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ORIGINAL ARTICLE

# Resveratrol and quercetin attenuate depressive-like behavior and restore impaired contractility of vas deferens in chronic stress-exposed rats: involvement of oxidative stress and inflammation

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## Abstract

Chronic stress is associated with male sexual problems including ejaculatory dysfunctions. The aim of this study was to determine whether resveratrol (RS) or quercetin (QE) has protective effects on vas deferens (VD) contractility in the unpredictable chronic mild stress (UCMS) rat model of depression. Animals were separated into six groups: control, control + RS and control + QE, stress, stress + RS, and stress + QE. Stress groups were subjected to UCMS procedure for 5 weeks. Animals in treatment groups were injected intraperitoneally with RS (20 mg/kg) or QE (30 mg/kg) for 5 weeks during UCMS period. UCMS caused depressive-like behaviors and enhanced systemic levels of corticosterone. The nerve-evoked contractile responses of VD significantly impaired and, noradrenaline- and ATP-induced contractile responses of VD significantly increased in stressed rats. UCMS exposure also markedly enhanced oxidative stress and inflammation in VD tissues. Treatment with RS or QE significantly ameliorated all the aforementioned parameters. The current study demonstrated that RS or QE protected against chronic stress-induced VD dysfunction by their antioxidant and anti-inflammatory effects on VD, suggesting that oxidative stress and inflammation may be synergistic parts in the development of VD dysfunction associated with chronic stress-induced depression.

**Keywords** Unpredictable chronic mild stress · Vas deferens · Oxidative stress · Inflammation · Resveratrol · Quercetin

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## Introduction

In the modern world, people are constantly exposed to unpredictable various stressors that have been suggested to be involved in the development of depression (Koolhaas et al. 1997). These stressors lead to changes in locomotor, emotional, and sexual behaviors in animals (Willner et al. 1992). Chronic stress has been indicated to cause impairment of erectile and ejaculatory functions and loss of sexual desire (Lenzi et al. 2003). We have previously revealed the association between chronic stress-induced depression and erectile dysfunction (Demirtaş Şahin et al. 2018). It has also been established that chronic mild stress disrupted sperm quality and testicular structure in rats (Demirci and Sahin 2019). In spite of the animal reports suggesting the damaging effect of chronic stress on male reproductive system, almost no study investigated the effect of stress on ejaculatory functions in animals.

Ejaculation, a complex process mediated by various biochemical events and mechanisms, is mainly dependent on regular contractions of the vas deferens (VD) which conveys the sperm cells from the epididymis to the urethra (Koslov and Andersson 2013). VD contractility is essentially controlled by the sympathetic nerve fibers that primarily release noradrenaline. Purinergic co-transmission, especially induced by ATP, also contributes to the contractions of the VD (Koslov and Andersson 2013). In this context, the impairment of the sympathetic innervation and/or contractile function of the VD may lead to ejaculatory dysfunctions and the mechanisms underlying the motor activity of the VD may therefore be of interest as pharmacological targets for the treatment of ejaculatory disorders.

Oxidative stress represents a disturbance in the balance between the formation of reactive oxygen species (ROS) and a biological system's ability to counteract or neutralize their destructive effects. It has been reported to be one of the mechanisms underlying the development of male sexual and reproductive disorders (Agarwal et al. 2006; Sakr et al. 2015). Animal studies reported that chronic stress exposure enhanced oxidative stress and impaired antioxidant defense status in blood, brain (Abd El-Fattah et al. 2018), and also testicular tissues (Sakr et al. 2015). Experimental evidence indicated that ROS were responsible for the impairment of sympathetic neurotransmission and contractile activity of VD in diabetic rats (Güneş et al. 2005). Also, a previous study demonstrated restoration of VD hypocontractility by the treatment with antioxidants in LPS-treated rats (Geyik et al. 2009). On the other hand, inflammation contributes to the development of chronic stress-induced male sexual and reproductive dysfunctions (Yazir et al. 2018; Fahim et al. 2019). Resveratrol (RS) and quercetin (QE) are two natural polyphenols, mainly present in grapes and red wine. Both polyphenols possess numerous pharmacological properties including antioxidant and anti-inflammatory effects (Zhao et al. 2017). It has been established that both RS and QE reversed the adverse effects of chronic stress on behavior by alleviating oxidative and inflammatory stress (Mehta et al. 2017; Abd El-Fattah et al. 2018). Additionally, in rats exposed to chronic stress, RS has been shown to have restorative effects on penile cavernosal and testicular dysfunctions (Sakr et al. 2015; Yazir et al. 2018).

Based on the information above, it might be expected that RS and QE may prevent a possible impairment of VD contractility in unpredictable chronic mild stress (UCMS)-exposed rats. However, to date, it was not demonstrated that UCMS results in any impairment of VD contractile activity. Therefore, in the current study, we aimed to clarify the effects of chronic stress on the contractile activity of VD and whether RS or QE could restore chronic stress-induced impairment of VD contractions by their antioxidant and/or anti-inflammatory effects.

## Methods

### Animals and housing

Adult male Wistar albino rats, 4 months of age, weighing 250–300 g, were procured from Kocaeli University, Experimental Medical Research and Application Center and housed under controlled conditions (12 h light/12 h dark cycle, humidity of 45%, and temperature of 22 ± 2 °C) in an animal colony (~ 5 to 6 per cage). The rats were allowed free access to tap water and chow.

Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011, published by The National Academies Press, USA) and were approved by the Animal Research Ethics Committee of Kocaeli University, Turkey (Decision No: KOÜ HADYEK 2/9-2017; decision date: 09/02/2017).

