



Trans, trans-2,4-decadienal impairs vascular endothelial function by inducing oxidative/nitrative stress and apoptosis



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ABSTRACT

Aldehydes are implicated in the development of hypertension. Trans, trans-2,4-decadienal (tt-DDE), a dietary α,β -unsaturated aldehyde, is widespread in many food products. However, the role of tt-DDE in the pathophysiology of hypertension remains unknown. This study was designed to investigate whether tt-DDE consumption evokes hypertension and to explore the mechanisms underlying such a role. Sprague-Dawley rats were administered different concentrations of tt-DDE. After 28 days, blood pressure and endothelial function of mesenteric arteries were measured. Results showed that tt-DDE treatment significantly increased blood pressure and impaired endothelial function based on endothelium-dependent vasorelaxation and p-VASP levels. Mechanistically, tt-DDE induced oxidative/nitrative stress in the arteries of rats as evidenced by overproductions of superoxide and peroxynitrite, accompanied with increased expressions of iNOS and gp91^{phox}. To further investigate the effects of tt-DDE on endothelial cells and underlying mechanisms, human umbilical vein endothelial cells (HUVECs) were treated with different concentrations of tt-DDE. tt-DDE induced oxidative/nitrative stress in HUVECs. Moreover, tt-DDE induced endothelial cells apoptosis through JNK-mediated signaling pathway. These results show, for the first time, that oral intake of tt-DDE elevates blood pressure and induces endothelial dysfunction in rats through oxidative/nitrative stress and JNK-mediated apoptosis signaling, indicating that excess ingestion of tt-DDE is a potential risk factor for endothelial dysfunction and hypertension.

1. Introduction

Hypertension is a well-known risk factor for various cardiovascular diseases [1,2]. Vascular endothelial dysfunction contributes to the initiation and progression of vascular injury and is an efficient independent predictor of hypertension [3,4]. Cumulative evidence reveals that oxidative stress directly alters endothelial function or causes changes in vascular tone, leading to the generation and/or maintenance of hypertension [5,6]. Oxidative stress-initiated lipid peroxidation of membrane phospholipids results in a large number of reactive

aldehydes, and the accumulation of endogenous aldehydes in human tissues is a major cause of cellular and tissue dysfunction [7,8]. Under pathological conditions such as vasculitis, diabetes, and atherosclerosis, vascular endothelium accumulates high levels of aldehydes [8–11]. Vascular endothelium is vulnerable to be attacked by aldehydes, and excess reactive aldehydes lead to endothelial dysfunction [6,8]. It has been proposed that excess reactive aldehydes contribute to progressive and deleterious changes in hypertensive by increasing cytosolic-free calcium levels, inducing endothelial dysfunction, and changing renal vascular function [7,8,12,13].

Abbreviations: ACh, Acetylcholine; Bax, BCL-2 associated X protein; Bcl-2, B-cell lymphoma 2; eNOS, Endothelial nitric oxide synthase; GSH, Reduced glutathione; GSSG, Oxidized glutathione; HUVECs, Human umbilical vein endothelial cells; iNOS, Inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; LDH, Lactate dehydrogenase; LDL, Low-density lipoprotein; L-NAME, N^o-nitro-L-arginine methylester; MAPK, Mitogen-activated protein kinase; MMP, Mitochondrial membrane potential; NADPH, Nicotinamide adenine dinucleotide phosphate; O₂⁻, Superoxide; ONOO⁻, Peroxynitrite; p-ERK1/2, p-eNOS^{Ser1177}; Endothelial nitric oxide synthase serine 1177 phosphorylation, p-VASP; Phosphorylation of vasodilator-stimulated phosphoprotein, ROS; Reactive oxygen species, SMT; S-methylisothiourrea, SNP; Sodium nitroprusside, tt-DDE; Trans, trans-2,4-decadienal

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Notably, reactive aldehydes are widely spread in the environment and commonly found in food and water [14,15]. They come from cigarette smoke, automobile exhaust, heated cooking oil, food additive/flavoring agents, and many other industrial products [16]. Studies have demonstrated that these aldehydes are absorbed by small intestines easily, distributed systemically, and accumulate in many tissues, leading to progressive cellular damage and nonspecific tissue injury, as well as other toxic effects related to oxidative stress [17–19]. Exogenous aldehydes can act synergistically with endogenous aldehydes to accelerate damage to human health [8,20]. However, to date, little information is available about the effects of exogenous aldehydes on blood pressure and endothelial function.

Trans, trans-2,4-decadienal (tt-DDE) is a highly reactive α,β -unsaturated aldehyde. It is not only a food additive/flavoring agent, a major byproduct of polyunsaturated lipid oxidation during storage and heating [21] but also an endogenously generated product of oxidative stress-mediated lipid peroxidation of cell membranes [22]. Dietary tt-DDE was reported to be absorbed and distributed to multiple organs and tissues, promoting enlargement of atherosclerotic lesions [23]. However, very limited data concerning the vasculotoxic effects of tt-DDE in experimental animals or humans are reported. Results of cytotoxic and lethal effects have indicated that tt-DDE is one of the most toxic breakdown products of lipid peroxidation in different experimental models such as human fibroblasts and erythroleukemia cells [9,24,25]. tt-DDE is known to react with DNA and cytochrome c, inhibiting cell growth, changing cellular glutathione levels, elevating intracellular reactive oxygen species (ROS) levels, and promoting mitochondrial oxidative damage and the production of proinflammatory cytokines [26,27]. Because of the high toxicity of tt-DDE and its widespread presence in food products, it is regarded as a high priority compound of concern by the U.S. National Cancer Institutes (NCI) and National Toxicology Program (NTP) at the National Institutes of Environmental Health Sciences (NIEHS) [26]. Therefore, elucidating the effects of tt-DDE on blood pressure and vascular endothelial function are urgently needed.

Accordingly, the present study was designed to investigate whether tt-DDE exposure increased blood pressure and impaired vascular endothelial function in rats, and if so, to further explore the underlying molecular involved.

2. Materials and methods

2.1. Animals and experimental design

All experimental procedures in this study were performed according to the National Institutes of Health Guidelines on the Use of Laboratory Animals, and were approved by the Animal Research Committee of Dalian Medical University. Male Sprague-Dawley (SD) rats were purchased at the age of 4 weeks from the Liaoning Changsheng Biotechnology Co., Ltd. (Benxi, China). These rats were housed under standard conditions with a 12/12 h light-dark cycle at a room temperature of 22 ± 2 °C and humidity $60 \pm 5\%$ during the experiment. After one week of acclimation, rats were randomly divided into three groups ($n = 8$ per group) and received tt-DDE in corn oil by gavage at doses of 0 (control group), 500 (low dose group; tt-DDE-L), or 1000 mg tt-DDE/kg body weight (high dose group; tt-DDE-H) once daily for 28 days. Food consumption and body weights were measured weekly. At the end of the exposure period, rats were intraperitoneally injected with 0.4 mL of 10% (w/v) chloral hydrate per 100 g. Serum and tissues were collected and stored at -80 °C until further analysis.

The doses of tt-DDE were determined based on prior dose-response results. In the preliminary experiments, rats were administered orally with 0, 50, 100, 200, 500, or 1000 mg/kg of tt-DDE for 28 consecutive days. No deaths or clinical abnormalities were observed in rats treated with 50, 100 or 200 mg/kg tt-DDE, including body weight, serum glucose, serum lipid, blood pressure, and liver and kidney functions

(data not shown). However, the blood pressure in the 500 and 1000 mg/kg treatment groups were significantly increased, suggesting that high-dose tt-DDE exposure is a potential risk factor of hypertension. Therefore, doses of 500 and 1000 mg/kg were selected as the low and high doses in this study.

2.2. Measurement of blood pressure and heart rate

Blood pressure was measured every week using the tail-cuff method. Before the blood pressure measurement, rats were placed in a dark, temperature-controlled chamber for three consecutive days (30 min per day). On the day of measurements, the rats were confined to the temperature-controlled chamber (37 °C) for 10–15 min. After the rats were habituated to the procedure, the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MBP) and heart rate (HR) were continuously monitored. Reported blood pressure values are the average of five sequential blood pressure measurements.

2.3. Measurement of blood biochemical parameters

Serum concentrations of alanine transaminase (ALT), creatinine, urea nitrogen, low-density lipoprotein (LDL), total cholesterol and triglycerides were quantified using commercially available diagnostic kits (Jiancheng BioEngineering, Nanjing, China) according to the manufacturer's instructions. Fasting blood glucose was measured using a diagnostic glucometer (SureStep, Lifescan, USA).

2.4. Evaluation of vasorelaxation *in vivo* and *ex vivo*

Endothelial function was determined by investigating the vasorelaxation response to acetylcholine (ACh) as described previously [28]. Briefly, the third branches of mesenteric arteries were carefully excised and cut into 1-mm ring segments in ice-cold physiological saline solution (PSS). The contractile force was recorded using a PowerLab Chart v 7.2.1 program (Danish MyoTechnology, Arhus, Denmark). After a 60 min equilibration period, the mesenteric artery segments were primed with 80 mM KCl to verify vascular smooth muscle viability. The cumulative dose responses of ACh (10^{-10} to 10^{-6} M) or sodium nitroprusside (SNP; 10^{-10} to 10^{-6} M) were recorded in phenylephrine (PE; 1 μ M)-precontracted mesenteric artery segments. For *ex vivo* experiments, the cultured mesenteric arteries of rats were treated with vehicle (phosphate buffer saline) or 10 μ M tt-DDE for 24 h, then the vasoactive response was determined. To evaluate NO availability, mesenteric arteries were pre-treated with N^{ω} -nitro-L-arginine methylester (L-NAME; 100 μ M, Sigma, USA), an NOS inhibitor, 30 min before ACh stimulation. In some experiments, ACh-induced relaxation was tested in tt-DDE-treated arteries co-incubated with the iNOS inhibitor S-methylisothiourea (SMT; 100 μ M, Sigma, USA), the NADPH oxidase inhibitor apocynin (10 μ M, MedChemExpress, USA), or the superoxide dismutase mimetic tempol (1 mM, Sigma, USA), respectively. The concentrations of tt-DDE and inhibitors were chosen based on preliminary dose-response experiments and previous studies [29].

