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Resveratrol improves vascular endothelial dysfunction in the unpredictable chronic mild stress model of depression in rats by reducing inflammation

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ABSTRACT

Chronic psychological stress may cause depression and it is a risk factor for vascular endothelial dysfunction. Inflammation may contribute to endothelial dysfunction. Resveratrol, which has antiinflammatory and vasculoprotective properties, has been reported its beneficial effects on endothelial dysfunction induced by hypertension, diabetes and, aging. The effects of resveratrol on stress-induced endothelial dysfunction is not investigated yet. This study aimed to investigate the efficacy of resveratrol on vascular function in the unpredictable chronic moderate stress (UCMS) model of rats and to examine the possible mechanisms of resveratrol by assessment of proinflammatory markers. Male rats were assigned to 4 groups (n = 8 for each group): Control, Control+Resveratrol, UCMS, UCMS+Resveratrol. UCMS and UCMS+Resveratrol groups were exposed to the UCMS procedure for 12 weeks. Resveratrol (20 mg/kg/day, i.p., during 12 weeks) was given to the Control+Resveratrol and UCMS+Resveratrol groups.Then depressive-like behaviors were evaluated by forced swimming test. After behavioral tests, systolic blood pressure was recorded. Endothelial function of the thoracic aorta was evaluated by isolated organ bath system. Vascular eNOS expression and inflammatory markers such as TNF-α, IL-1β, IL-6, CRP, ICAM1, MCP in serum and vascular tissue were analyzed to explore the mechanisms of resveratrol. UCMS resulted in depressive-like behavior, endothelial dysfunction and increased inflammatory cytokines in both serum and tissue samples. Resveratrol treatment improved depressive-like behavior, ameliorated vascular dysfunction, and reversed stress-induced inflammation. Our findings suggest that resveratrol exerted antidepressant-like effect and prevented vascular endothelial dysfunction by reducing systemic and peripheral inflammation in UCMS-induced depression in rats. Therefore, resveratrol may be a therapeutic option with a vasculoprotective effect in depression.

1. Introduction

Psychological stress causes stress reactions for adaptation of the organism to this condition via stimulation of the sympathetic nervous system and hypothalamic pituitary axis (HPA) axis. Adaptive physiological, cardiovascular and behavioral responses occur as an result of the stress reactions [1]. However, if the stimulation of the sympathetic nervous system and HPA axis is maintained without adaptation under the chronic stress condition, this may cause depressive disorder [2,3]. Alongside the behavioral effects of chronic stress, it is well known that it also leads to cardiovascular complications such as increased heart rate and blood pressure [4]. Recent studies have commonly shown that neuronal and systemic inflammation are also accompanied by chronic stress-induced depression [5,6]. It has been shown that atherosclerosis, another consequence of chronic stress, may be due to vascular wall inflammation [7]. Vascular inflammation may contribute to impairment of vascular endothelial function, which has a crucial role in the circulatory homeostasis, and thus cardiovascular effects of stress [8,9]. Although there are several studies on the treatment of stress-induced cardiovascular diseases, new treatment options are still being

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investigated.

The unpredictable chronic mild stress model (UCMS) in rodents, which gives reliable and valid results for the development of depression-like behaviors, is widely used to investigate the human clinical depression. With this animal model, rodents exhibit anhedonia and learned helplessness behaviors seen in human depression [10,11]. In addition, it was shown that cardiovascular complications such as hypertension, atherosclerosis and increased heart rate were induced by the UCMS procedure [3,12–14]. Vascular endothelial dysfunction and vascular inflammation has been exhibited besides behavioral changes in the UCMS model. In our previous study, it was reported that UCMS caused impairment of vascular function by vascular inflammation [15]. There are some other studies using this animal stress model to assess vascular function [13,16,17]. It has been shown that the key role of endothelial dysfunction in the adverse effect of stress on cardiovascular system in the animal studies using UCMS protocol. The animal studies using this model have reported that various cardiovascular complications due to chronic stress occurs such as hypertension, arrhythmias, heart rate variability, impairment of endothelium-dependent dilation. In these previous studies, increased inflammation and corticosterone levels were investigated as the underlying mechanisms leading to CVD complications [18]. In this study, the reason to select the UCMS model is compatible findings to the human depression in terms of behavioral and cardiovascular changes in previous studies.

Resveratrol, is a natural polyphenol, that has antiinflammatory, cardiovascular protective and neuroprotective properties [19–21]. Beneficial effect of resveratrol on vascular dysfunction has been exhibited in hypertensive-, diabetic- and hypercholesterolemic-animal models [20,22,23]. Antidepressant effect of resveratrol was also shown in animals [24,25]. However, the effects of resveratrol on stress-induced vascular dysfunction is not yet investigated. The aim of this study was to evaluate the efficacy of resveratrol on the chronic unpredictable moderate stress model of rats and to examine the possible mechanisms of resveratrol by assessment of thoracic endothelial function and proinflammatory cytokines.

2. Materials and methods

2.1. Animals

Wistar albino rats which are adult and male were supported by Kocaeli University Experimental Medical Research and Application Center and utilized to create the stress model in this research. Male rats (n = 32) aged 8–12 weeks were housed under the standard animal colony (8 animals per cage) and the humidity (45%), temperature (22 ± 2 °C) and lightning (12 h light/dark cycle) controlled experiment conditions. The animals were fed with standard commercial chow diet and water in the absence of any restrictions during the experiment. The experiments reported in this study were conducted in accordance with the Regulation of Animal Research Committee in Turkey (July 6, 2006, Number 26220). All experimental protocols were approved by the Kocaeli University Animal Research Ethics Committee (Project number: KOU HADYEK 11/3-2013).

2.2. Experimental design

Experiment animals were divided into four different groups (n = 8 per group): control group (animals not exposed to UCMS), control+Resveratrol group (animals not exposed to UCMS while treated with resveratrol), UCMS group (animals exposed to UCMS), UCMS+Resveratrol group (animals exposed to UCMS while treated with resveratrol). Resveratrol (Sigma Chemical Co., St. Louis, USA) was administered at a dose of 20 mg/kg/day to control+Resveratrol and UCMS+Resveratrol groups during 12 weeks of stress, intraperitonally [18]. At end of 12-week period of UCMS, to examine the impacts of UCMS, behavioral tests (locomotor activity, forced swimming test) were evaluated. Then systolic blood pressure and heart rate were examined with tail cuff method.