### Treatment schedule

Rats were randomly separated into two main groups: control groups and stress groups. Control groups were separated into three subgroups ($n = 16$ for each subgroup): control, control + RS, and control + QE. Stress groups, subjected to UCMS procedure for 5 weeks, were also assigned into three subgroups ($n = 16$ for each subgroup): stress, stress + RS, and stress + QE. Control and stress groups received 5% DMSO. Animals in treatment groups were injected intraperitoneally with RS (20 mg/kg) and QE (30 mg/kg) for 5 weeks during UCMS period. RS and QE were dissolved in 5% DMSO. All injections were administered in a volume of 2 ml/kg body weight of the rats. After 5-week UCMS period, the animals were evaluated in the locomotor activity test, forced swimming test, sucrose consumption test, and sucrose preference test. Then, they were sacrificed under intraperitoneal ketamine/xylazine (90 mg/kg/10 mg/kg, respectively) anesthesia to collect the blood and VD tissue samples for the functional, biochemical, and molecular analyses.

### Stress protocol

Animals were subjected to a series of unpredictable chronic stimuli for 5 weeks as previously described in our reports (Demirtaş et al. 2014; Demirtaş Şahin et al. 2018). Each week consisted of cold water swimming at 4 °C for 5 min, tail clamping for 1 min, cage tilting for 24 h, hot water swimming at 45 °C for 5 min, wet bedding for 24 h, level shaking for 10 min, restraint for 4 h and 24 h of reversed light/dark cycle, and pairing with another stressed rat for 48 h. All stressors were applied randomly to be unpredictable to the rats. Water and food were freely available in this procedure.

## Behavioral assessment

### Locomotor activity test

A Plexiglas chamber (Commat Ltd., Ankara, Turkey) consisted of 15-beam array of infrared horizontal (mounted every 2.5 cm, bottom) and vertical (mounted every 4.5 cm, upper) movement detectors was used to measure locomotor activity in this test. Ambulatory, vertical, and stereotypic activities were recorded during 10 min, and these activities were evaluated as total locomotor activity. Prior to test, the animals were allowed to adapt to the test room.

### Forced swimming test

The depressive-like behavior was evaluated using forced swimming test as previously reported by Porsolt et al. (1977). This test was conducted in an identical cylinder (height: 38 cm, diameter: 47 cm), containing of water at 22 ± 1 °C) for two consecutive sessions (the pretest and the test). During the pretest session, the rats were placed into the cylinder and submitted to the forced swimming for 15 min. Then, animals were dried and returned to their home cages. Twenty-four hours later, all rats were put back inside of the same cylinder for a 5-min test trial. The behavioral parameter as immobility time was recorded.

### Sucrose consumption and preference test

This test was performed as described by Sarkisova et al. (2010). All rats were trained to consume 20% sucrose solution in an individual cage for 15 min during 5 days and their sucrose intake (g) was measured. Following the sucrose intake training, the animals were deprived of water and food for 23 h, and then placed individually again into a two-bottle, free-choice cage. They were exposed to these two bottles with 20% sucrose (100 ml) and tap water (100 ml) for a period of 1 h. Water and sucrose intake were measured and the sucrose preference was determined using the formula:

Sucrose preference (%) = ((sucrose intake [g]/total fluid intake [g]) × 100).

## Functional studies by in vitro organ baths

After the animals were sacrificed, VD tissues were excised and placed in a Petri dish containing fresh Krebs solution consisting of (in mmol/l) NaCl, 118.4; KCl, 4.7; $MgSO_4$, 1.2; $KH_2PO_4$, 1.2; $NaHCO_3$, 25; $CaCl_2$, 1.9; and glucose, 11. The tissues were mounted under 1 g tension in an organ bath containing Krebs solution maintained at 37 °C and bubbled with a gas mixture of 95% $O_2$ and 5% $CO_2$. Each preparation was allowed to equilibrate for 1 h and washed with Krebs solution at 15-min intervals. The responses were recorded for each tissue with an isometric force-displacement transducer (FDT 05, Commat Ltd) connected to a data acquisition system (MP30, Commat Ltd.).

After the equilibration period, electrical field stimulation (EFS) (2–32 Hz, 50 V, 0.5 ms) was applied to tissues for 10 s via two platinum wire electrodes by a stimulator (ST 95 PT, Commat Ltd.). In the other experiments, prior to the electrical stimulation, we used prazosin (an α1-adrenergic receptor antagonist-0.3 μmol/l) or suramin (a P2-purinergic receptor antagonist-100 μmol/l) to isolate purinergic or adrenergic contractions in response to EFS, respectively. In separate experiments, the preparations were contracted with noradrenaline ($10^{-8}$–$10^{-4}$ mol/l), or ATP ($10^{-8}$–$10^{-4}$ mol/l) to obtain adrenergic or purinergic responses, respectively. At the end of the experiments, tissues were contracted with 80 mmol/l KCl. The contraction responses elicited by EFS, noradrenaline, and ATP were expressed as percentage of 80 mM KCl-induced contraction responses for each VD preparation.

## Measurement of oxidative stress

### Measurement of lipid peroxidation (MDA) level

MDA was measured using QuantiChrome TBARS Assay Kit (DTBA-100, BioAssay Systems, Hayward, CA, USA) according to the manufacturer's protocol. Tissue was homogenized in cold PBS buffer and centrifuged for 10 min at 14,000 rpm. Ice-cold 10% trichloroacetic acid was added into the supernatant to deproteinize. After the neutralization, the clear sample was mixed with thiobarbituric acid (TBA) solution and incubated at 100 °C for 60 min. The optical absorbance was determined at 535 nm using the VIS-Spectrophotometers (Molecular Devices). The results were given as nmol/ml.