2.5. Measurement of total NO production

Total NO production (NOx) from arteries of rats was estimated by measuring the concentrations of NO and its oxidative metabolic products, nitrite and nitrate, using a modified Griess reaction method according to the manufacturer's instructions (Beyotime Biotechnology, Shanghai, China). Intracellular NO was examined in human umbilical vein endothelial cells (HUVECs) loaded with the NO-sensitive fluorescent dye diaminofluorescein-2 diacetate (DAF2-DA; 5 μ M, Sigma, USA) at 37 °C for 15 min. Samples were monitored under a Leica DM 14000B confocal microscope (Wetzlar, Germany).

2.6. Determination of superoxide production

Superoxide (O_2^-) production from arteries of rats and viable HUVECs were quantified by lucigenin-enhanced chemiluminescence as previously described [30]. Data are expressed as relative light units (RLU) per second per mg protein (RLU/s/mg protein). Dihydroethidine (DHE, Wako Chemical, Osaka, Japan) staining was used to detect the formation of superoxide *in situ* in both aortic segments and HUVECs as previously described [31]. Fluorescence was measured using an inverted fluorescence microscope (Olympus, USA).

2.7. Determination of reduced and oxidized glutathione

The amounts of reduced (GSH) and oxidized (GSSG) glutathione from arteries of rats were determined by the colorimetric method using a total glutathione/oxidized glutathione assay kit (Beyotime Biotechnology, Shanghai, China), and the GSH and GSSG ratio was calculated.

2.8. Determination of nitrotyrosine

Nitrotyrosine contents in arteries and cells, an index of peroxynitrite ($ONOO^-$) formation and nitrate stress, was established using a nitrotyrosine ELISA assay kit (Northwest Life Science, CA, USA) as previously described [32].

2.9. Determination of NOS activity

Total NOS activity and inducible NOS (iNOS) activity from arteries of rats were determined using an NOS activity assay kit (tNOS, Jiancheng BioEngineering, Nanjing, China) according to the manufacturer's instructions. Endothelial NOS (eNOS) activity from arteries of rats was obtained by deducting iNOS activity from total NOS activity, and results were normalized to the mean value of controls.

2.10. Cell culture and drug treatment

HUVECs were purchased from ScienCell (Carlsbad, CA, USA) and cultured in endothelial cell medium (ECM; ScienCell, Carlsbad, CA, USA) containing 5% (v/v) fetal bovine serum, 1% (v/v) endothelial cell growth supplement, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 °C under a humidified atmosphere with 5% CO_2 . Cells at passages 4–8 were used for all experiments. A stock solution of tt-DDE (100 mM) in dimethyl sulfoxide (DMSO) was prepared and maintained at –20 °C. tt-DDE was diluted in culture medium to obtain the desired concentration, which was stable at DMSO concentrations less than 0.1%. Cells were pretreated with tempol (100 μ M), SB203580 (p38 inhibitor, 10 μ M, Calbiochem, USA), or SP600125 (JNK specific inhibitor, 20 μ M, Calbiochem, USA) for 30 min before exposure to tt-DDE [33,34].

2.11. Cell viability

Cell viability was determined using the CCK-8 assay. Briefly, 7×10^3 cells were seeded into 96-well culture plates and allowed to adhere for 24 h. Then, the culture medium was changed to fresh medium containing different concentrations of tt-DDE (5, 10, 15, 20, 25, or 50 μ M). After incubation for the indicated time (12, 24, and 48 h), CCK-8 was added, and absorbances were measured at 450 nm using an *EnSpire*® Multimode Plate Reader (PerkinElmer, MA, USA). Effects on cell viability were assessed as the percent cell viability compared with that in the untreated control group, which was arbitrarily considered 100% viability. tt-DDE concentration that caused 50% cell growth inhibition (IC_{50}) was determined by interpolation from dose-response curves.

2.12. Lactate dehydrogenase (LDH) release

LDH leakage from cells into media, an indicator of cell injury, was detected using an assay kit (KeyGEN Biotech, Nanjing, China) according to the manufacturer's instructions.

2.13. Determination of caspase-3 activity

Caspase-3 activity in freshly isolated arteries of rats and HUVECs was evaluated using a caspase-3 colorimetric assay kit (Chemicon International, Temecula, CA, USA) according to the manufacturer's instructions.

2.14. Apoptosis analysis

Apoptosis of HUVECs was assessed using the Annexin V-FITC Apoptosis Detection Kit according to the manufacturer's instructions (KeyGEN Biotech, Nanjing, China). In brief, cells in 70% confluent plates were treated with different doses of tt-DDE. After treatment for 24 h, the cells were collected and stained simultaneously with FITC-labeled annexin V and propidium iodide (PI). The stained cells were analyzed by FACS Accuri C6 flow cytometer (Becton Dickinson, CA, USA).

2.15. Analysis of mitochondrial membrane potential

Mitochondrial membrane potential (MMP, $\Delta\psi_m$) was measured with a unique cationic dye of JC-1 (Beyotime Biotechnology, Shanghai, China). Briefly, cells (2×10^5 cells/mL) were seeded in 6-well plates overnight and treated with different concentrations of tt-DDE for 24 h, then incubation with JC-1 (10 μ g/mL) at 37 °C for 15 min. Monomeric JC-1 green fluorescence emission and aggregate JC-1 red fluorescence emission were monitored under an inverted fluorescence microscope (Olympus, USA).

2.16. Confocal immunofluorescence

Cells were cultured and immunofluorescence stained in chamber slides. After exposure to tt-DDE, cells were incubated with Mito-Tracker® Red (Life Science, CA, USA) for 30 min, and then fixed with 4% (w/v) paraformaldehyde. After permeabilizing and blocking, they were incubated with antibodies against cytochrome *c* overnight and then secondary fluorescein isothiocyanate (FITC)-labeled antibody at ambient temperature for 1 h. Subsequently, the chamber slides were mounted with 4', 6-diamidino-2-phenylindole (DAPI)-containing Vectashield solution (Vector Labs, Burlingame, CA, USA) to counterstain cell nuclei. The samples were monitored under a Leica DM 14000B confocal microscope (Wetzlar, Germany).

2.17. Western blot analysis

Arteries and HUVECs were lysed in lysis buffer or using a cytoplasmic and nuclear protein extraction kit (Beyotime Biotechnology, Shanghai, China). Protein concentrations were determined using a BCA protein assay kit (Beyotime Biotechnology, Shanghai, China). Equal amounts of proteins were separated by sodium dodecyl sulfide-polyacrylamide gel electrophoresis and electrotransferred onto a polyvinylidene fluoride membrane (PVDF, 0.45 μ m, Millipore, Molsheim, France). After blocking, the membranes were incubated with respective primary antibodies at the manufacturer's recommended dilutions overnight at 4 °C. Then the membranes were incubated with the secondary antibodies, and blots were detected with chemiluminescent substrate (ThermoFisher, Shanghai, China). The primary antibodies for Bax, Bcl-2, cleaved caspase-3, cleaved caspase-9, cytochrome *c*, JNK, p-JNK, ERK1/2, p-ERK1/2, p38, p-p38, eNOS, p-eNOS^{Ser1177}, iNOS, β -actin, and all of the secondary antibodies were obtained from Cell

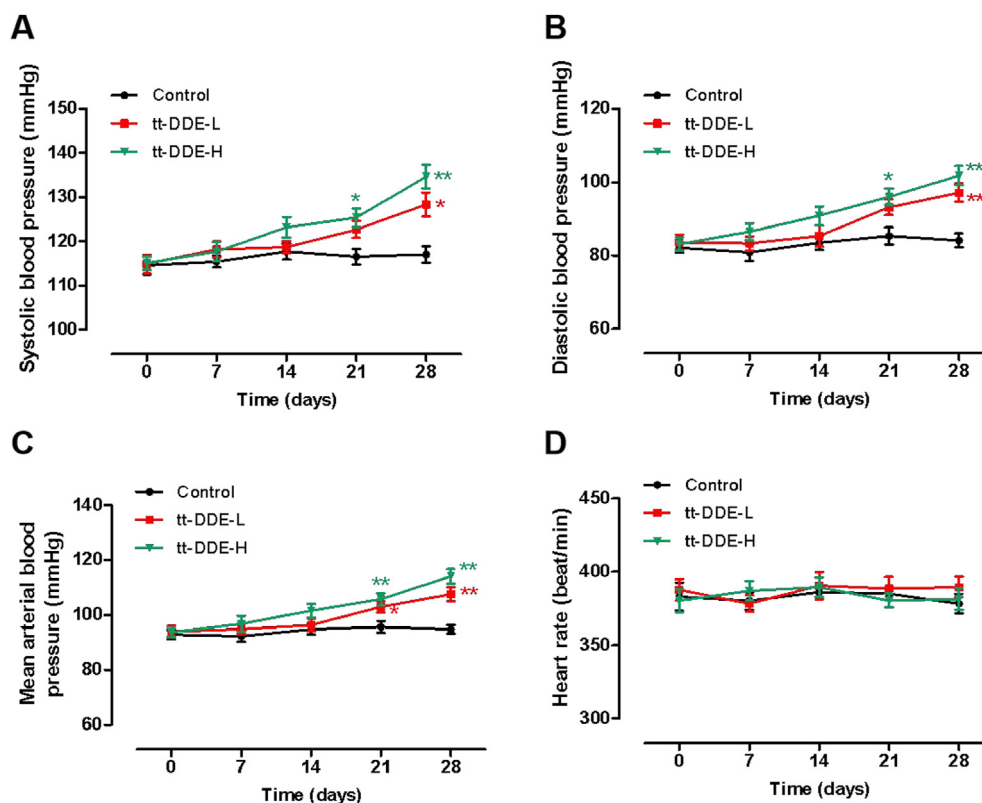


Fig. 1. The effects of tt-DDE on blood pressure and heart rate in rats. (A) Systolic blood pressure (SBP). (B) Diastolic blood pressure (DBP). (C) Mean arterial blood pressure (MAP). (D) Heart rate (HR). All values are presented as mean \pm SEM; n = 8 in each group. * $p < 0.05$, ** $p < 0.01$ vs. Control.