The body weights of the animals were recorded at the beginning and the end of the stress procedure.

Then the rats were sacrificed under anesthesia (created by injection of 90 mg/kg ketamine and 10 mg/kg xylazine). Blood and thoracic aorta samples of animals were extracted for vascular reactivity, immunohistochemical, biochemical and molecular analyses.

2.3. Unpredictable chronic mild stress (UCMS) model

Rats were exposed to different stressors to generate the UCMS in a daily and unpredictable manner in terms of experiments animals as previously described [19]. Once applied stressors type was not used for 2 following days to maintain the unpredictability. These so-called stressors were; restraint for 4 h, cage tilting for 24 h, wet bedding for 24 h, swimming in 4 °C water maze for 5 min, swimming in 45 °C water maze for 5 min, level shaking for 10 min, pairing the animal with another stressed animal for 48 h, nip tail for 1 min and inversion of light/dark cycle for 24 h. Utilization of these stressors was random for a 12-week schedule. One of these stressors was exposed to the animals per day without application of the same stressor for 2 consecutive days to prevent the comprehension of stimulation by rats. The UCMS modeling was not involved in food and water shortage and animals of all 2 groups access to food and water in case of need.

2.4. Behavioral tests

2.4.1. Locomotor activity

Locomotor activity was measured in a plexiglass locomotor activity chamber (May Commat Ltd, Ankara, Turkey). This chamber was consisited of 15-beam array of infrared horizontal (mounted every 2.5 cm, bottom) movement detectors were used to measure locomotor activity in test. The total distance moved was assessed for 5 min before the forced swimming test. Prior to the test, the animals were allowed to adapt to the test room. The apparatus was cleaned after each rat session [25]. The total distance moved was assessed for 5 min before the forced swimming test [26].

2.4.2. Forced swimming test (FST)

FST was performed for the assessment of depressive-like behavior of rats as previously described [26]. A cylinder (height, 47 cm; inside diameter, 38 cm) containing 38 cm of tap water maintained at 22 ± 1 °C was used as the FST apparatus. FST included two days trials: conditioning and test. On the first day, rats were put into the test apparatus and left in the cylinder for 15 min for the conditioning trial. 24 h after this trial, rats were put again into the cylinder and left in the water for a 5 min test trial. The immobility time was recorded during the test trial. The immobility time is defined as the lack of motion of the whole body except for the small movement necessary to keep the animal's head above the water according to Porsolt et al. (1977) [27].

2.5. Systolic blood pressure (SBP) measurement tail cuff

At the end of the behavioral tests, indirect SBP and heart rate (HR) were recorded using by the tail-cuff plethysmography (MAY-COM BPHR 200; Commat Iletisim, Ankara, Turkey) as previously described [28]. The rats were put in a restraining holder to measure SBP, consciously. To obtain vasodilatation, the tails of the rats were taken out of the holder and warmed with an infrared light bulb, locally. A cuff and pulse sensor were placed around the tail and used to obtain the value of SBP. The cuff was inflated until the pulse disappeared, then deflated and the point of the reappearance of the pulse was recorded as the value of SBP. To obtain the average value of SBP and HR, inflation-deflation cycles of the cuff were repeated three times for each rat, sequentially.

2.6. Organ bath studies

Vascular endothelial function was assessed as previously described [28]. Following the thoracic aorta samples were excised surgically, they were placed in Krebs Solution (KS) composed of (mM): NaCl 118, KCl 4.71, MgCl2 1.05, NaH2PO4 1.33, NaHCO3 25, CaCl2 2.7, and glucose 5.6. The thoracic aorta rings were prepared by dissection as approximately 3–5 cm long. The rings were suspended in the 20- mL organ bath chambers containing KS (pH 7.4) maintained at 37 °C. The bath chambers were aerated with the carbogen (95% O2 and 5% CO2). The measurement of isometric force of each ring was provided by a connected force-displacement transducer (MAY-COM FDT 10 A; Commat Iletisim). A four-channel transducer data-acquisition system (MP30B-CE; Biopac Systems, Santa Barbara, CA, USA) was recorded the isometric force of the rings on a personal computer by using a software (BSL Pro 3.7; Biopac Systems) that could analyze the data. Before the beginning of experiments, the thoracic aorta rings were allowed to equilibrate with 1 g resting tension for 60 min with changing the bath solution every 15 min and this resting tension was not altered during the experiment. After the equilibration, the viability of the aortic ring preparations was tested by 80 mM KCl in KS. Then, tissues were washed and allowed to equilibrate for 30 min. Based on our preliminary studies, 10^{-6} or 10^{-5} M of phenylephrine resulted in approximately 90% of the max response to phenylephrine (submaximal concentration). These phenylephrine concentrations were used for precontraction before the relaxant responses with carbachol (10^{-8}-10^{-5} M), sodium nitroprusside (SNP; 10^{-8}-10^{-4} M), or papaverine (10^{-5}-10^{-4} M) by addition of one of these agents to the bath with 30 min intervals (in a cumulative manner). The agonist concentration was increased after the response to the previous concentration had reached completely a plateau. After a concentration-response curve was completed, tissues were washed for a further 30 min and they were allowed to return basal tension. The drugs, which were used in organ bath studies, were obtained from Sigma Chemical Co. (St. Louis, USA).

2.7. Enzyme-linked immunosorbent assay (ELISA)

For cytokines measurements, blood and thoracic aorta samples were collected at the end of the 12-week resveratrol treatment. The serum was segregated by centrifugation at 4000 rpm for 15 min and kept at − 80 °C before the start of the experiments. Protein levels of tumor necrosis actor alpha (TNF-α), Interleukine 1 beta (IL-1β), Interleukine 6 (IL-6), C-reactive Protein (CRP), Intracellular cell adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) were assessed by ELISA kit (Biosource, Invitrogen, Carlsbad, CA) according to the recommendations of the manufacturer. The absorbance was measured at 450 nm using a microtiter ELISA reader (VERSAmax Molecular Devices, Sunnyvale, CA, USA). ELISA results were given as picograms of the measured molecule per mL of serum (pg/mL).