### Estimation of catalase activity

Catalase activity in tissue was measured with EnzyChrom Catalase Assay Kit (ECAT-100, BioAssay Systems), according to the manufacturer's instructions. Briefly, tissue was homogenized in 200-μl cold PBS buffer and centrifuged for 10 min at 14,000 rpm to remove any debris. Clear supernatant (10 μl) was used for the direct measurement of catalase degradation of $H_2O_2$. The change in color intensity was estimated at 570 nm. The results were presented in U/l. One unit is defined as the amount of catalase decomposing 1 μmole of $H_2O_2$ per min at pH 7 and room temperature.

### Estimation of superoxide dismutase activity

SOD activity was assessed by EnzyChrom SOD Assay Kit (ESOD-100, BioAssay Systems), according to the manufacturer's protocol. Briefly, 20 μl of each sample was mixed with 160-μl working solution containing xanthine and WST-1. Absorbance was measured immediately at 440 nm and the reagent was incubated at 25 °C for 60 min. Following the incubation, the absorbance was measured at 440 nm. The activity of SOD was estimated by subtracting the two consequent absorbance reading (before and after the incubation) and comparing this value with the SOD concentration standard curve. The activity was represented as U/ml.

### Estimation of GSH level

The GSH level was determined using QuantiChrom GSH Assay kit (DIGT-250, BioAssay Systems) based on the DTNB (5,5′-dithiobis-[2-nitrobenzoic acid]) method. After tissue homogenization in ice-cold PBS, the samples were deproteinized and mixed with DTNB. After the incubation for 25 min at room temperature, the optical density at 412 nm was determined. The results were presented in mmol/l.

### Determination of total protein

After the homogenization of tissue for enzyme activities or metabolites, all measurements were normalized with respect to the protein level by bicinchoninic acid (BCA) assay. Total protein concentration was determined by SMART BCA Protein Assay Kit (Intron Biotechnology, Gyeonggi-do, Korea) according to manufacturer's protocol.

## Gene expression analysis

VD tissue samples were collected to determine the gene expressions of TNF-α, NF-κB, IL-1β, and IL-6. Total RNA was isolated by GeneJet RNA Purification Kit (Thermo Scientific, Vilnius, Lithuania). Genomic DNA was removed by DNaseI (Thermo Scientific). The quality of RNA samples was determined by absorbance at 260 and 280 nm, and cDNA was synthesized using Thermo Scientific cDNA synthesis kit (Thermo Scientific, Rockford, IL, USA), according to the manufacturer's instructions. The cDNA products were subjected to PCR amplification with gene-specific primers (Table 1). A total of 50 ng of cDNA was amplified in each well using PowerUp SYBR Green Master Mix by LC480-II (Roche, Basel, Switzerland). Actin-β (ActB) was used as the internal control for normalization.

## Enzyme-linked immunosorbent assay

The concentration of corticosterone in the VD tissues was analyzed by an ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA) according to the manufacturer's instructions. The results were expressed as pg/ml.

## Statistical analysis

Data was expressed as mean ± standard error of the mean (SEM) and assessed using GraphPad Prism software (San Diego, CA, USA). Responses induced by EFS, noradrenaline, or ATP were expressed as the percentage (%) of the contractile response to 80 mmol/l KCl for each VD preparation. $E_{max}$ and $pD_2$ values were calculated to evaluate the effects of agonists. In order to determine significant differences, one-way and two-way ANOVA post hoc Bonferroni's test was used. $p < 0.05$ was considered to be statistically significant.

# Results

## Resveratrol and quercetin did not alter locomotor activity in stressed rats

As illustrated in Fig. 1a, no significant difference among all experimental groups was observed in terms of locomotor activity.

## Resveratrol and quercetin enhanced depressive-like behavior in stressed rats

Forced swimming test results showed that stressed rats displayed more immobility than control rats ($p < 0.0001$; Fig. 1b), whereas RS and QE treatment significantly decreased the immobility time in stressed rats ($p < 0.0001$; Fig. 1b). However, RS and QE treatment showed no effects on immobility time in control rats (Fig. 1b). In the sucrose consumption test, the consumed sucrose in stressed rats was less than control rats ($p < 0.0001$; Fig. 2a). RS and QE treatments markedly increased the sucrose consumption in stressed rats ($p < 0.0001$ and $p < 0.01$, respectively; Fig. 2a), while they had no effects on the sucrose consumption in control rats (Fig. 2a). Additionally, sucrose preference was lower in stressed rats compared with control rats ($p < 0.0001$; Fig. 2b), while RS and QE significantly enhanced the sucrose preference in stressed rats ($p < 0.001$ and $p < 0.01$, respectively; Fig. 2b). However, treatment with RS and QE in control rats had no influence on the sucrose preference (Fig. 2b).

**Table 1** Gene-specific primers used in real-time PCR

| Gene name | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|---|---|---|
| TNFα | TGAACTTCGGGGTGATCG | GGGCTTGTCACTCGAGTTTT |
| NF-κB | AGAGAAGCACAGATACCACTAAG | CAGCCTCATAGAAGCCATCC |
| IL-1β | TGTGATGAAAGACGGCACAC | CTTCTTCTTTGGGTATTGTTTGG |
| IL-6 | CCTGGAGTATGTGAAGAACAACT | GGAAGTTGGGGTAGGAAGGA |
| ActB | AGAGAAGCTGTGCTATGTTG | GTACTCCTGCTTGCTGATCC |

## Resveratrol and quercetin improved vas deferens contractile activity in stressed rats

The contractile responses to all frequencies of EFS were markedly reduced in the VD of stressed rats as compared to those of controls ($p < 0.05$; Fig. 3). The impairment of EFS-induced contractile responses in the VD of stressed rats significantly restored by RS treatment ($p < 0.05$ at all frequencies; Fig. 3). QE also increased EFS-induced contractions in stressed rats ($p < 0.05$ at all frequencies; Fig. 3). However, the contractions induced by EFS in the control rats did not change by RS or QE treatment (Fig. 3). In separate experiments, chronic stress also caused a marked decrease in nerve-evoked contractions in the presence of suramin or prazosin ($p < 0.05$ at all frequencies; Fig. 4a, b). Treatment with RS or QE significantly restored the impairment of EFS-induced contractile responses in the VD of stressed rats ($p < 0.05$ at all frequencies; Fig. 4a, b) and the contractions induced by EFS in the control rats did not change by RS or QE in the presence of suramin or prazosin (Fig. 4a, b). Besides, as we expected, the magnitude of EFS-induced contractile responses significantly reduced