Signaling Technology (Danvers, MA, USA). The primary antibodies for p-VASP S239, VASP and gp91^{phox} were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Protein densities were quantified using Quantity-One Software (Bio-Rad, CA, USA) and normalized with β -actin as an internal control.

2.18. Statistical analysis

Data are represented as means \pm standard error of mean (SEM). Statistical significance was determined by one-way ANOVA, followed by Bonferroni's correction where appropriate. Differences were considered significant when $p < 0.05$. Statistical tests were performed using GraphPad Prism software version 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Basic parameters in tt-DDE-treated rats

As shown in Fig. 1, the systolic, diastolic, and mean arterial blood pressure (SBP, DBP, and MAP, respectively) in tt-DDE-treated rats were all significantly increased compared with those from vehicle-treated rats after 28 days of treatment, but no difference in heart rate was observed among all groups. tt-DDE consumption also resulted in decreased body weight and food intake, and increased serum LDL levels (Table 1). In addition, there was no statistical significance in fasting blood glucose, cholesterol, triglycerides, ALT, creatinine and urea nitrogen among all groups (Table 1), and no obvious histological abnormalities were observed in liver and kidney tissues (Fig. S1).

3.2. tt-DDE impaired endothelial function in mesenteric arteries of rats in vivo and ex vivo

Endothelial dysfunction plays a vital role in the genesis of hypertension [35]. To determine the effects of tt-DDE on endothelial function, relaxation of mesenteric arteries in response to ACh, an endothelium-dependent vasodilator, was investigated. As shown in Fig. 2A and B, concentration-dependent vasodilation in response to ACh was impaired in vascular segments from the tt-DDE-treated rats compared with those from the vehicle-treated rats. However, endothelium-independent relaxation in response to SNP was identical in all groups. These results indicated that tt-DDE induced impairment of endothelial function rather than smooth muscle function in rats. Then, phosphorylation of vasodilator-stimulated phosphoprotein at serine 239 (p-VASP), a novel marker for NO/cGMP/cGK signaling and a biochemical indicator of NO availability in vascular tissues, was examined [36]. Results showed that p-VASP levels were significantly decreased in arteries from tt-DDE-treated rats (Fig. 2C). Additionally, mesenteric arteries incubated with tt-DDE showed significant impairment in ACh-induced vasorelaxation, while SNP-induced vasorelaxation was normal, indicating that tt-DDE directly impaired endothelial function (Fig. 2D and E). In arteries from vehicle control, L-NAME significantly blunted ACh-induced vasorelaxation. However, in tt-DDE incubated arteries, the inhibitory effect exerted by L-NAME on ACh-induced relaxation was lower compared to the vehicle control (Fig. 2D). Together, these results suggested that tt-DDE-induced endothelial dysfunction through reducing NO bioavailability.

3.3. tt-DDE induced oxidative/nitrative stress

Considerable evidence suggests that increased superoxide generation and interactions between superoxide and NO are the major reasons for endothelial dysfunction [28]. As summarized in Fig. 3A and B, tt-

Table 1
General parameters in SD rats after 28-day oral consumption of tt-DDE.

Parameters	Control	tt-DDE-L	tt-DDE-H
Initial body weight (g)	132.65 ± 9.43	132.25 ± 11.17	132.65 ± 12.10
Final body weight (g)	290.60 ± 22.03	260.30 ± 15.98*	244.06 ± 11.20**
Body weight gain (g)	157.95 ± 20.59	128.07 ± 16.62**	111.41 ± 16.05**
Total food intake (g)	1133.12 ± 37.32	934.30 ± 57.52*	825.35 ± 39.04**
Cholesterol (mmol/L)	3.22 ± 0.79	3.65 ± 0.86	3.49 ± 0.64
Tryglycerides (mmol/L)	0.51 ± 0.16	0.66 ± 0.37	0.52 ± 0.13
LDL (mmol/L)	0.74 ± 0.13	0.92 ± 0.21	1.03 ± 0.21*
Fasting blood glucose (mg/dl)	6.3 ± 0.47	5.8 ± 0.38	7.1 ± 0.51
ALT (U/L)	46.90 ± 11.73	57.38 ± 10.48	52.31 ± 9.04
Creatinine (μmol/L)	49.76 ± 8.63	52.51 ± 11.03	56.28 ± 10.42
Urea nitrogen (mmol/L)	3.51 ± 0.79	3.95 ± 0.88	4.45 ± 1.12

Values are means ± SEM (n = 8). LDL: Low-density lipoprotein; ALT: Alanine transaminase. **p* < 0.05, ***p* < 0.01 vs. Control.

DDE ingestion induced a dramatic increase in basal superoxide production in arteries, as evidenced by both DHE and lucigenin-enhanced chemiluminescence analysis. tt-DDE also significantly reduced GSH

levels and increased GSSG levels, resulted in reduced GSH/GSSG ratio (an important marker of cellular redox balance) (Fig. 3C-E). These results indicated that tt-DDE induced significant oxidative stress in

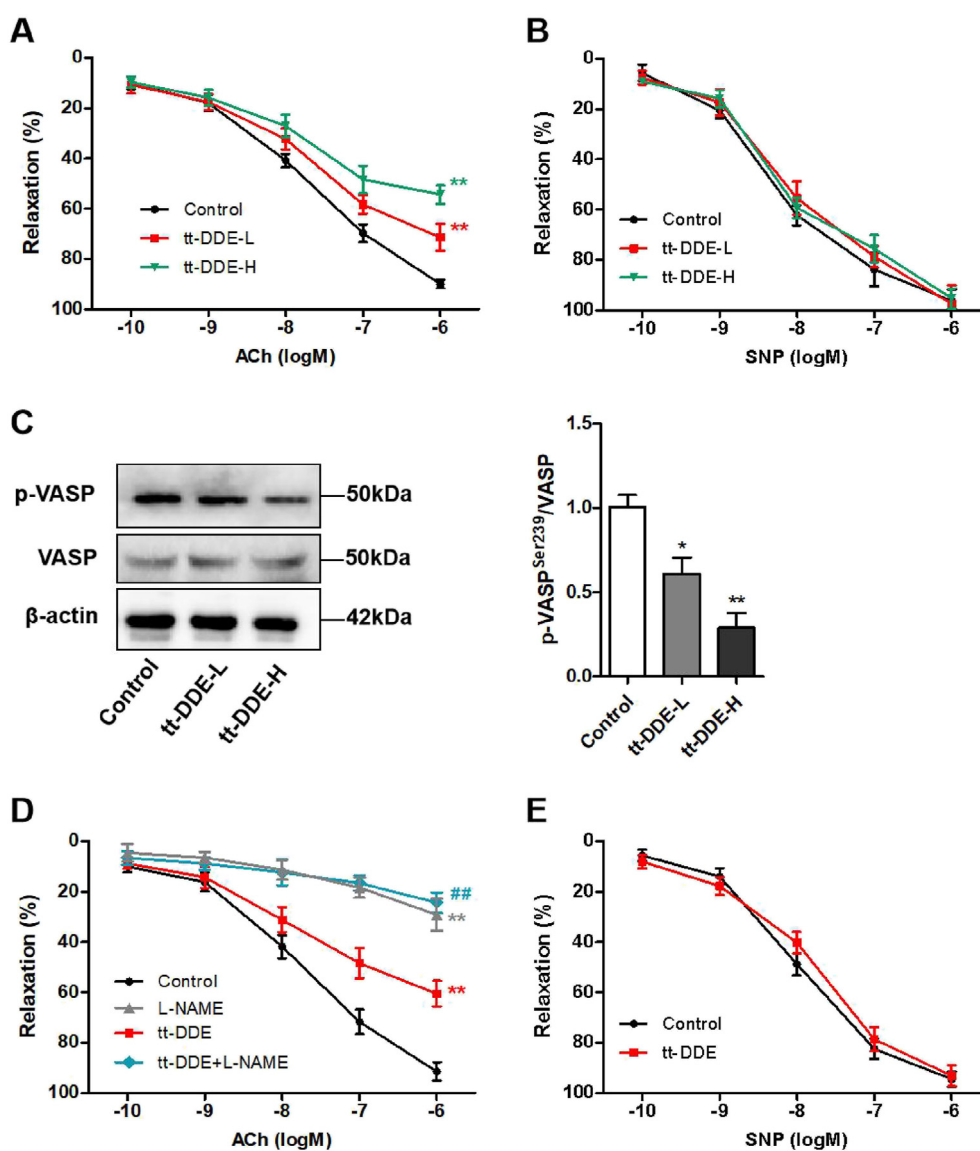


Fig. 2. The effects of tt-DDE on endothelial function *in vivo* and *ex vivo*. (A) Dose-response curves for ACh-induced relaxation of mesenteric arteries in vehicle and tt-DDE treated rats. (B) Dose-response curves for SNP-induced relaxation of mesenteric arteries in vehicle and tt-DDE treated rats. (C) Representative blots and quantified data of p-VASP expression. (D) Dose-response curves for ACh-induced relaxation of cultured mesenteric arteries. (E) Dose-response curves for SNP-induced relaxation of cultured mesenteric arteries. All values are presented as mean ± SEM; n = 6–8 in each group. **p* < 0.05, ***p* < 0.01 vs. Control; ##*p* < 0.01 vs. tt-DDE.

