the control group ($P<0.05$). In terms of MDA, CAT and CuZn-SOD tissue levels, no significant differences were observed among the studied groups ($P>0.05$).

Histopathology

As the descending aorta samples were microscopically examined, no significant pathological changes were seen in any of the study groups. In all

Table 1 — Aortic tissue levels of MDA, GSH, CuZn-SOD, and CAT

Parameters	Control (n=7)	CAPE (n=7)	DOX (n=7)	CAPE+DOX (n=7)	*P*-value
MDA (nmol/gwt)	37.74[a] (2.67)	41.43[a] (9.37)	38.12[a] (1.40)	38.25[a] (1.53)	0.712
GSH (nmol/gwt)	840[ac] (70.87)	868.87[a] (95.81)	750.75[bc] (19.68)	735[b] (18.37)	0.018
CuZn-SOD (U/g protein)	440.38[a] (76.14)	315.83[a] (29.99)	285.69[a] (160.47)	313.24[a] (116.43)	0.054
CAT (k/g protein)	218.57[a] (36.21)	192.91[a] (72.84)	216.44[a] (68.04)	188.83[a] (55.31)	0.377

[Data are summarized as median (interquartile range). Values with same superscripts in a line for a parameter indicates no significant difference between the groups (P>0.05). CAPE: Caffeic acid phenethyl ester, DOX: Doxorubicin, MDA: malondialdehyde, GSH: reduced glutathione, CuZn-SOD: Copper, zinc superoxide dismutase, and CAT: catalase]

Table 2 — The histopathological scores of tunica media thickness of descending aorta for each group

Groups	Tunica media thickness (μm)
Control	118.1±20.9[a]
CAPE	111.1±19.4[a]
DOX	98.6±17.1[b]
CAPE+DOX	116.7±18.3[a]

[Data are expressed as mean ± SD. Values with same superscripts in column for a parameter indicates no significant difference between the groups (P>0.05). CAPE: Caffeic acid phenethyl ester, DOX: Doxorubicin]

groups, the vessel samples showed intact endothelial lining and with no cellular degeneration. No significant changes were also noted in tunica media and tunica adventitia. Distinct elastic laminae were arranged in wavy and concentric patterns in all groups with no prominent pathologies. The results of histomorphometric analysis for the thickness of tunica media layer of the aorta were presented in Table 2. Tunica media thickness was significantly reduced in DOX group (Fig. 1C) as compared to the other groups (P<0.05) (Fig. 1A, 1B & 1D). There were no detectable differences among Control, CAPE, and CAPE+DOX groups.

Anthracyclines are widely used chemotherapeutic agents in cancer treatments. A member of these agents is doxorubicin and commonly used in treatments of various types of neoplasia. However, doxorubicin is known to induce cardiotoxicity and hepatotoxicity. Doxorubicin can also impair endothelial homeostasis by IL-10 upregulation and hence may cause vessel damage and dysfunction[23]. Anthracyclines can cause endothelial damage by triggering overexpression of inflammatory cytokines and thus cause aortic stiffness. They may cause aortic stiffness through increased production of free radicals and inducing oxidative stress and damage. Oxidative damage can cause structural changes in the vascular matrix. Vascular endothelial damage further reduces nitric oxide synthesis and also increases vascular stiffness, leading to endothelial cell dysfunction[24,25]. Oxidative stress related pathways seem to be mainly responsible from doxorubicin induced cardiotoxicity and hepatotoxicity. Reactive oxygen species are produced in large amounts in the metabolically active myocardium. Organisms have developed a complex antioxidant defense system consisting of enzymatic and non-enzymatic components to defend against ROS formation[26]. Oxidative stress occurs with excessive ROS production. Reactive oxygen species can react directly or indirectly with many molecules such as proteins, lipids and nucleic acids[26,27]. Molecules such as singlet oxygen, hydroxyl radical and superoxide anion are reactive oxygen species[28]. Since the oxidative stress related cellular degeneration is the main mechanism in various cellular toxicants and agents, anti oxidant based studies are commonly in use. Flavonoids are a group of substances derived from secondary metabolites of plants and have antioxidant properties besides other beneficial effects on cellular survival. They have also been shown to improve cardiovascular outcomes by attenuating endothelial dysfunction and atherosclerosis[29].