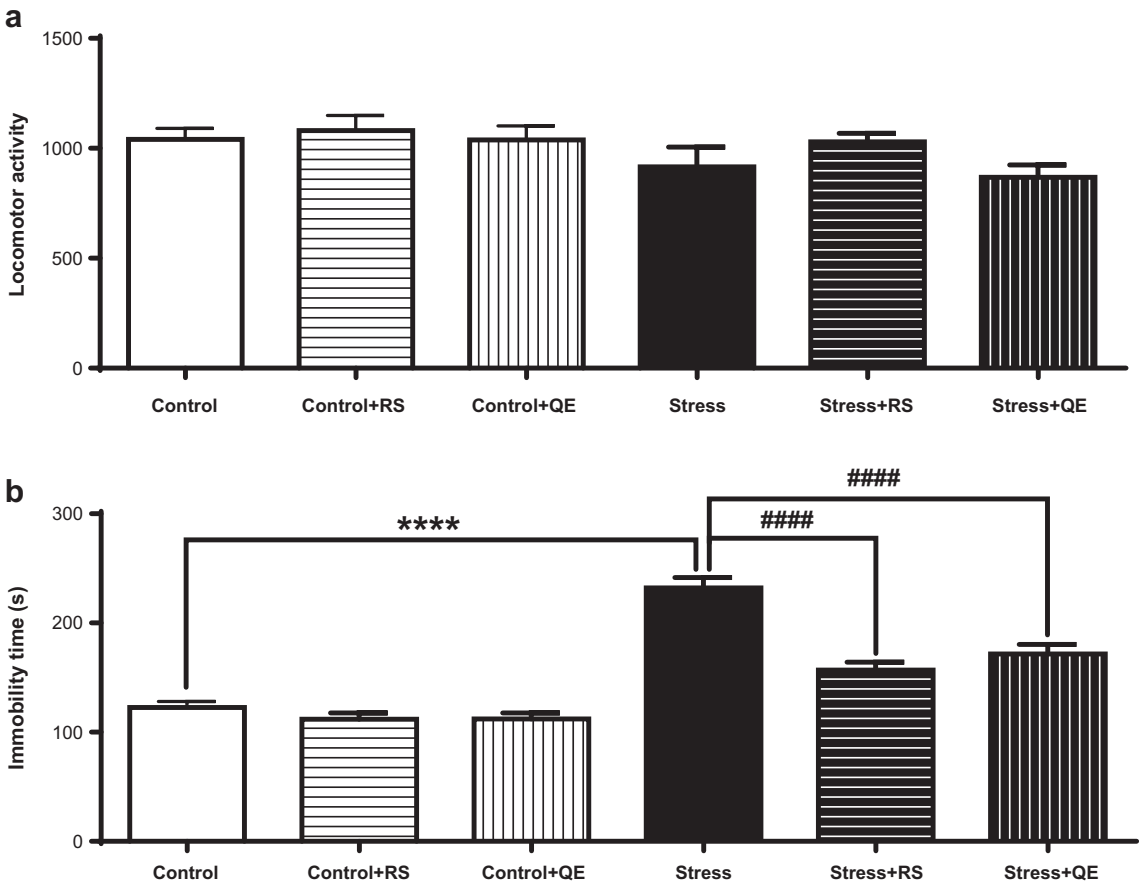



**Fig. 1** Total locomotor activity (**a**) and immobility times in the forced swimming test (**b**) in control, control + RS, control + QE, stress, stress + RS, and stress + QE groups. ****$p < 0.0001$ vs. control group; $^{\#\#\#\#}p < 0.0001$ vs. stress group. Values represent the mean ± SEM. $n = 10$ for all groups