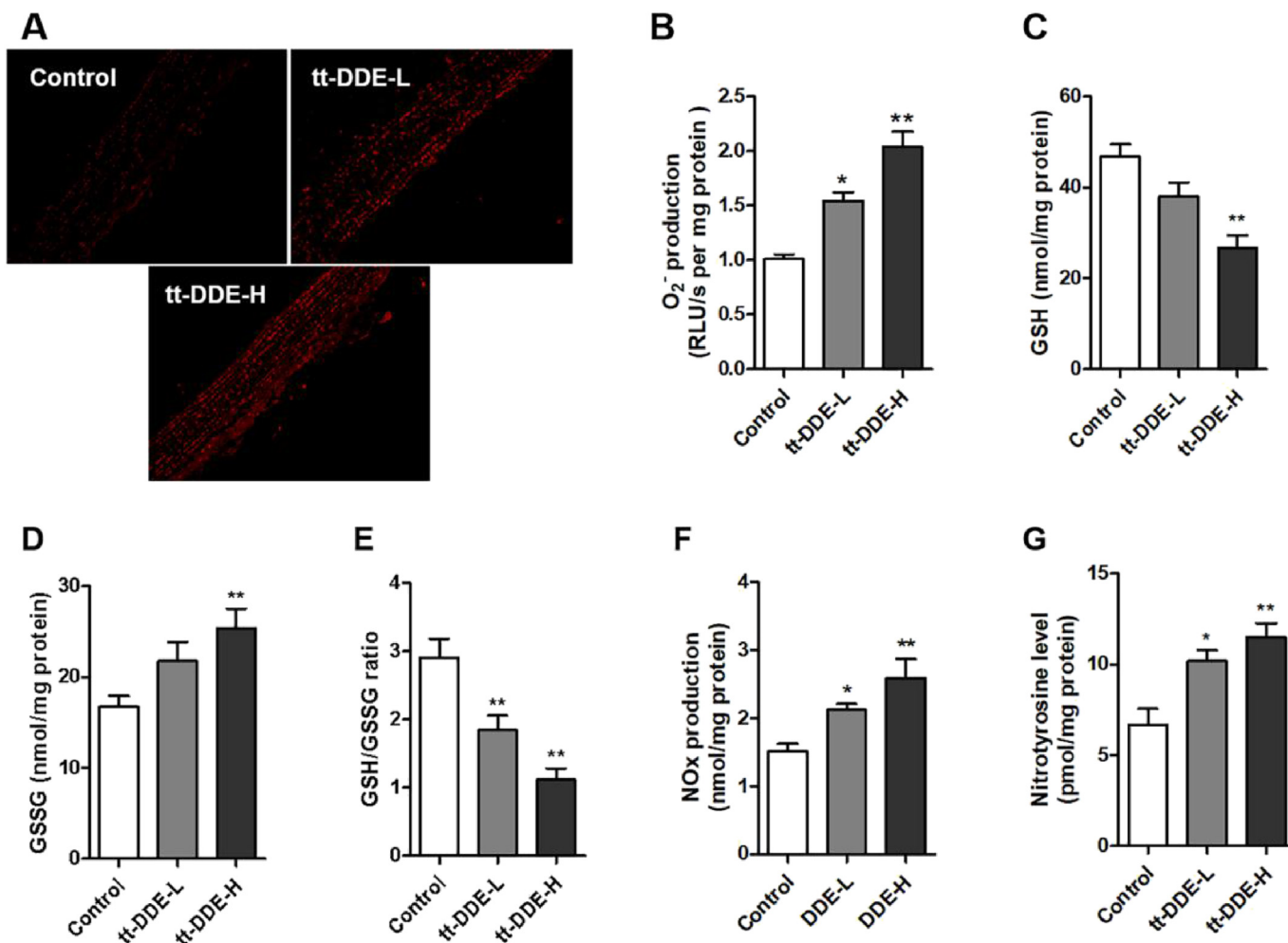


Fig. 3. The effects of tt-DDE on oxidative/nitrative stress in arteries of rats. (A) *In situ* superoxide production as detected by DHE staining in aortic segments of rats (original magnification, $100\times$). (B) Superoxide production in arteries determined by lucigenin-enhanced chemiluminescence. (C–E) Glutathione (GSH) levels, oxidized glutathione disulfide (GSSG) levels, and ratios of GSH/GSSG in arteries of rats. (F) NOx levels and (G) Nitrotyrosine formation in arteries of rats. All values are presented as mean \pm SEM; $n = 6-8$ in each group. * $p < 0.05$, ** $p < 0.01$ vs. Control.

arteries of rats. Moreover, NOx production was markedly increased in arteries of tt-DDE-treated rats (Fig. 3F). Reaction of superoxide with NO leads to the formation of ONOO⁻, resulting in diminished NO bioavailability and aggravated vascular injury [32,37]. As expected, the ONOO⁻ content was increased in tt-DDE-treated arteries compared with those from vehicle controls (Fig. 3G), suggesting a significant nitrative stress.

3.4. tt-DDE regulated eNOS phosphorylation and expressions of iNOS and gp91^{phox}

To identify the enzymatic sources contributing to tt-DDE-induced oxidative/nitrative stress, the levels of p-eNOS, iNOS, and NADPH oxidase in arteries of rats were determined. As summarized in Fig. 4A–C, the eNOS activity and phosphorylation expression were significantly reduced in arteries of tt-DDE-treated rats, while the iNOS activity and expression were significantly increased, which indicated that iNOS is the source of enzymes causing NOx overproduction. NADPH oxidase gp91^{phox} is the most important enzyme responsible for superoxide production in vasculature [28]. Results showed that gp91^{phox} expression in arteries of tt-DDE-treated rats was dose-dependently increased (Fig. 4B and C). These results suggested that tt-DDE induced oxidative/nitrative stress by stimulating the expressions of iNOS and NADPH oxidase. Additionally, the contributions of iNOS, NADPH oxidase and ROS production to tt-DDE induced endothelial

dysfunction were further evaluated. As shown in Fig. 4D, inhibition of iNOS, NADPH oxidase or ROS production significantly improved endothelium-dependent relaxation in tt-DDE-treated arteries. These results indicated that NADPH oxidase and iNOS-mediated oxidative/nitrative stress plays an important role in tt-DDE induced endothelial dysfunction.

3.5. tt-DDE induced oxidative/nitrative stress in HUVECs

Vascular endothelial cells play a crucial role in maintaining vascular homeostasis. To further investigate direct toxic effect of tt-DDE on vascular endothelium, we cultured HUVECs. As shown in Fig. 5A, tt-DDE significantly reduced cell viability in a dose- and time-dependent manner. The IC₅₀ value of tt-DDE was 15.37 μ M after 24 h of treatment, and the doses of 5, 10 and 15 μ M were used for further study. As shown in Fig. 5B, tt-DDE significantly increased the LDH release in endothelial cells, but there was no significant cytotoxicity to human vascular smooth muscle cells. tt-DDE also induced endothelial cell dysfunction as evidenced by impaired HUVEC angiogenesis and migratory capacities (Fig. S3). Furthermore, the results of DHE staining and lucigenin-enhanced chemiluminescence analysis showed that tt-DDE significantly increased O₂⁻ production (Fig. 5B and C). DAF2-DA fluorescence analysis in HUVECs confirmed an increase in NO production after 24 h of tt-DDE incubation (Fig. 5D). Consistent with the results from rat arteries, ONOO⁻ levels were also increased in HUVECs (Fig. 5E).

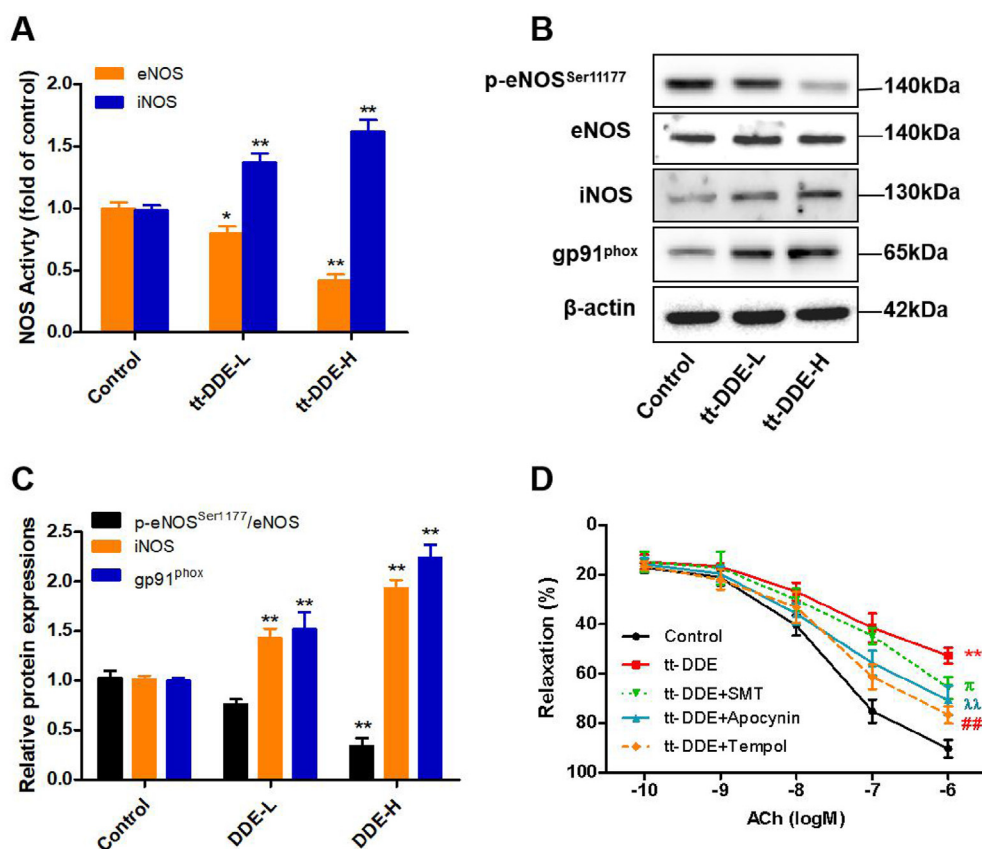


Fig. 4. The effects of tt-DDE on enzymes responsible for oxidative/nitrative stress in arteries of rats. (A) eNOS and iNOS activities in arteries of rats. (B) Representative blots and (C) quantified data for p-eNOS, iNOS, and gp91^{phox} in arteries of rats. (D) The effects of S-methylisothiourea (SMT), apocynin and tempol on ACh-induced relaxation of tt-DDE incubated mesenteric arteries. All values are presented as mean \pm SEM; n = 6–8 in each group. * p < 0.05, ** p < 0.01 vs. Control; $^{\pi}p$ < 0.05, $^{\lambda\lambda}p$ < 0.01, $^{##}p$ < 0.01 vs. tt-DDE.