2.8. Real-time PCR analyses

Thoracic aorta samples were collected to determine gene expression levels of, endothelial nitric oxide synthase (eNOS), TNF-α, IL-1β, Il6, CRP, and ICAM-1. Total RNA was isolated by JenaBioscience RNA Isolation Kit (JenaBioscience, Jena, Germany) from tissue samples and the quality and quantity of RNA was determined by absorbance at 260 and 280 nm with the picodrop spectrophotometer (Picodrop Technology, Cambridge) and same amount of RNA was converted to cDNA by RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Vilnius, Lithuania) according to the manufacturer's instructions. Target gene amplifications were tracked with gene specific probes and primers (Universal ProbeLibrary, Roche), according to the manufacturer's recommendations on the instrument, LightCycler (LC) 480II (Roche, Mannheim, Germany). Fold changes in mRNA expression between resveratrol treatments and controls were determined by the ΔCT method. Fluorescence threshold cycle (Ct) values were calculated using LC-480II System Software. Results were normalized to the average Ct for the Gapdh gene run in the Quantitative real-time PCR. ΔΔCt values were calculated with control groups to determine expression changes.

2.9. Immunohistochemical analyses for eNOS

After behavioral tests and SBP measurement, collected thoracic aorta samples from animals were fixed with 10% neutral buffered formalin fixation and immunohistochemical analyses were applied according to our previous study [28]. These samples were embedded into paraffin to prepare sections with routine histological tissue procedures. Paraffin-embedded samples were cut at a thickness of 4–5 μm and transferred to poly-L-Lysine slides. The sections were deparaffinized in xylene, hydrated with different and serial dilutions of ethanol in the range from %50 to %100 alcohol concentration and then washed with phosphate-buffered saline (PBS). After that, the tissue sections were placed into 1 mM citrate buffer (pH 6.0) in a microwave oven for antigen retrieval. Antigen recovered tissue sections were cooled at room temperature for 20 min and incubated with 3% H_20_2 containing methanol to prevent from endogenous peroxidase activity. Then, sections were blocked with specific serum for 10 min at room temperature to avoid nonspecific binding. The tissue sections were incubated with primary antibody against eNOS at room temperature. Negative controls were formed with the antibody diluent solution (Ab-diluent reagent solution, Invitrogen, Carlsbad, CA, USA) at the same concentration instead of primary antibody. After primary antibody incubation was done, tissue sections were washed and incubated with a biotinylated secondary antibody for 1 h at room temperature. When secondary antibody incubation was done, diaminobenzidine (DAB) (DAB Substrate Kit, Invitrogen, Carlsbad, CA, USA) was applied to section for visualization. All samples were exposed to the same tissue processing protocol.

2.10. Statistical analyses

The results were presented as mean ± standard error of the mean (SEM). The relaxant responses to carbachol and SNP were expressed as the percentage of the precontraction response to phenylephrine. pD2 (-logEC50) values were determined to evaluate the changes in thoracic aorta sensitivity to carbachol and SNP. Statistical differences were determined by one-way ANOVA followed by Tukey's post hoc test for all experiments, except immunohistochemistry analyses. eNOS immunoreactivity was measured by ImageJ programme and then analyzed with SPSS. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Bodyweight

Average body weights at the beginning of the experiments were similar in all groups (Table 1). After 12 weeks of exposure to UCMS, alterations in body weight were observed in the UCMS group compared to the controls ($p < 0.0001$). The rat's body weights were similar with control animals in UCMS+Resveratrol group.

3.2. Effects of stress and resveratrol on behavioral tests

The total locomotor activity was not affected by stress or resveratrol treatment (Fig. 1A). In the second day test trial of the FST, the immobility time of the UCMS group was significantly longer than the control, which demonstrated that there were depression-like behaviors in rats (Fig. 1B, $p < 0.001$). The immobility time in CUMS group was reversed to the controls by resveratrol treatment for 12 weeks during the UCMS procedure (Fig. 1B, $p < 0.0001$).

Table 1
Body weights (g), Emax values (% of 10^{-5} M Phenylephrine) for carbachol, SNP and papaverine, Emax values (g) for 80 mM KCl in control, control+Resveratrol, UCMS and UCMS+Resveratrol groups. Values are expressed as mean ± S.E.M. n = number of animals used. Values are expressed as mean ± S.E.M. *$p < 0.05$, **** $p < 0.0001$ compared with control group, $^{\#}p < 0.05$, $^{\#\#\#\#}$ $p < 0.0001$ compared with UCMS group (n = 8 for each group).

	Control	Control+Resveratrol	UCMS	UCMS+Resveratrol
Body Weight (g) – initial	290.3 ± 23.0	287.6 ± 2.56	288.6 ± 19.2	292.1 ± 26.7
Body Weight (g) – last	321.1 ± 4.47	315.8 ± 1.3	277.8 ± 6.14****	318.5 ± 4.65$^{\#\#\#\#}$
Blood Pressure (mmHg)	105.4 ± 1.8	106.9 ± 1.2	111.4 ± 6.5	107.7 ± 4.4
Heart Rate (bpm)	301.9 ± 4.8	300.3 ± 0.5	305.1 ± 8.6	298.2 ± 4.4
KCl Emax (mg)	879.7 ± 308.0	903.4 ± 2.6	905.1 ± 333.2	921.1 ± 359.1
Carbachol E_{max} (phe%)	84.4 ± 7.5	82.0 ± 2.8	63.4 ± 9.2*	83.5 ± 7.5$^{\#}$
Carbachol pD_2 (-logEC_{50})	6.72 ± 0.07	6.69 ± 0.03	6.57 ± 0.03	6.68 ± 0.08
SNP E_{max} (phe%)	99.5 ± 0.72	99.5 ± 0.5	98.9 ± 0.6	99.7 ± 0.7
SNP pD_2 (-logEC_{50})	6.58 ± 0.08	6.53 ± 0.02	6.57 ± 0.09	6.55 ± 0.03
Papaverine E_{max} (phe%)	100.1 ± 0.39	100.0 ± 0.5	100.2 ± 0.58	99.63 ± 0.53