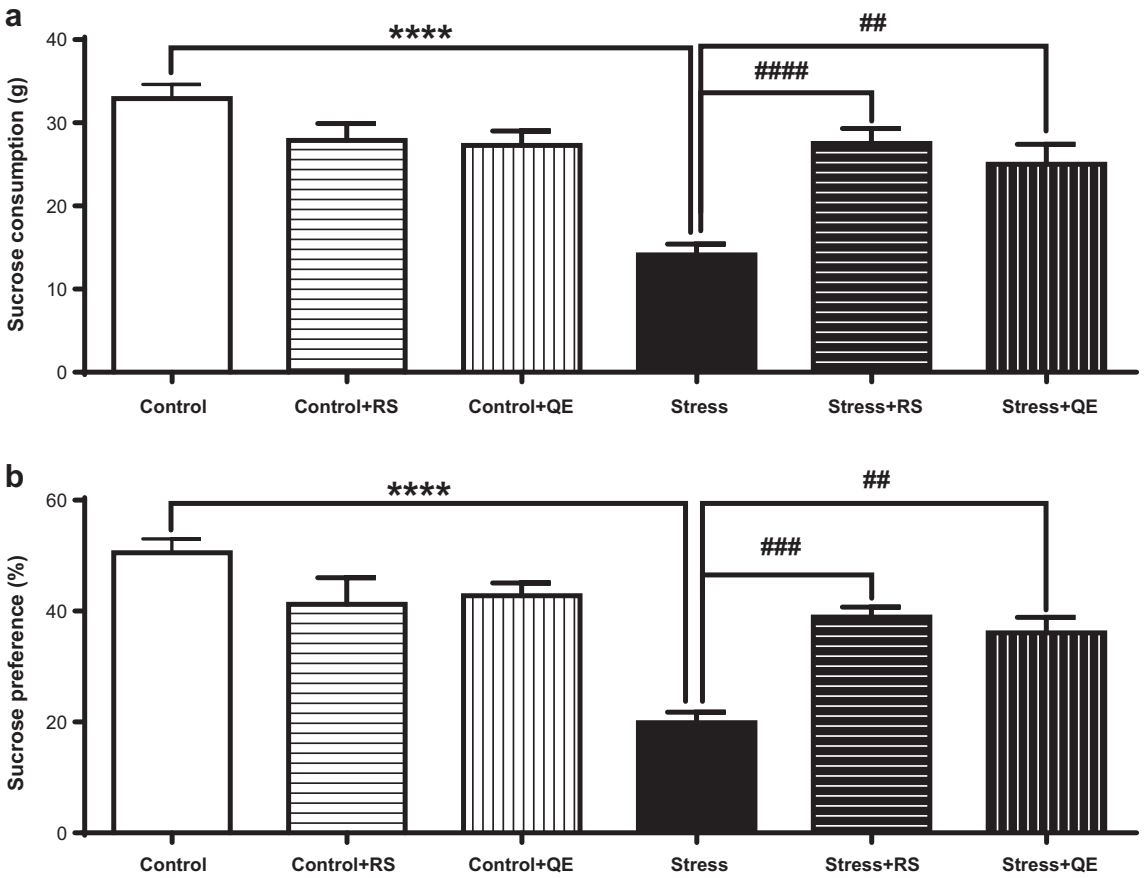



**Fig. 2** Sucrose consumption (**a**) and sucrose preference (**b**) scores in control, control + RS, control + QE, stress, stress + RS, and stress + QE groups. ****$p < 0.0001$ vs. control group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, and $^{\#\#\#\#}p < 0.0001$ vs. stress group. Values represent the mean ± SEM. $n = 10$ for all groups

in the presence of suramin or prazosin compared to those obtained in the absence of these agents. Additionally, these nerve-evoked contractions were dramatically impaired in the presence of suramin + prazosin (data not shown).

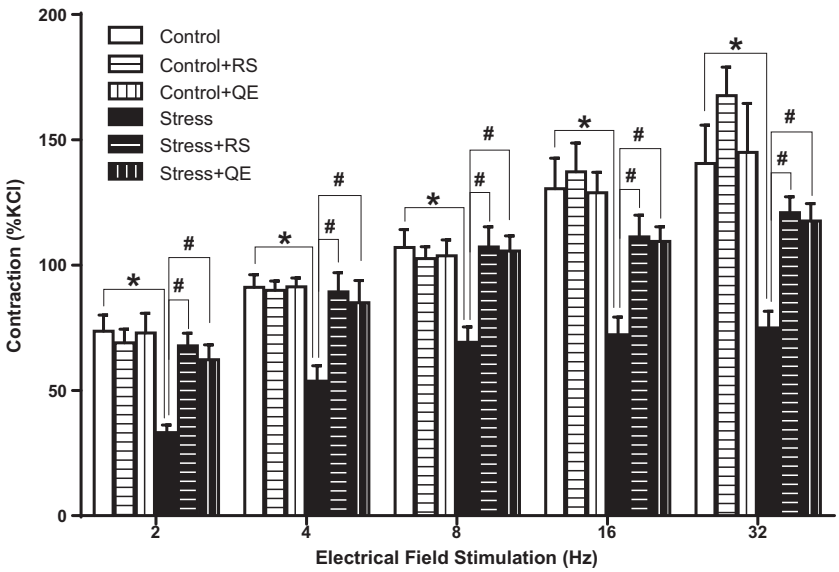



**Fig. 3** EFS-induced contractile response of vas deferens tissues obtained from control, control + RS, control + QE, stress, stress + RS, and stress + QE groups. $^{*}p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. stress group. Values are expressed as percentage of 80 mmol/l KCl-induced contraction and represent the mean ± SEM. $n = 8$ for all groups