Moreover, decreased p-eNOS levels and increased iNOS and NADPH oxidase were observed in tt-DDE-treated HUVECs (Fig. S4). Together, these results indicated that tt-DDE induced cytotoxicity and oxidative/nitrative stress in HUVECs.

3.6. Tempol restored tt-DDE-induced apoptosis

Oxidative/nitrative stress of vascular walls is closely related to excessive apoptosis of endothelium, and apoptosis reflects deterioration after endothelial cell injury [28]. Therefore, we further tested whether tt-DDE induced apoptosis of endothelial cells and explored underlying mechanisms. Flow cytometry assay showed that exposure to tt-DDE significantly increased apoptotic HUVEC death in a dose-dependent manner (Fig. 6A and B). In addition, tt-DDE dose-dependently increased expressions of cleaved caspase-3 and caspase-9 in HUVECs (Fig. 6C). Similarly, the caspase-3 activity was significantly increased in the arteries of tt-DDE-treated rats (Fig. 6D). To explore whether tt-DDE-induced O₂⁻ overproduction is related to apoptosis in endothelial cells, levels of LDH and caspase-3 with tempol were estimated. As shown in Fig. 6E–G, tempol pretreatment significantly reduced LDH release, and caspase-3 activity and expression in comparison with those in tt-DDE-treated HUVECs alone. These results suggested that tt-DDE-induced apoptosis in endothelial cells were partly mediated by an increase in superoxide anion generation.

3.7. tt-DDE activated mitochondria apoptosis signaling in HUVECs

Mitochondria is a critical regulator of cell death, and mitochondrial insults can cause an imbalance between ROS production and removal, resulting in net ROS production [38]. As shown in Fig. 7A, tt-DDE significantly reduced MMP in endothelial cells. Specific protein expressions in the mitochondrial signaling pathway are shown in Fig. 7B, with exposure to tt-DDE dose-dependently attenuating the Bcl-2/Bax

ratio compared to that in the control cells. In addition, exposure to tt-DDE markedly increased cytochrome *c* release from mitochondria to the cytosol (Fig. 7B). Simultaneously, an immunofluorescence imaging analysis was performed to confirm the co-localization of cytochrome *c* and mitochondria (Fig. 7C). These results demonstrated tt-DDE-induced mitochondria dysfunction and activation of mitochondria apoptosis signaling through regulation of the Bcl-2/Bax ratio and triggering of cytochrome *c* release in HUVECs.

3.8. tt-DDE-induced apoptosis by MAPK signaling pathways

Oxidative stress-activated MAPK is involved in apoptosis [39]. The roles of the JNK, ERK1/2, and p38 pathways in tt-DDE-induced apoptosis were investigated. As shown in Fig. 8A, exposure of cells to tt-DDE significantly increased phosphorylation of JNK and p38 in a dose-dependent manner, whereas there was no significant difference in phosphorylation of ERK1/2. Consistently, tt-DDE significantly increased phosphorylations of JNK and p38 without affecting ERK1/2 phosphorylation in arteries of rats (Fig. S5). To clarify the involvement of MAPKs in modulation of apoptosis by tt-DDE, the effects of JNK inhibitor SP and p38 inhibitor SB on caspase-3 activities were assayed. As shown in Fig. 8B, the JNK inhibitor effectively blocked the stimulatory role of tt-DDE in cleaved caspase-3 expression. However, the p38 inhibitor showed no obvious effect on cleaved caspase-3 levels (Fig. 8C). These results indicated that tt-DDE-induced endothelial cells apoptosis was closely related to activation of JNK phosphorylation.

4. Discussion

In the present study, we demonstrated for the first time that tt-DDE consumption increased blood pressure through impairing vascular endothelial function. Additionally, our data provided evidence that oxidative/nitrative stress and apoptosis were causally linked to the tt-DDE

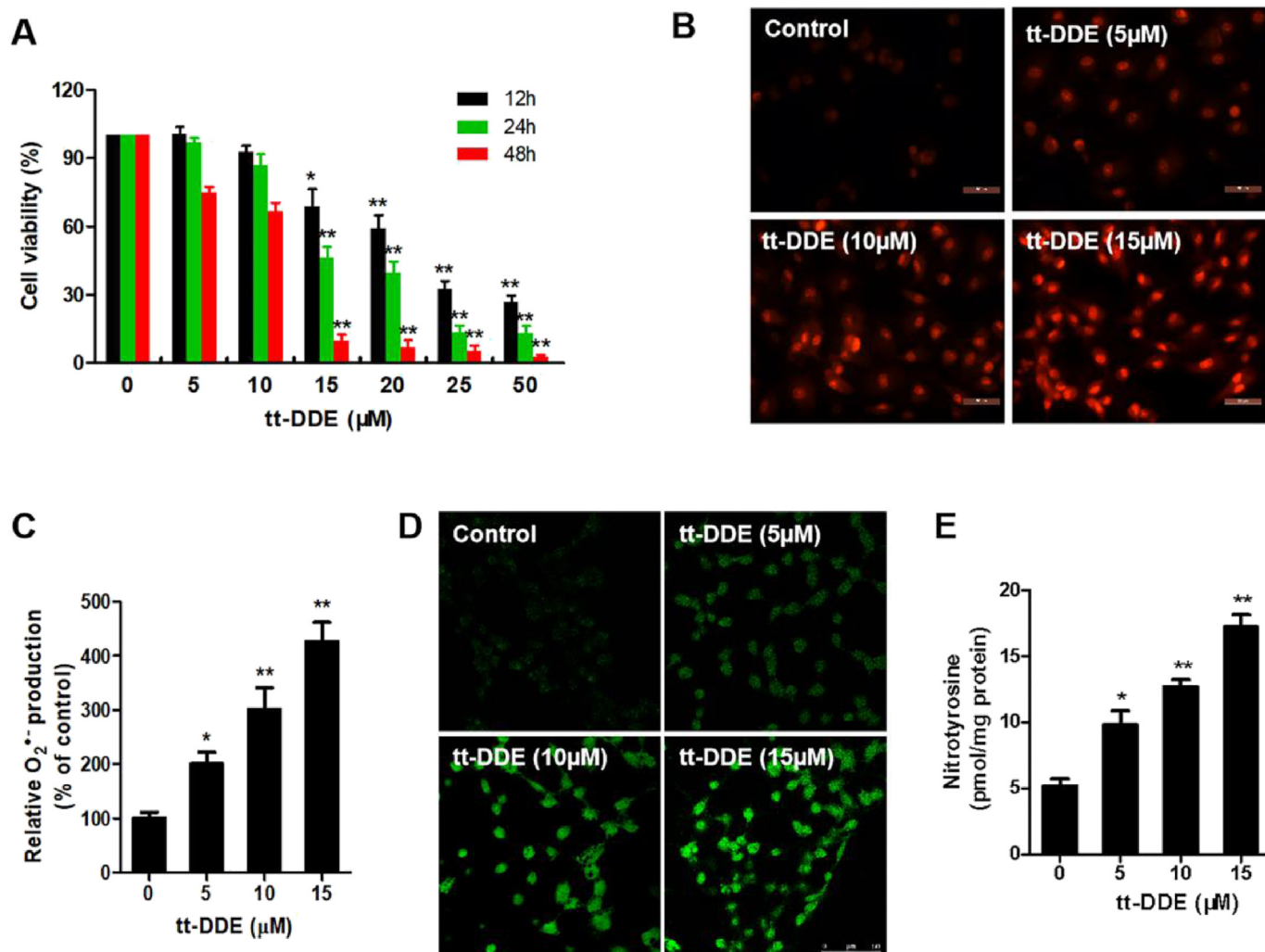


Fig. 5. The effects of tt-DDE on cell viability and oxidative/nitritive stress in HUVECs. (A) Cells viability was determined by CCK-8 assay. (B) Representative images of DHE staining (scale bar: 50 μm). (C) Superoxide production determined by lucigenin-enhanced chemiluminescence in HUVECs. (D) NO production as detected by DAF2-DA fluorescence in HUVECs (scale bar: 50 μm). (E) Nitrotyrosine level in HUVECs. All values are presented as the means ± SEM of three separate experiments. * $p < 0.05$, ** $p < 0.01$ vs. Control.

induced endothelial dysfunction, leading to the blood pressure elevation. Our results suggest that tt-DDE exposure is a potential risk factor for endothelial dysfunction and hypertension.

Numerous studies have reported that reactive aldehydes as products of lipid peroxidation are implicated in cardiovascular diseases, including hypertension [6,13,16,23]. tt-DDE is one of the most abundant and cytotoxic aldehydes among lipid oxidation products [40]. It is widely spread in the environment and food products, making human exposure virtually universal. However, scientific studies on the effects of tt-DDE exposure on the cardiovascular system of humans or animals are largely unknown. In the present study, we reported that tt-DDE intake for 28 days induced blood pressure elevation in rats. Importantly, we found that tt-DDE impaired the vasodilatory function of mesenteric arteries *in vivo* and *ex vivo* via reduction of NO bioavailability. These results suggested that tt-DDE exposure induced endothelial dysfunction by impairing NO availability, and subsequently led to elevated blood pressure in rats. In the future, it is necessary to conduct large cohort studies in general population to investigate the effects of dietary intake, occupational exposure, or environmental concentrations of tt-DDE on human blood pressure.