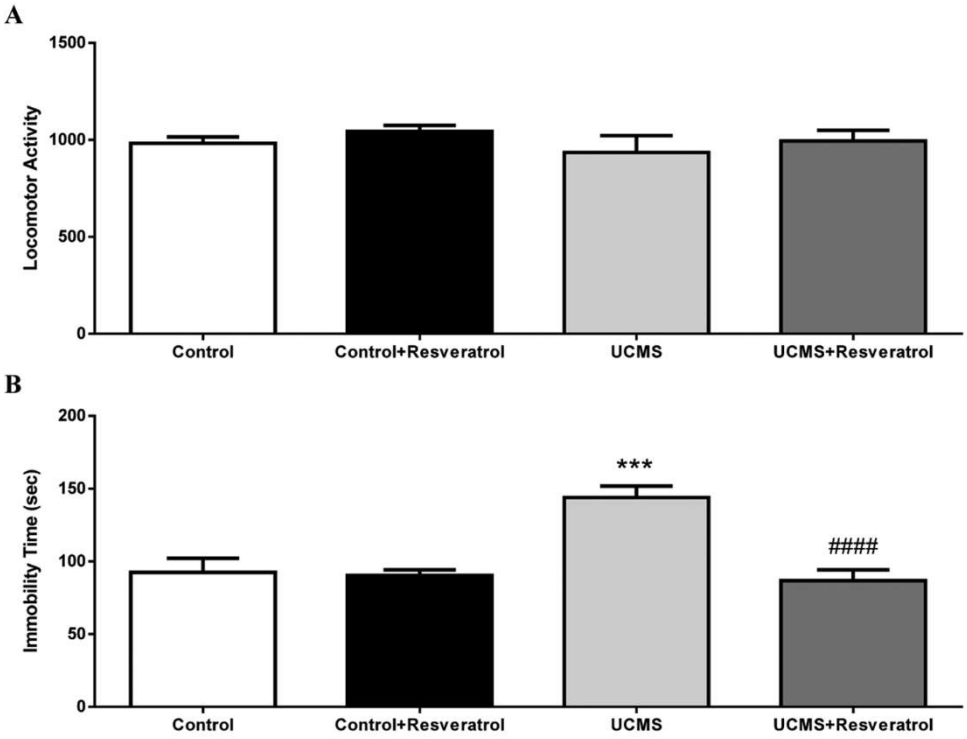


Fig. 1. Total locomotor activity (A) and immobility time (B) scores for control, control+Resveratrol, UCMS and UCMS+Resveratrol groups. Values are expressed as mean ± S.E.M. n = 8 for each group. ***$p < 0.001$ statistically different from control and $^{\#\#\#}p < 0.001$ statistically different from UCMS group (n = 8 for each group).

3.3. Effects of stress and resveratrol on SBP and vascular reactivity

There were no significant differences in the systemic blood pressure (mmHg) and heart rate (bpm) between all groups (Table 1).

After precontraction with phenylephrine, endothelium-dependent (in response to carbachol) and independent (in response to SNP) relaxations were investigated in thoracic aorta rings. Precontractile tone obtained by submaximal concentration of phenylephrine was similar in all groups (data not shown). In the UCMS group, endothelium-dependent relaxant response to carbachol was decreased whereas this response was returned to the control group after resveratrol treatment (Fig. 2A, $p < 0.05$). There were no significant differences in endothelium-independent relaxant responses to SNP and papaverine among the groups (Fig. 2B, Table 1). The contractile responses to 80 mM KCl were similar in control, control+Resveratrol, UCMS and UCMS+Resveratrol groups (Table 1). eNOS immunoreactivity significantly decreased in UCMS groups, whereas there were no significant changes in the resveratrol treatment group compared with controls (Fig. 6).

3.4. Effects of stress and resveratrol on levels of proinflammatory markers in the thoracic aorta

The gene expression levels of proinflammatory markers; TNF-α ($p < 0.0001$), IL-1β ($p < 0.01$), IL-6 ($p < 0.0001$), CRP ($p < 0.01$), ICAM1 ($p < 0.01$) were significantly increased in the UCMS group compared to the control group (Fig. 3B,C,D,E,F). These results were significantly decreased in UCMS+Resveratrol group after resveratrol treatment (for TNF-α ($p < 0.0001$), IL-1β ($p < 0.0001$), IL-6 ($p < 0.0001$), CRP ($p < 0.001$), ICAM1 ($p < 0.05$), respectively) (Fig. 3A, B, C, D, E). Similarly, proinflammatory markers in thoracic aorta; TNF-α ($p < 0.0001$), IL-1β ($p < 0.0001$), IL-6 ($p < 0.0001$), CRP ($p < 0.001$), ICAM1 ($p < 0.01$) and MCP1 ($p < 0.0001$) were significantly elevated in the UCMS group (Fig. 4A, B, C, D, E, F)), and resveratrol treatment in UCMS group decreased the proinflammatory cytokines.

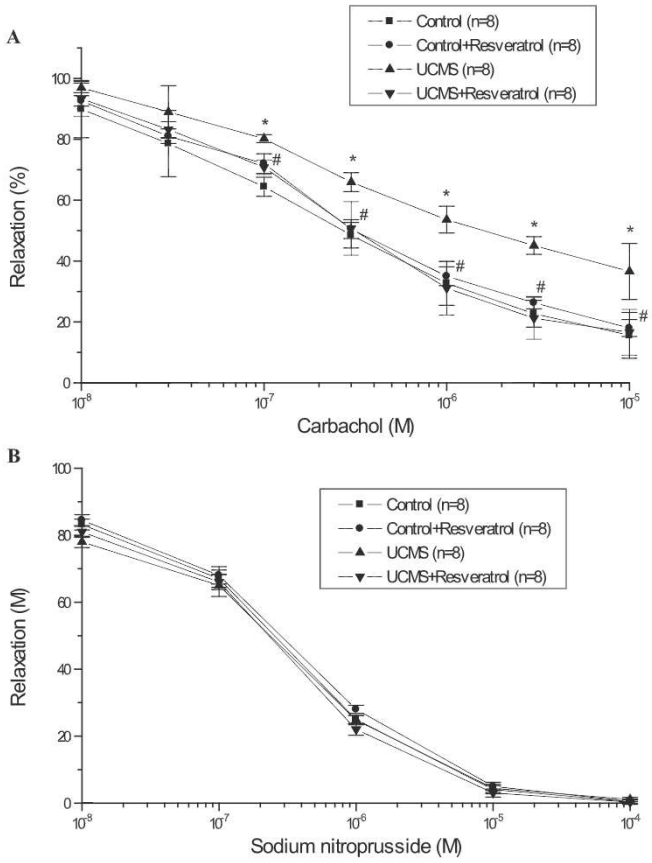


Fig. 2. Carbachol (A) and SNP (B) concentration-response curves in isolated thoracic aorta precontracted with phenylephrine (10^{-5} M) for control, control+Resveratrol, UCMS and UCMS+Resveratrol groups. Values are expressed as mean ± S.E.M. * $p < 0.05$ compared with control group; # $p < 0.05$ compared with UCMS group (n = 8 for each group).