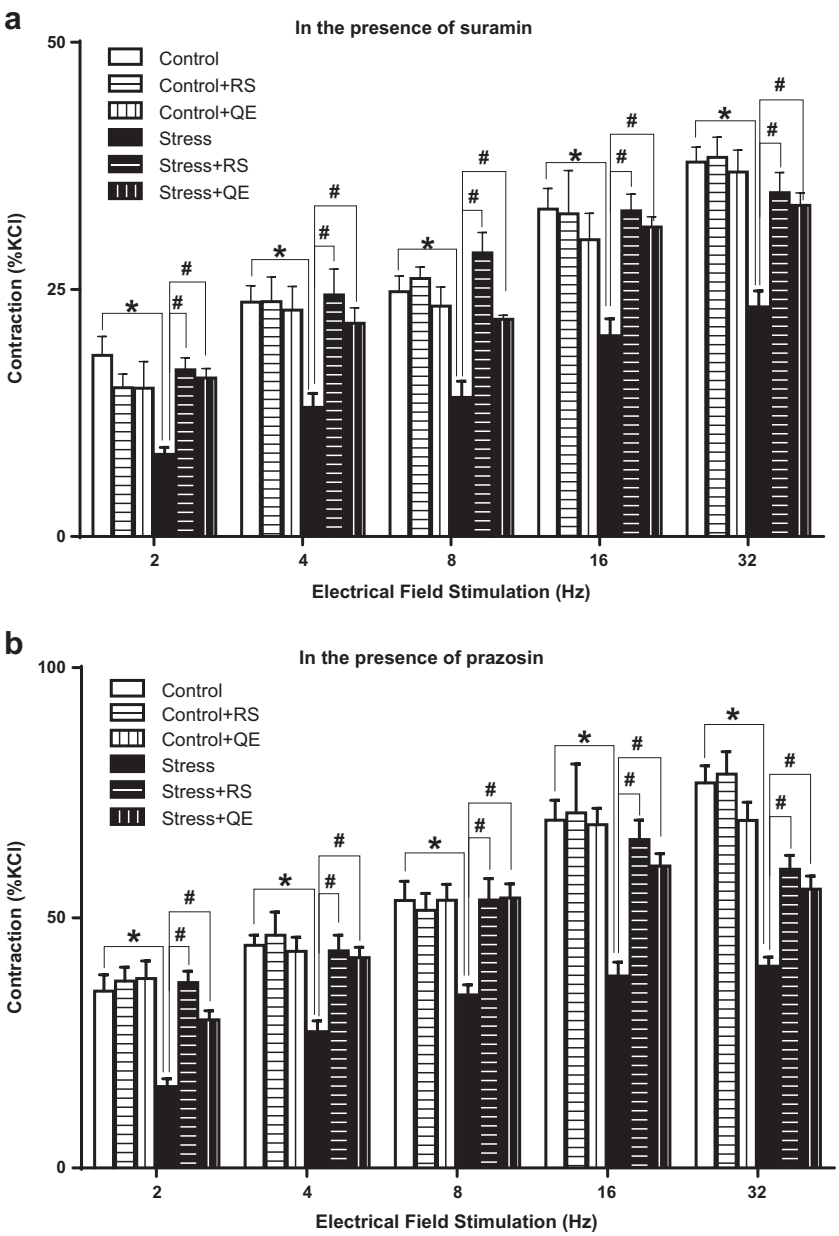



**Fig. 4** EFS-induced contractile response of vas deferens tissues obtained from control, control + RS, control + QE, stress, stress + RS, and stress + QE groups in the presence of suramin (**a**) and prazosin (**b**). $^{*}p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. stress group. Values are expressed as percentage of 80 mmol/l KCl-induced contraction and represent the mean ± SEM. $n = 8$ for all groups

The noradrenaline ($10^{-8}$ to $10^{-4}$ mol/l)-induced concentration-dependent contraction responses of obtained from the VD tissues of all experimental groups are presented in Fig. 5a. In stressed rats, the contractile response of the tissues to noradrenaline significantly increased with an $E_{max}$ of 35.33 ± 1.96% compared to the control rats ($E_{max} = 22.70 \pm 1.79\%$) ($p < 0.05$). RS treatment markedly reversed this increment in stressed rats ($E_{max} = 25.73 \pm 0.83\%$) ($p < 0.05$). QE was also able to restore the noradrenaline-induced responses of the tissues obtained from stressed rats ($E_{max} = 28.69 \pm 0.67\%$) ($p < 0.05$). However, treatment with RS and QE did not cause any significant change in the contractile response of the VD to noradrenaline ($E_{max} = 22.43 \pm 1.60\%$ and $E_{max} = 23.02 \pm 1.36\%$, respectively). Also, no significant difference in $pD_2$ values was found between the groups ($pD_2$ for control group = 5.28 ± 0.05; $pD_2$ for control + RS group = 5.42 ± 0.06; $pD_2$ for control + QE group = 5.29 ± 0.05; $pD_2$ for stress group = 5.34 ± 0.07; $pD_2$ for stress + RS group = 5.40 ± 0.10; $pD_2$ for stress + QE group = 5.38 ± 0.03).

The contraction of the VD tissues in response to ATP ($10^{-8}$ to $10^{-4}$ mol/l) of all experimental groups is shown in Fig. 5b. Chronic stress caused a significant increase in the ATP-induced contractions with an $E_{max}$ of 20.67 ± 1.58% when compared with the controls ($E_{max} = 12.62 \pm 1.08\%$) ($p < 0.05$). RS markedly restored the impairment in contractile responses to ATP in VD obtained from stressed rats ($E_{max} = 15.12 \pm 0.58\%$) ($p < 0.05$). QE treatment also caused a significant decrease on the contractile