Oxidative stress is an important cause of hypertension by quenching vasodilator NO, directly damaging endothelial cells, altering the redox state, and stimulating inflammatory and growth-signaling events [6]. In

Chang's study, tt-DDE significantly attenuated glutathione status and accumulated ROS in human bronchial epithelial cells [26]. Consistently, the present study showed that exposure to tt-DDE significantly induced oxidative stress in rats. We also demonstrated that tt-DDE triggered O₂^{•-} formation by stimulation of NADPH oxidase gp91^{phox} expression. Under physiological conditions, eNOS produced low concentrations of NO, which protects the cardiovascular system, whereas, in pathological conditions, iNOS promotes high concentrations of NO, which leads to harmful effects on the cardiovascular system [41]. Increased superoxides rapidly react with NO, resulting in *in situ* formation of peroxynitrite, a strong oxidative/nitritive molecule that aggravates vascular injury [37]. In this regard, 4-hydroxy-2-nonenal (4-HNE), another α,β-unsaturated aldehyde, was reported to induce endothelial dysfunction by reducing NO bioavailability via modulation of NO synthase activity and elevating peroxynitrite [42]. In the present study, tt-DDE induced obviously nitritive stress by inhibited eNOS phosphorylation and activated iNOS expression in both tt-DDE-treated rats and HUVECs. More importantly, inhibition of iNOS, NADPH oxidase or ROS production greatly restored tt-DDE-induced endothelial dysfunction, suggesting that iNOS and NADPH oxidase contribute to tt-DDE-induced endothelial dysfunction. Together, we showed for the first time that ingestion of tt-DDE impaired endothelial function by reducing NO bioavailability and exacerbating oxidative/nitritive stress via

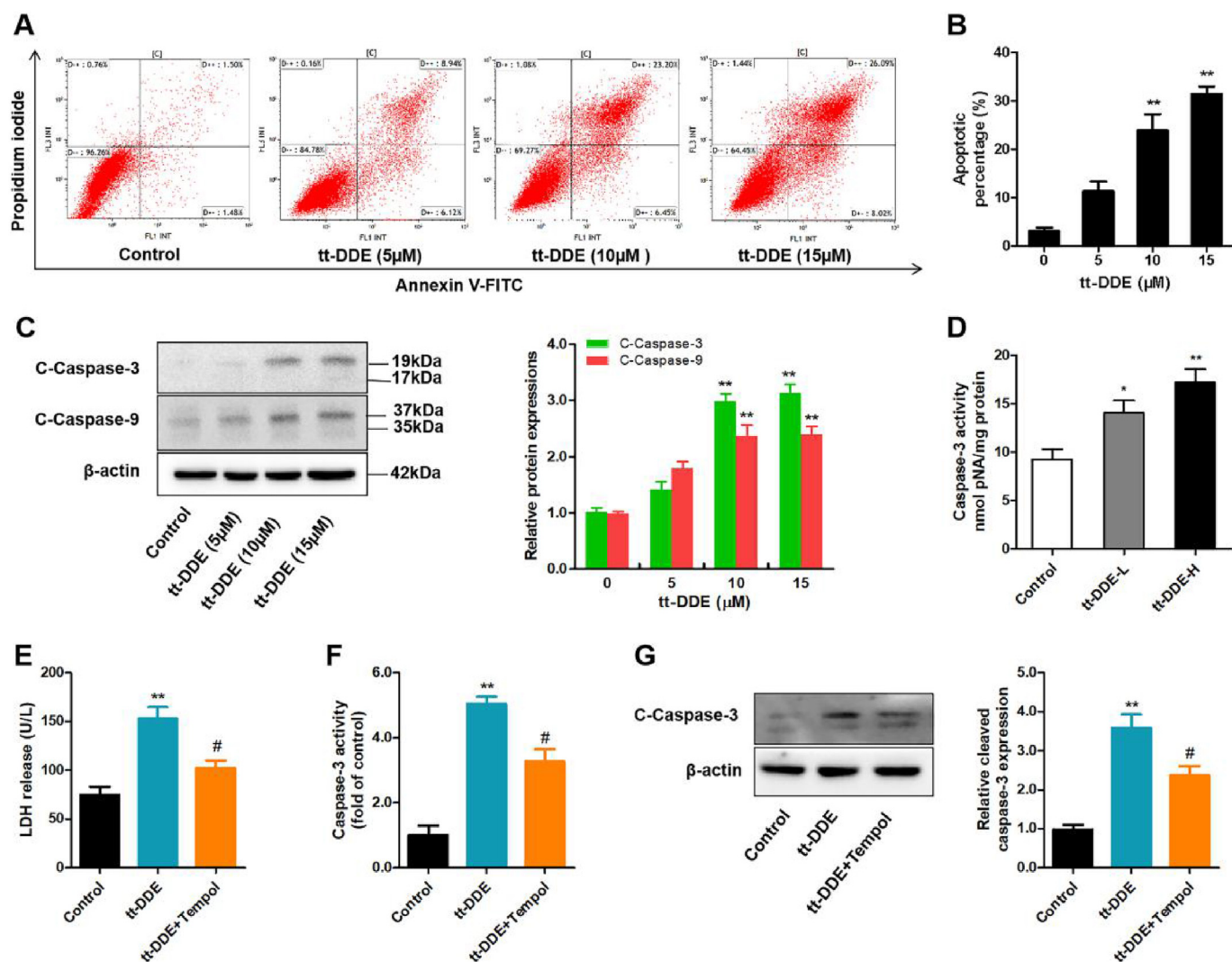


Fig. 6. The effects of tt-DDE on apoptosis in HUVECs. (A) Flow cytometric analysis of tt-DDE-induced apoptosis in HUVECs and (B) the percentages of apoptotic cells based on total cell population. (C) Representative blots and quantified data for cleaved caspase-3 and caspase-9. (D) Caspase-3 activity in arteries from vehicle and tt-DDE treated rats was detected by an assay kit. (E) LDH release and (F) caspase-3 activity in HUVECs treated with vehicle control, tt-DDE (10 µM), and tempol (100 µM) in combination with tt-DDE (10 µM). (G) Representative blots and quantified data for cleaved caspase-3. All values are presented as the means \pm SEM of three separate experiments. * $p < 0.05$, ** $p < 0.01$ vs. Control; # $p < 0.05$ vs. tt-DDE.

activation of NADPH and iNOS oxidase expression and subsequent production of cytotoxic peroxynitrite.

Both excessive intracellular ROS generation as well as high pathological NO levels induce cell apoptosis, which mediates endothelial injury [43]. Endothelial cells are crucial both for vascular homeostasis and protection of vasculature against oxidant species, and damage to vascular endothelial cells is usually considered the initial step in endothelial dysfunction [5]. Evidence indicates that both tt-DDE and precursors of tt-DDE such as hydroperoxides of unsaturated fatty acids can induce cellular apoptosis in HUVECs [9]. This study provided evidence that tt-DDE induced apoptosis in HUVECs, while tempol significantly reduced this cytotoxicity and apoptosis, suggesting that tt-DDE-induced oxidative stress was closely related to endothelial cells apoptosis. Mitochondria are both generators and targets of ROS. The mitochondrial dysfunction is an important contributor to hypertension and related complications [44,45]. Mitochondrial membranes are rich in protein thiols, which are potential targets for reactive aldehydes [46–48]. In this regard, tt-DDE was reported to induce mitochondrial dysfunction by reducing mitochondrial membrane potential and modifying cytochrome c [14,16,27]. Consistent with previous findings, this study showed that tt-DDE induced mitochondrial function deterioration

and triggered a mitochondrial apoptotic cascade. These results indicated that tt-DDE induced vascular endothelial injury by promoting mitochondrial apoptosis of endothelial cells.

MAPK are involved in early signaling mechanisms after cells are exposed to various stimuli, including growth factors or toxic substances [49]. α , β -unsaturated aldehydes were reported to directly conjugate with JNK following histone modification, and activation of p38 may be largely related to cell type and environment, exposure time, and specific cell stressors [22]. In this study, we found that exposure to tt-DDE activated JNK as well as p38 in both arteries and HUVECs. Persistent activation of JNK is known to be involved in the development of apoptosis [50]. The present study showed that tt-DDE-activated caspase-3 was abolished by a JNK inhibitor in HUVECs. This novel result revealed that JNK activation is critical for tt-DDE-mediated apoptosis of endothelial cells, which act as an important cause of endothelial dysfunction.