3.5. Effects of stress and resveratrol on protein and gene expression levels of eNOS in the thoracic aorta

eNOS immunoreactivity was detected in the cytoplasm of the endothelial cells of the thoracic aorta in the control group (Fig. 6). The level of eNOS protein was lowered in the UCMS group compared to the control group ($p < 0.05$, Fig. 6). It was reversed to the control level in the resveratrol-treated UCMS group (Fig. 6). In line with the level of eNOS protein results, eNOS gene expression was significantly decreased in UCMS ($p < 0.05$), whereas it was significantly different from the UCMS group in resveratrol treated UCMS group ($p < 0.001$) and similar to the control group (Fig. 7).

3.6. Effects of stress and resveratrol on proinflammatory markers in serum

Serum concentration of proinflammatory markers; TNF-α ($p < 0.0001$), IL-1β ($p < 0.01$), CRP ($p < 0.0001$), ICAM1 ($p < 0.05$) and MCP1 ($p < 0.0001$) were significantly increased in the UCMS group compared to the control group (Fig. 5A, B, C, D, E). All markers levels were decreased in the UCMS+Resveratrol group when compared to the UCMS group and returned to the controls (Fig. 5A, B, C, D, E).

3.7. Effects of stress and resveratrol on the serum corticosterone

Serum concentration of corticosterone was significantly increased in the UCMS group ($p < 0.01$). It was significantly decreased in the UCMS+Resveratrol group than the UCMS group ($p < 0.001$), whereas it was reversed to the controls after resveratrol treatment (Fig. 5F).

4. Discussion

This study showed that UCMS induced depressive-like behavior which was demonstrated by increased immobility time in FST. Elevated serum levels of corticosterone in the UCMS group was also confirmed in the stress model used. Impairment of the endothelium-dependent vasorelaxation in the UCMS group indicated that this animal model of depression caused vascular endothelial dysfunction in this study. The reduction of eNOS protein and gene expression levels in the UCMS group supported the endothelial dysfunction. Elevated levels of proinflammatory markers such as TNF-α, IL-6, IL-1β, CRP, ICAM1, MCP1 in serum and thoracic aorta samples were accompanied by these impairments. Resveratrol treatment during UCMS model of depression prevented depressive-like behavior and endothelial dysfunction. Moreover, the elevated levels of proinflammatory markers in serum and thoracic aorta in UCMS rats were prevented by resveratrol treatment. Resveratrol administration did not cause any change in non-stressed control animal. The fact that resveratrol alone did not have any effect in healthy animals suggest that the effects in resveratrol administration to the stress group were stress-specific effects. In the present study, the most appropriate animal model to mimic human clinical depression, the UCMS model was used to reproduce depressive-like behaviors including learned helplessness and anhedonia [10,11]. This animal model of stress was selected because of the previous studies investigating stress-induced vascular diseases after the UCMS procedure [13,16,17]. The effectiveness of this model was confirmed by the measurement of corticosterone level in the serum and the immobility time in the FST. We reported that CUMS in rats leads to depression-like behaviors such as classic anhedonia behavior by sucrose preference test in our previous study. In this study, we have also shown that anhedonia behavior was prevented by chronic resveratrol treatment [29]. In the present study, we tested only the immobility behavior in FST and also measured the corticosterone level as confirmation of the animal model of stress in the same condition. In addition, a change of the bodyweight at the end of the 12 weeks may have exhibited as a result of anhedonia, indirectly. The UCMS protocol in this study was confirmed by the elevated corticosterone level, the increased immobility time, and decreased bodyweight in the stress group. After treatment with resveratrol, the level of corticosterone decreased in the UCMS rats treated with resveratrol. Besides the immobility time in the UCMS+Resveratrol group was shorter than the UCMS group. These findings are in line with the previous studies indicating the antidepressant effect of resveratrol [24,25]. Neither UCMS protocol nor resveratrol treatment has any effect on locomotor activity test results, which suggested that the antidepressant effect of resveratrol is not dependent on the change of motor performance.

Vascular endothelial function was evaluated by isolated tissue bath experiments, in this study. These experiments have shown the stress-induced vascular endothelial dysfunction illustrated by impaired endothelium-dependent vasorelaxation to carbachol in the thoracic aorta preparation isolated from the UCMS group in comparison with the control group. However, the endothelium-independent responses to SNP were similar in all groups. The impairment of the endothelium-dependent relaxations without any change of the endothelium-independent responses indicated that UCMS may have led to decreased NO production in vasculature. In line with the in vitro vascular functional findings, the reduction of the eNOS immunoreactivity and the eNOS protein levels in the thoracic aorta supported the decreased NO production after 12 weeks of stress exposure. There are a lot of studies suggested that decreased NO production in this stress model [13,15,30]. In our previous study, it was shown that UCMS caused reduction of eNOS expression and vascular endothelial dysfunction due to vascular inflammation [15]. Similarly, the animal studies using UCMS model have been focused on investigation of stress-induced increased inflammation and corticosterone levels as the