The use of apitherapeutic products can provide positive results in the treatment of cardiovascular diseases[30]. CAPE with its strong antioxidant capacity has been shown to be effective in protecting cells from various injuries[31]. As a non-enzymatic endogenous defense mechanism, GSH can partially indicate the health status of cells[32]. In our research, GSH level of heart tissues in DOX group decreased as compared to Control group, but this decrease was not statistically significant. In addition, no significant changes were detected in the levels of MDA, CuZn-SOD and CAT, indicating that doxorubicin did not cause significant cellular damage in the aortic vessel wall in the present experimental conditions. Tissue GSH level was significantly higher in CAPE group as compared to CAPE+DOX and DOX groups, however no significant difference was observed between DOX and CAPE+DOX groups, indicating that CAPE administration did not cause significant changes in

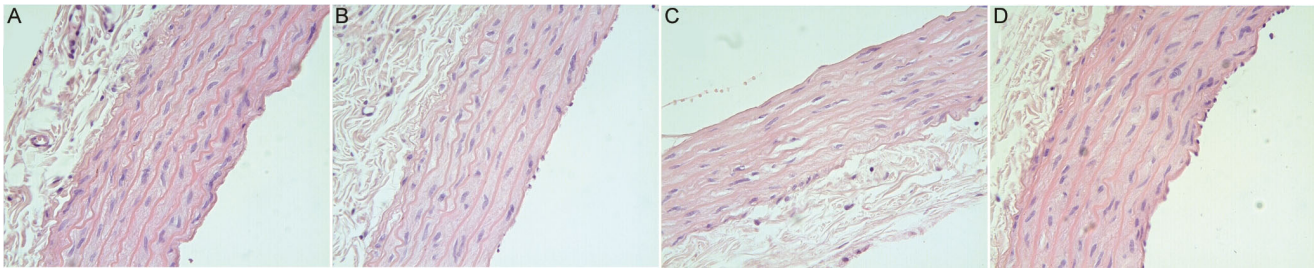

Fig. 1 — Photomicrographs of the aortic vascular walls. The vascular wall histology appears normal in the control (A) and CAPE (B) groups. Tunica media thickness seems reduced in DOX group (C). The vascular wall thickness in the CAPE+DOX group (D) are similar to the Control group. H&E; ×400.

terms of protecting cells from doxorubicin induced oxidative stress, though no significant cellular damage were detected biochemically. All biochemical parameters investigated in the current research suggests that doxorubicin treated groups did not have lipid peroxidation in the vascular tissue and CAPE had no effect on the antioxidant status of the vascular tissue of rats in all groups. Lipid peroxidation is known to be one of the most important parameters in determining oxidative damage[33].

In microscopic examination, the most prominent change in the aorta was the thinning of the tunica media in DOX group as compared to Control and CAPE groups. Most significantly, CAPE+DOX group showed nearly normal vascular histology. Similarly, focal thinning of the tunica media as well as elastication of the aortic lamina were reported in doxorubicin administered rats[34]. On the other hand, there are studies reporting increased thickening of the coronary arterial walls. Eckman *et al*.[35] reported that rats routinely given doxorubicin showed greater thickening of the coronary arterial walls at low doses, and this increase was correlated with the observed smooth muscle hypertrophy within the walls of artery segments with anthracycline-mediated increased NADPH oxidase activation in the adventitial layer.

Doxorubicin can also impair cellular functions by inducing increased expression of nitric oxide synthase (NOS). Olukman *et al*.[36] reported an increase in the expression of both endothelial and inducible NOS isoforms in rats given doxorubicin, and interpreted that the increase in NOS activity causes increased nitric oxide (NO) production and the simultaneous increase in oxygen radicals may cause a decrease in vascular relaxation, and resveratrol, an antioxidant agent, can reverse these negative effects. In another study, 12 mg/kg total dose doxorubicin for a period of 4 weeks in rats were reported to have no significant effects on body and heart weights as well as left ventricular and aortic water and calcium contents[37]. However, in the same study, they also reported that contraction responses of the thoracic aortic strips significantly reduced in doxorubicin given animals as compared to the control group indicating that doxorubicin may have physiological effects on vascular smooth muscle function at doses that do not cause any signs of toxicity in the heart muscle. In our investigation, we did not observe any significant changes in biochemical parameters though vessel thickness were noted to reduce with doxorubicin treatment.

Conclusion

We investigated the effect of CAPE treatment on aortic vessels in doxorubicin given rats. The results indicated that CAPE treatment ameliorates the histopathological changes that are characterized by the reduced vessel wall thickness induced by doxorubicin. However, CAPE treatment did not seem to effect biochemical parameters that are indicative of oxidative stress. Therefore, overall results suggest that the beneficial effects of CAPE observed in vessel walls may be due to some other pathways not investigated in the current study, which requires further detailed investigations.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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