In conclusion, our results showed that tt-DDE consumption elevated blood pressure and impaired endothelial function through inducing oxidative/nitrative stress and JNK-mediated apoptosis signaling. These results contribute to a better understanding of the cardiovascular toxic mechanisms associated with aldehyde compounds and provide new

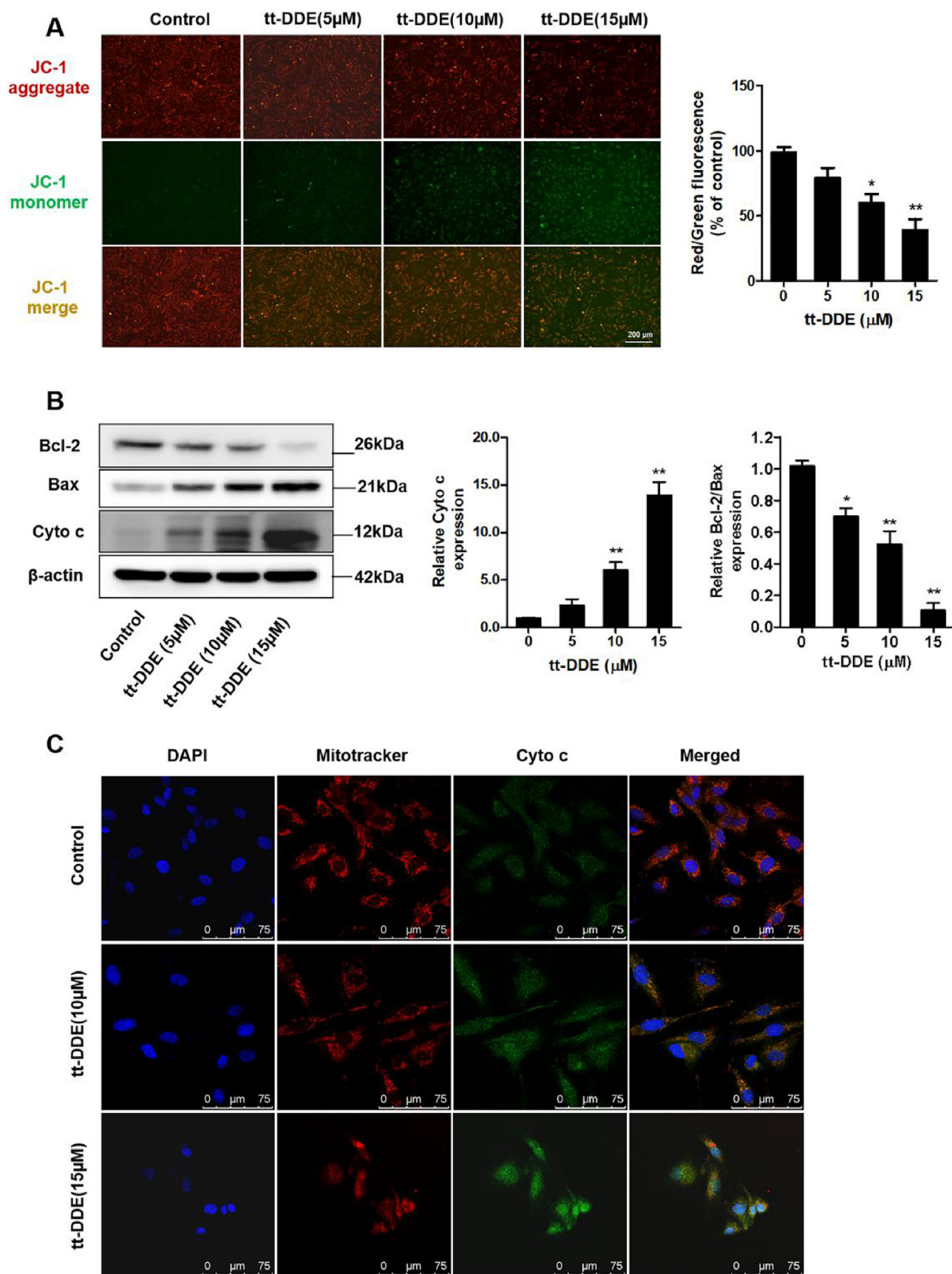


Fig. 7. The effects of tt-DDE on mitochondrial membrane potential and mitochondria apoptosis signaling in HUVECs. (A) Representative images of JC-1 staining (scale bar: 200 μ m) and the ratio of red/green fluorescent. (B) Representative blots and quantified data for Bcl-2, Bax, and cytochrome c expression. (C) Representative confocal microscopy images of cytochrome c release from mitochondria to the cytoplasm in HUVECs (scale bar: 75 μ m). All values are presented as the means \pm SEM of three separate experiments. * p < 0.05, ** p < 0.01 vs. Control. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

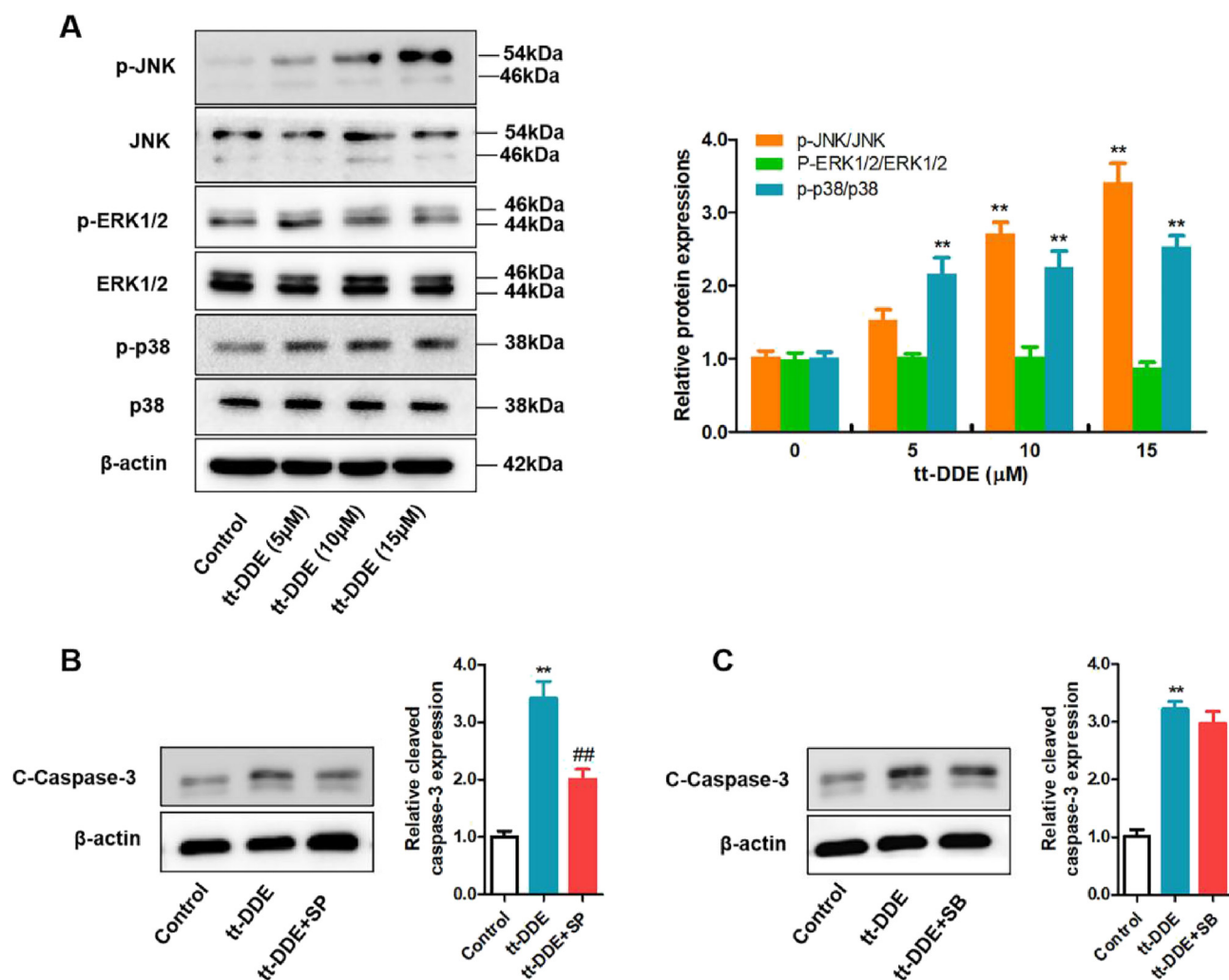


Fig. 8. The effects of MAPK pathway signaling on tt-DDE-induced apoptosis in HUVECs. (A) Representative blots of total and phosphorylations of JNK, ERK1/2, and p38, and quantified data showing the ratios of phosphorylation to total protein expression in HUVECs. (B) Effects of JNK inhibitor (SP) on cleaved caspase-3 expression. (C) Effects of p38 inhibitor (SB) on cleaved caspase-3 expression. All values are presented as the means \pm SEM of three separate experiments. * $p < 0.05$, ** $p < 0.01$ vs. Control; ## $p < 0.01$ vs. tt-DDE.

information that informs the use of tt-DDE in food production and processing.

Author contributions

Y.Y. Hu and D.Y. Zhou designed the research and wrote the manuscript. Y.Y. Hu and F.W. Yin conducted the experiment and analyzed the data. Z.L. Yu, Y.L. Peng, G.H. Zhao and Z.Y. Liu participated in the experiment. D.Y. Zhou supervised the experiment. X.C. Ma, F. Shahidi, and B.W. Zhu made suggestions for revision.

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Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2020.101577>.

References

- [1] D. Agarwal, et al., Role of proinflammatory cytokines and redox homeostasis in exercise-induced delayed progression of hypertension in spontaneously hypertensive rats, *Hypertension* 54 (2009) 1393–1400.
- [2] A. Caillon, M.O.R. Mian, J.C. Fraulob-Aquino, K.G. Huo, E.L. Schiffrin, $\Gamma\delta$ T cells mediate angiotensin II-induced hypertension and vascular injury, *Circulation* 135 (2017) 2155–2162.
- [3] D.H. Endemann, E.L. Schiffrin, Endothelial dysfunction, *J. Am. Soc. Nephrol.* 15 (2004) 1983–1992.
- [4] U. Herbst, M. Toborek, S. Kaiser, M.P. Mattson, B. Hennig, 4-Hydroxynonenal induces dysfunction and apoptosis of cultured endothelial cells, *J. Cell. Physiol.* 181 (1999) 295–303.
- [5] L. Xie, et al., SIRT3 mediates the antioxidant effect of hydrogen sulfide in endothelial cells, *Antioxid. redox sign* 24 (2016) 329.
- [6] A. Hatzitolios, F. Iliadis, N. Katsiki, M. Baltatzis, Is the anti-hypertensive effect of dietary supplements via aldehydes reduction evidence based? A systematic review, *Clin. Exp. Hypertens.* 30 (2008) 628–639.
- [7] F. Guéraud, et al., Chemistry and biochemistry of lipid peroxidation products, *Free Radic. Res.* 44 (2010) 1098.
- [8] S.E. Lee, Y.S. Park, Role of lipid peroxidation-derived α , β -unsaturated aldehydes in vascular dysfunction, *Oxid. med. cell. longev.* 2013 (2013).
- [9] T. Kaneko, K. Kaji, M. Matsuo, Cytotoxicities of a linoleic acid hydroperoxide and its