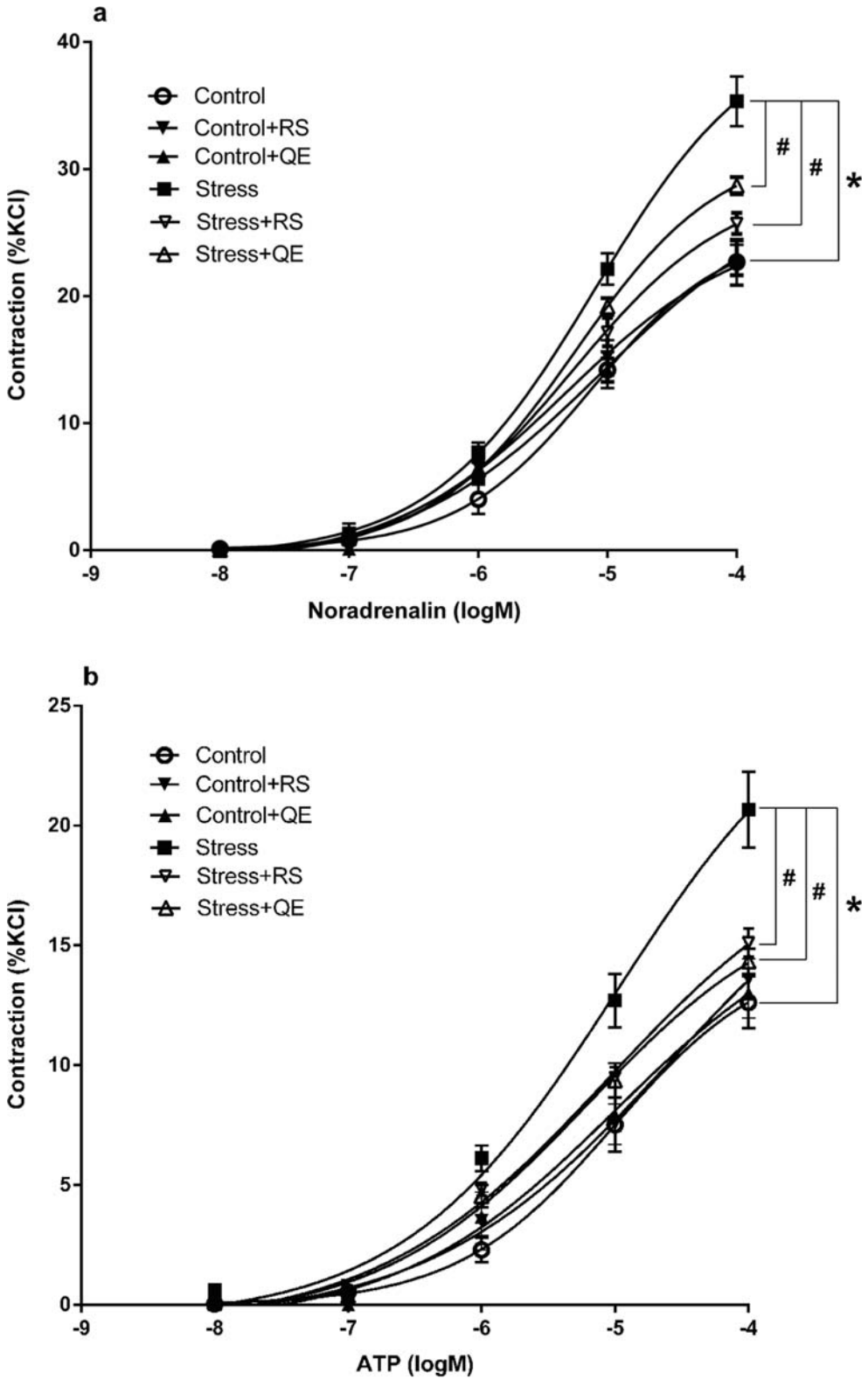



**Fig. 5** Noradrenaline-induced (**a**) and ATP-induced (**b**) contractile response of vas deferens tissues obtained from control, control + RS, control + QE, stress, stress + RS, and stress + QE groups. $^{*}p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. stress group. Values represent the mean ± SEM. $n = 8$ for all groups

responses of the tissues to ATP in stressed rats with an $E_{max}$ of 14.33 ± 0.51%) ($p < 0.05$). On the other hand, RS or QE did not affect the contractions in response to ATP in control tissues with no differences in $E_{max}$ ($E_{max}$ for control + RS group = 13.58 ± 0.85%, $E_{max}$ for control + QE group = 13.01 ± 1.03%). Additionally, no significant difference was observed in $pD_2$ values between the groups ($pD_2$ for control group = 5.20 ± 0.20; $pD_2$ for