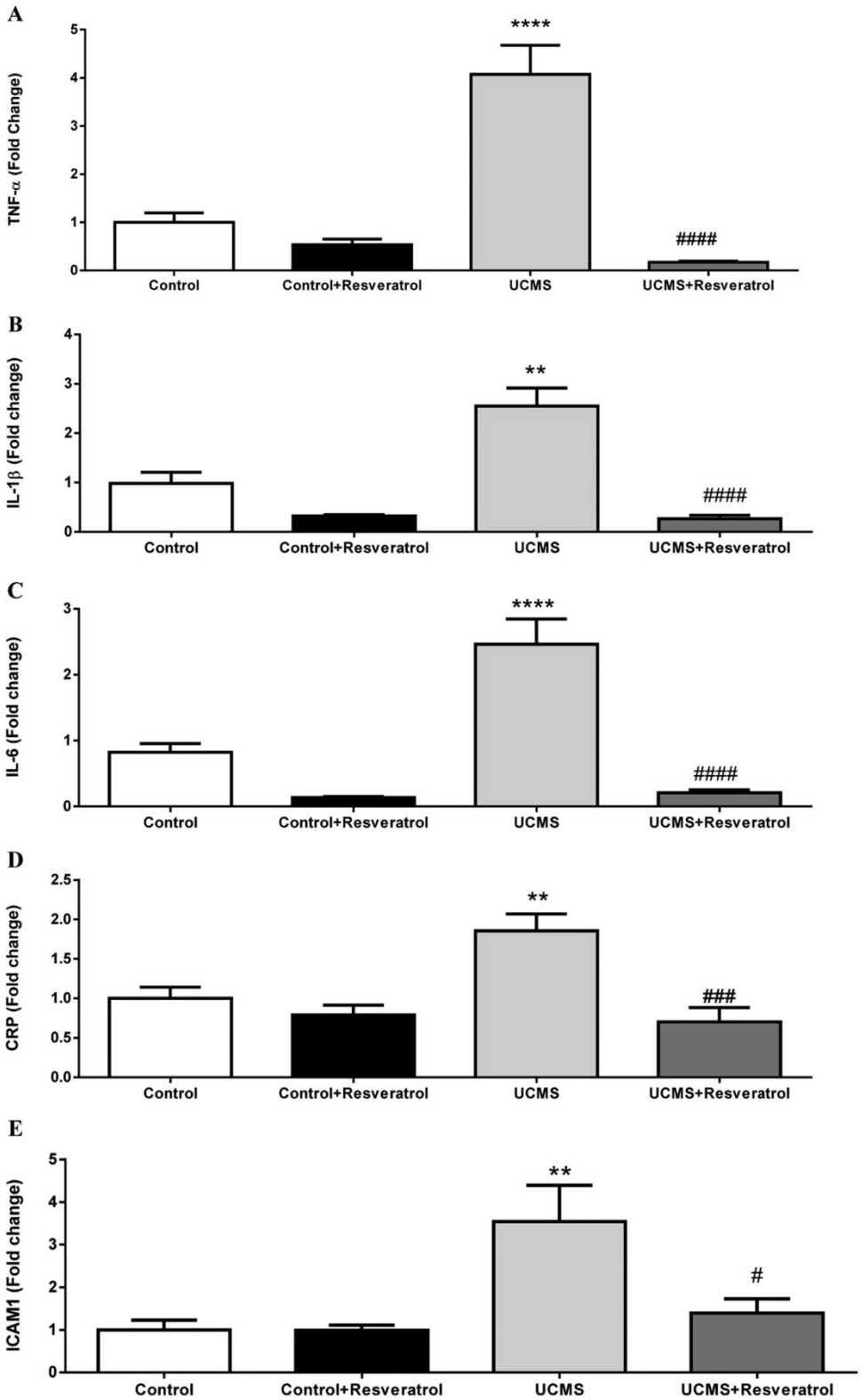


Fig. 3. Aortic gene expressions of, TNF-α (A), IL-1β (B), IL-6 (C), CRP (D), ICAM1 (E) from control, control+Resveratrol, UCMS and UCMS+Resveratrol groups. Values are expressed as mean ± S.E.M. * $p < 0.05$, * * $p < 0.01$, * **$p < 0.001$, * ** * $p < 0.0001$ compared with control group; $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$, $^{\#\#\#\#}$ $p < 0.0001$ compared with UCMS group (n = 8 for each group).

underlying mechanisms leading to endothelial dysfunction [18]. In the present study, the reverse of the decreased eNOS level in the stress group to the controls after chronic treatment of resveratrol has shown that resveratrol may have beneficial effects in preventing endothelial dysfunction in this stress model. In accordance with our data, some previous studies in different medical conditions also corroborate the protective effect of resveratrol on vascular endothelial dysfunction [20,22,23,31]. Previous studies demonstrated that chronic psychological stress is an important factor causing cardiovascular diseases such as increased heart rate, coronary atherosclerosis, and vascular endothelial