- related aliphatic aldehydes toward cultured human umbilical vein endothelial cells, *Chem. Biol. Interact.* 67 (1988) 295–304.
- [10] A. Negre-Salvayre, et al., Pathological aspects of lipid peroxidation, *Free Radic. Res.* 44 (2010) 1125–1171.
- [11] B.G. Hill, P. Habberzettl, Y. Ahmed, S. Srivastava, A. Bhatnagar, Unsaturated lipid peroxidation-derived aldehydes activate autophagy in vascular smooth-muscle cells, *Biochem. J.* 410 (2008) 525–534.
- [12] D.J. Conklin, et al., Acrolein generation stimulates hypercontraction in isolated human blood vessels, *Toxicol. Appl. Pharmacol.* 217 (2006) 277–288.
- [13] S. Vasdev, C.A. Ford, L. Longerich, V. Gadag, S. Wadhawan, Role of aldehydes in fructose induced hypertension, *Mol. Cell. Biochem.* 181 (1998) 1–9.
- [14] C.A.O. Sigolo, D.M. Paolo, M.H.G. Medeiros, Covalent modification of cytochrome c exposed to trans,trans-2,4-decadienal, *Chem. Res. Toxicol.* 20 (2007) 1099–1110.
- [15] P.P. Lin, et al., Metabolomic profiling of mice urine and serum associated with trans-trans 2, 4-decadienal induced lung lesions by liquid chromatography-mass spectrometry, *Anal. Bioanal. Chem.* 406 (2014) 4287–4297.
- [16] D.J. Conklin, et al., Acrolein consumption induces systemic dyslipidemia and lipoprotein modification, *Toxicol. Appl. Pharmacol.* 243 (2010) 1–12.
- [17] E.n. Goicoechea, et al., Use of an in vitro digestion model to study the bioaccessibility of 4-Hydroxy-2-nonenal and related aldehydes present in oxidized oils rich in omega-6 acyl groups, *J. Agric. Food Chem.* 56 (2008) 8475–8483.
- [18] K. Kanazawa, E. Kanazawa, M. Nataka, Uptake of secondary autoxidation products of linoleic acid by the rat, *Lipids* 20 (1985) 412–419.
- [19] P.C. Chan, NTP Toxicity Studies of Toxicity Studies of 2,4-decadienal (CAS No. 25152-84-5) Administered by Gavage to F344/N Rats and B6C3F1 Mice, Toxicity report series, 2011, pp. 1–94.
- [20] J.S. Miles, et al., Synergistic effect of endogenous and exogenous aldehydes on doxorubicin toxicity in yeast, *BioMed Res. Int.* 2018 (2018) 1–11.
- [21] M.D. Guillén, P.S. Uriarte, Aldehydes contained in edible oils of a very different nature after prolonged heating at frying temperature: presence of toxic oxygenated α , β unsaturated aldehydes, *Food Chem.* 131 (2012) 915–926.
- [22] U.C.S. Yadav, K.V. Ramana, Regulation of NF- κ B-Induced inflammatory signaling by lipid peroxidation-derived aldehydes, *Oxid. Med. Cell. Longev.* 2013 (2013) 690545.
- [23] A. Cabré, J. Girona, J.C. Vallvé, M. Heras, L.S. Masana, Cytotoxic effects of the lipid peroxidation product 2,4-decadienal in vascular smooth muscle cells, *Atherosclerosis* 169 (2003) 245–250.
- [24] T. Kaneko, S. Honda, S. Nakano, M. Matsuo, Lethal effects of a linoleic acid hydroperoxide and its autoxidation products, unsaturated aliphatic aldehydes, on human diploid fibroblasts, *Chem. Biol. Interact.* 63 (1987) 127–137.
- [25] C. Nappez, S. Battu, J.L. Beneytout, trans,trans-2,4-decadienal: cytotoxicity and effect on glutathione level in human erythroleukemia (HEL) cells, *Cancer Lett* 99 (1996) 115–119.
- [26] L.W. Chang, W.S. Lo, P. Lin, Trans, trans-2,4-decadienal, a product found in cooking oil fumes, induces cell proliferation and cytokine production due to reactive oxygen species in human bronchial epithelial cells, *Toxicol. Sci.* 87 (2005) 337–343.
- [27] C.A.O. Sigolo, P.D. Mascio, A.J. Kowaltowski, C.C.M. Garcia, M.H.G. Medeiros, trans,trans -2,4-decadienal induces mitochondrial dysfunction and oxidative stress, *J. Bioenerg. Biomembr.* 40 (2008) 103–109.
- [28] D. Zhu, et al., Irisin improves endothelial function in type 2 diabetes through reducing oxidative/nitrative stresses, *J. Mol. Cell. Cardiol.* 87 (2015) 138–147.
- [29] A. Virdis, et al., Vascular generation of tumor necrosis factor- α reduces nitric oxide availability in small arteries from visceral fat of obese patients, *J. Am. Coll. Cardiol.* 58 (2011) 238–247.
- [30] D. Yao, et al., Chemiluminescence detection of superoxide anion release and superoxide dismutase activity: modulation effect of *Pulsatilla chinensis*, *Anal. Bioanal. Chem.* 379 (2004) 171.
- [31] L. Yi, et al., Advanced glycation end products accelerate ischemia/reperfusion injury through receptor of advanced end product/nitrative thioredoxin inactivation in cardiac microvascular endothelial cells, *Antioxid. Redox Sign* 15 (2011) 1769–1778.
- [32] R. Li, et al., Adiponectin improves endothelial function in hyperlipidemic rats by reducing oxidative/nitrative stress and differential regulation of eNOS/iNOS activity, *Am. J. Physiol. Endocrinol. Metab.* 293 (2007) 1703–1708.
- [33] R. Zhang, et al., Thioredoxin-2 inhibits mitochondria-located ASK1-mediated apoptosis in a JNK-independent manner, *Circ. Res.* 94 (2004) 1483–1491.
- [34] J.L. Figarola, J. Singhal, S. Rahbar, S. Awasthi, S.S. Singhal, LR-90 prevents methylglyoxal-induced oxidative stress and apoptosis in human endothelial cells, *Apoptosis* 19 (2014) 776–788.
- [35] A.G. Filho, A. Kinote, D.J. Pereira, D.S. Rennó, R.C. Ferreira-Melo, Infliximab prevents increased systolic blood pressure and upregulates the AKT/eNOS pathway in the aorta of spontaneously hypertensive rats, *Eur. J. Pharmacol.* 700 (2013) 201–209.
- [36] M. Oelze, et al., Vasodilator-stimulated phosphoprotein serine 239 phosphorylation as a sensitive monitor of defective nitric oxide/cGMP signaling and endothelial dysfunction, *Circ. Res.* 87 (2000) 999.
- [37] Z. Hou, Y. Hu, X. Yang, W. Chen, Antihypertensive effects of Tartary buckwheat flavonoids by improvement of vascular insulin sensitivity in spontaneously hypertensive rats, *Food Funct* 8 (2017) 4217–4228.
- [38] M.T. Lin, M.F. Beal, Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases, *Nature* 443 (2006) 787–795.
- [39] X. Xia, et al., Dietary canolol induces apoptosis in human cervical carcinoma HeLa cells through ROS-MAPK mediated mitochondrial signaling pathway: in vitro and in vivo, *Chem. Biol. Interact.* 300 (2019) 138–150.
- [40] C.K. Wang, et al., Pulmonary changes induced by trans,trans-2,4-decadienal, a component of cooking oil fumes, *Eur. Respir. J.* 35 (2010) 667–675.
- [41] L. Ji, F. Fu, L. Zhang, W. Liu, F. Gao, Insulin attenuates myocardial ischemia/reperfusion injury via reducing oxidative/nitrative stress, *Am. J. Physiol. Endocrinol. Metab.* 298 (2010) E871–E880.
- [42] J. Whitsett, P.M. Sr, J. Vasquez-Vivar, 4-Hydroxy-2-nonenal increases superoxide anion radical in endothelial cells via stimulated GTP cyclohydrolase proteasomal degradation, *Arterioscl. Throm. Vas.* 27 (2007) 2340.
- [43] H. Cai, D.G. Harrison, Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress, *Circ. Res.* 87 (2000) 840–844.
- [44] Q. Marisol, S.L. Colombo, G. Andrew, M. Salvador, Mitochondria as signaling organelles in the vascular endothelium, *Proc. Natl. Acad. Sci. U.S.A.* 103 (2006) 5379–5384.
- [45] S.J. Chapple, X. Cheng, G.E. Mann, Effects of 4-hydroxynonenal on vascular endothelial and smooth muscle cell redox signaling and function in health and disease, *Redox Biol* 1 (2013) 319–331.
- [46] L. Tsu-Kung, et al., Specific modification of mitochondrial protein thiols in response to oxidative stress: a proteomics approach, *J. Biol. Chem.* 277 (2002) 17048–17056.
- [47] A.L. Isom, et al., Modification of Cytochrome c by 4-hydroxy-2-nonenal: evidence for histidine, lysine, and arginine-aldehyde adducts, *J. Am. Soc. Mass Spectrom.* 15 (2004) 1136–1147.
- [48] H. Zhong, H. Yin, Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: focusing on mitochondria, *Redox Biol* 4 (2015) 193–199.
- [49] K. Uchida, et al., Activation of stress signaling pathways by the end product of lipid peroxidation. 4-hydroxy-2-nonenal is a potential inducer of intracellular peroxide production, *J. Biol. Chem.* 274 (1999) 2234–2242.
- [50] D. Grassi, N. Diaz-Perez, L.A. Volpicelli-Daley, C.L. Lasmézas, P α -syn* mitotoxicity is linked to MAPK activation and involves tau phosphorylation and aggregation at the mitochondria, *Neurobiol. Dis.* 124 (2019) 248–262.