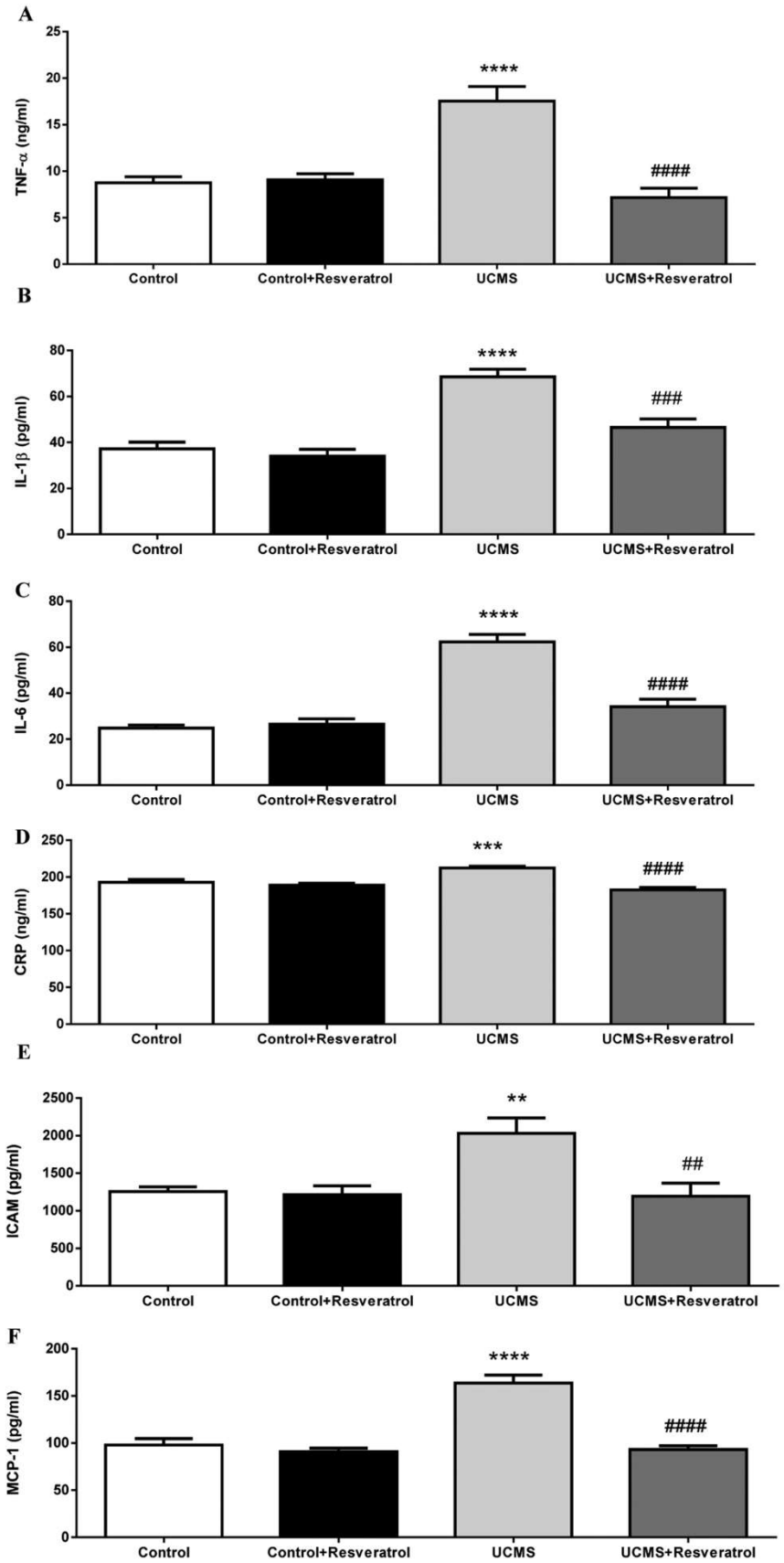


Fig. 4. TNF-α (A), IL-1β (B), IL-6 (C), CRP (D), ICAM1 (E) and MCP1 (F) levels in thoracic aorta for control, control+Resveratrol, UCMS and UCMS+Resveratrol groups. Values are expressed as mean ± S.E.M. * * $p < 0.01$, * ** * $p < 0.0001$ compared with control group; ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared with UCMS group (n = 8 for each group).

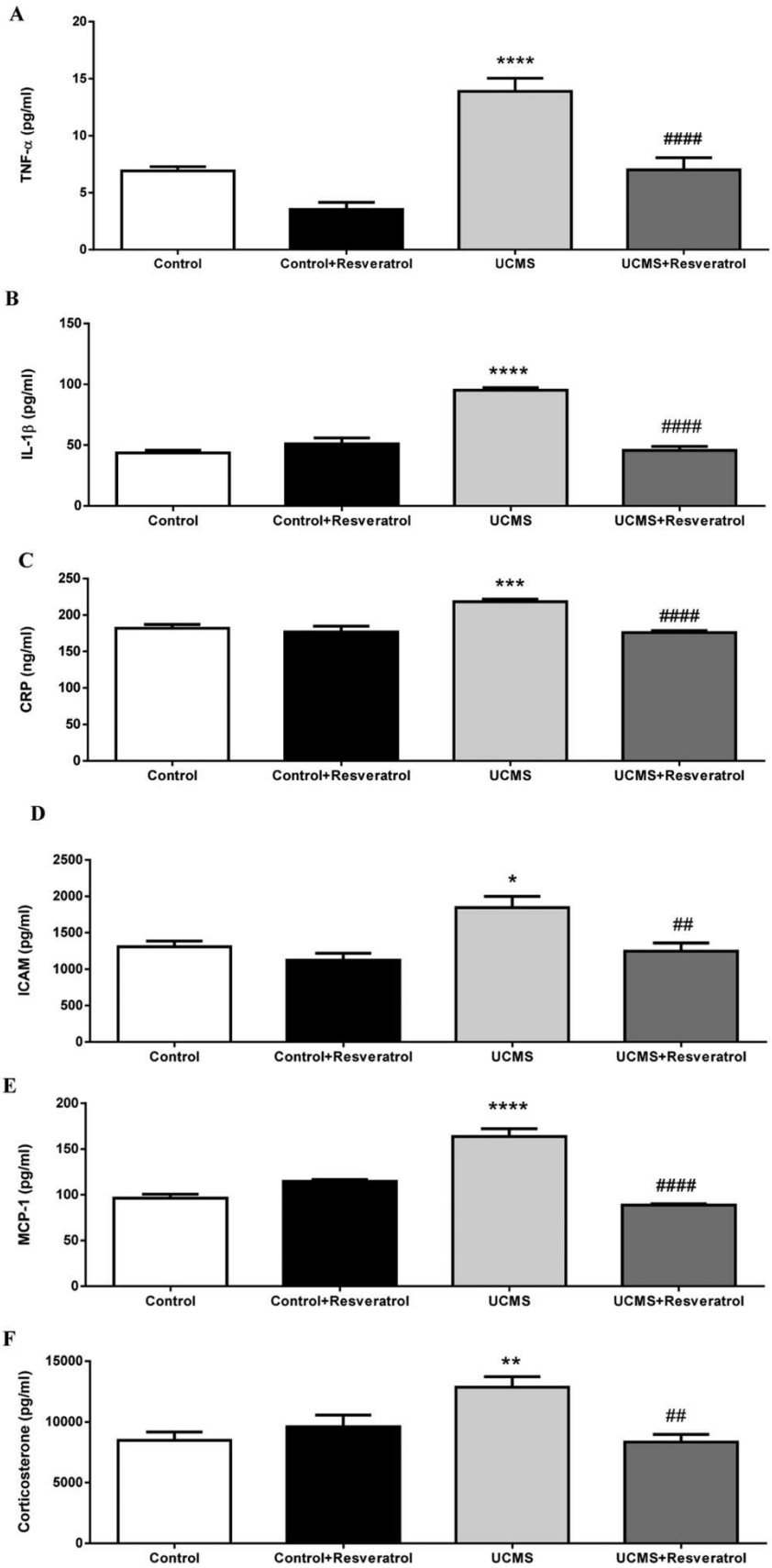


Fig. 5. TNF-α (A), IL-1β (B), CRP (C), ICAM1 (D), MCP1 (E) and corticosterone (F) levels in serum for control, control+Resveratrol, UCMS and UCMS+Resveratrol groups. Values are expressed as mean ± S.E.M. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ compared with control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ compared with UCMS group ($n = 8$ for each group).

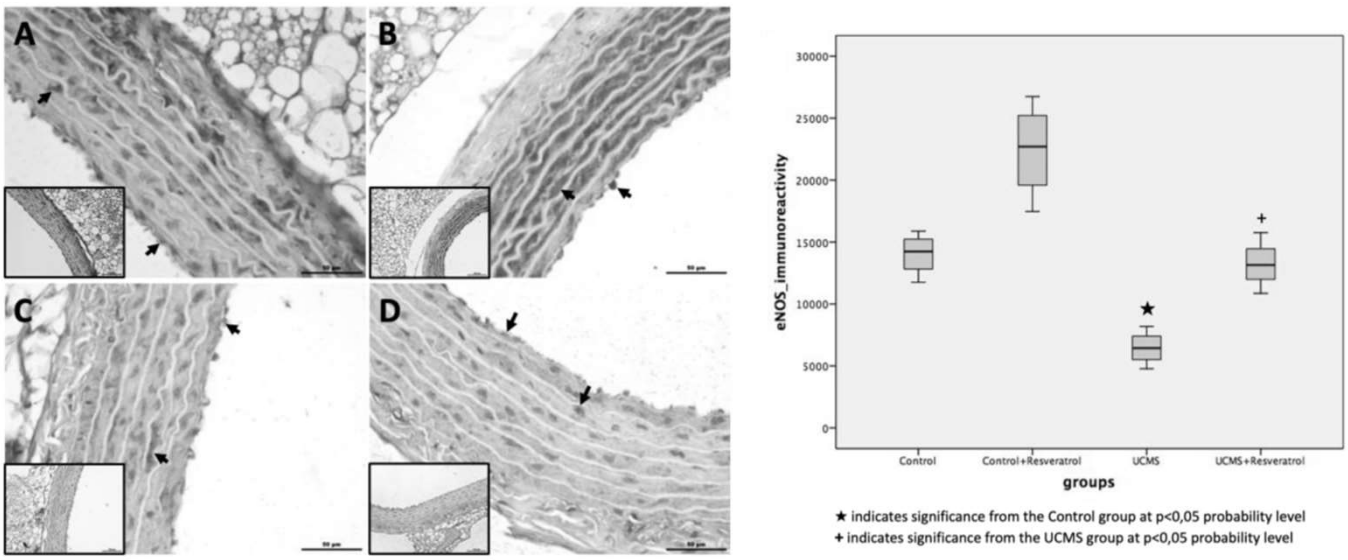


Fig. 6. eNOS immunoreactivity of control (A), control+Resveratrol (B), UCMS (C), and UCMS+Resveratrol (D) groups. Arrows indicate eNOS positive cells in T. intima and T.media. 400X magnification.

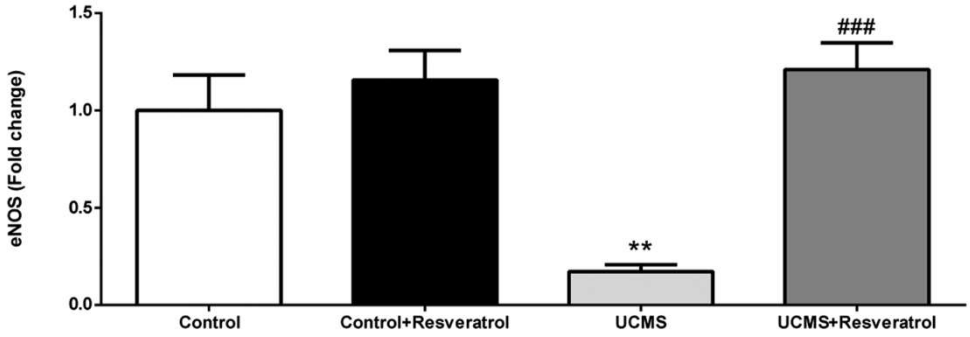


Fig. 7. Aortic gene expression of eNOS from control, control+Resveratrol, UCMS and UCMS+Resveratrol groups. Values are expressed as mean ± S.E.M. ** $p < 0.01$ compared with control group; $^{###}$ $p < 0.001$ compared with UCMS group (n = 8 for each group).

dysfunction [4]. The chronic inflammatory process in the vascular wall contributes to these cardiovascular complications during chronic stress [7]. There are some studies reported that TNF-α and IL-1b increased in the vascular tissue led to endothelial dysfunction by inhibition of the NO synthesis or availability [30]. On the other hand, the chronic stress-induced excessive sympathetic system and HPA activity are other factors that contributed to vascular endothelial dysfunction by releasing catecholamines and corticosteroids [3,4]. It was shown that excessive glucocorticoid level resulted in decreased NO availability and so impaired vasorelaxation to acetylcholine by inducing reactive oxygen species overproduction [32]. Following these studies, our data suggest that the UCMS procedure induced a significant elevation in the levels of corticosterone and proinflammatory biomarkers and so decreased endothelium-dependent relaxation and eNOS expression levels in the UCMS group may be dependent on these alterations. Based on our study, the antiinflammatory property of resveratrol may be contributed to its vasculoprotective effect in this rat model of stress. The corticosterone-lowering effect of resveratrol may also have a role in this effect. This UCMS model was resulted in peripheral and systemic inflammation because of increased proinflammatory cytokines were not only in the thoracic aorta but also in serum. Therefore, the antidepressant effect of resveratrol may, at least in part, be dependent on the prevention of systemic inflammation by the antiinflammatory effect of resveratrol.

5. Conclusion

The present study showed that resveratrol restored UCMS-induced vascular endothelial dysfunction and ameliorated depression-like behavior in UCMS-exposed rats. Resveratrol has therapeutic potential to prevent psychological stress-induced depression and vascular changes by its antiinflammatory effect. Further studies to investigate the other mechanisms of resveratrol in this animal model are needed.

CRediT authorship contribution statement

Gülçin Gacar: Conceptualization, methodology, Funding acquisition, Project administration, **Semil Selcen Gocmez:** investigation, methodology, Formal analysis,Writing - original draft, Writing - review & editing, **Zehra Seda Halbutoğulları:** methodology, visualization, **Kamil Can Kılıç:** methodology, data curation, **Aysenur Kaya:** methodology, data curation, **Yusufhan Yazir:** methodology, supervision, **Tijen Utkan:** Conceptualization, investigation, supervision, Validation.

Data availability

Data will be made available on request.

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Berlin, Germany.

Declaration of Competing Interest

The authors declare that there is no conflict of interest associated with this publication.

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