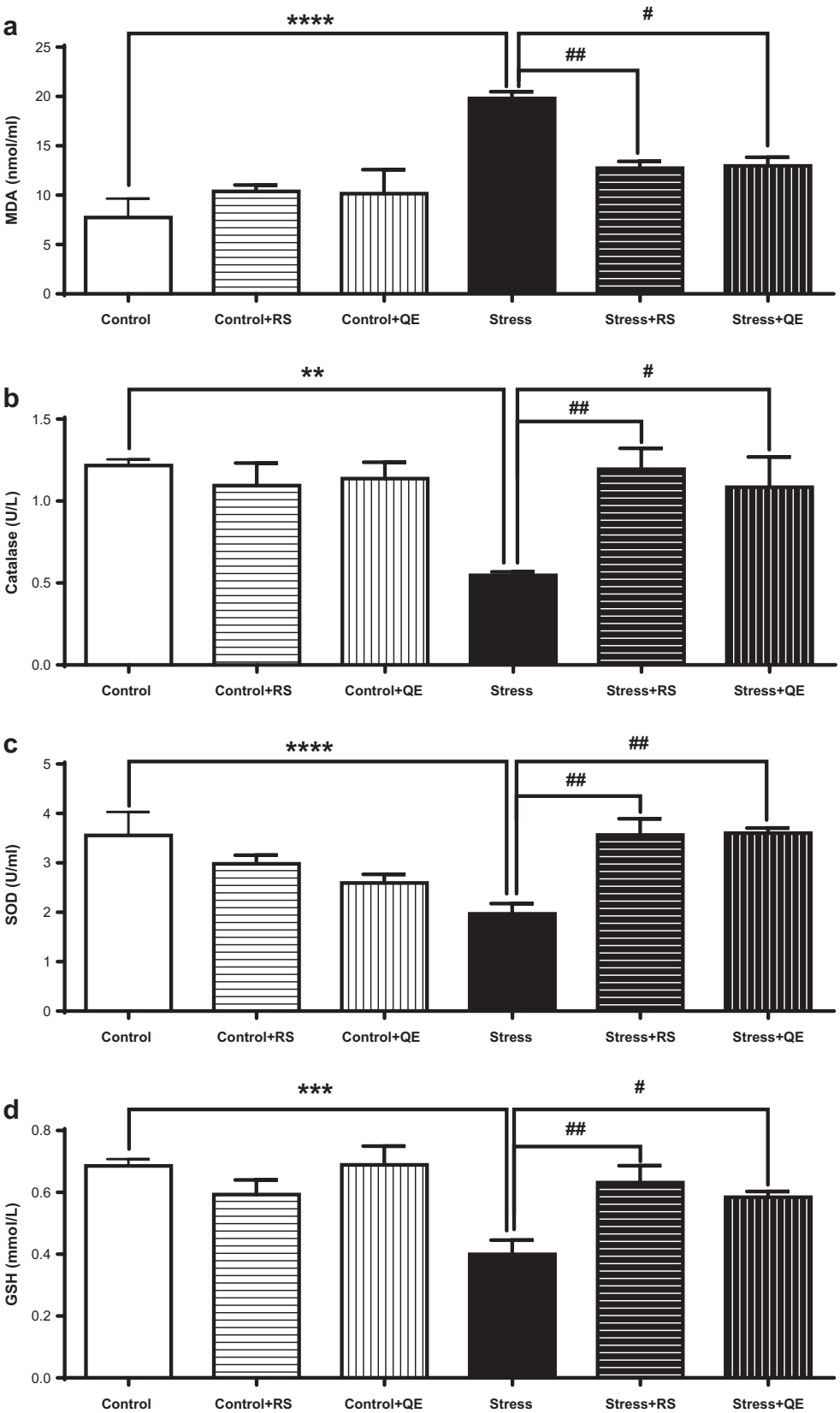
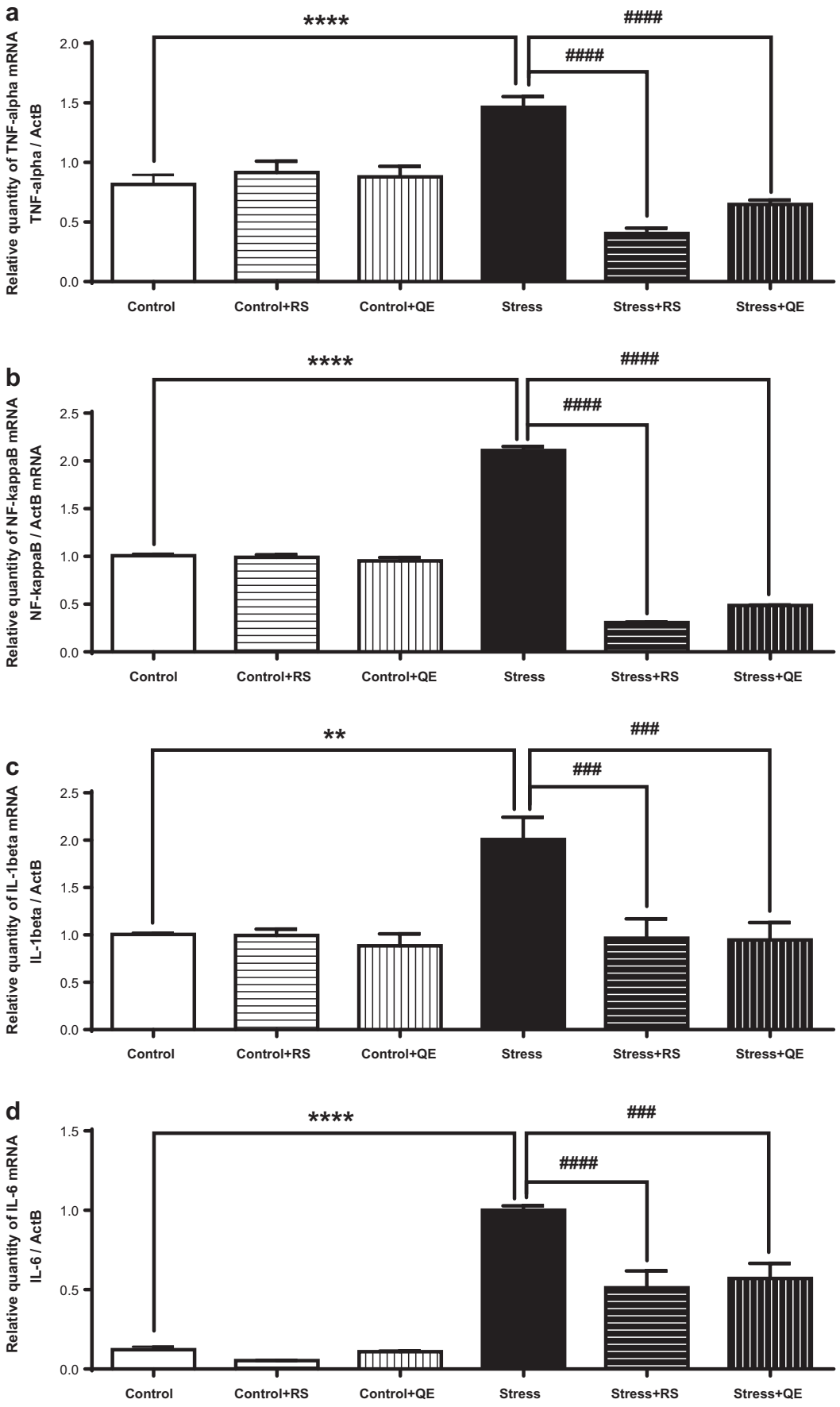



**Fig. 6** MDA levels (**a**), CAT and SOD activities (**b**, **c**), and GSH levels (**d**) in vas deferens tissues obtained from control, control + RS, control + QE, stress, stress + RS, and stress + QE groups. $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ vs. control group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. stress group. Values represent the mean ± SEM. $n = 8$ for all groups

control + RS group = 5.29 ± 0.23; $pD_2$ for control + QE group = 5.31 ± 0.06; $pD_2$ for stress group = 5.25 ± 0.12; $pD_2$ for stress + RS group = 5.45 ± 0.03; $pD_2$ for stress + QE group = 5.44 ± 0.08).


a
Relative quantity of TNF-alpha mRNA
TNF-alpha / ActB
****
####
####
2.0
1.5
1.0
0.5
0.0
Control
Control+RS
Control+QE
Stress
Stress+RS
Stress+QE
b
Relative quantity of NF-kappaB mRNA
NF-kappaB / ActB mRNA
****
####
####
2.5
2.0
1.5
1.0
0.5
0.0
Control
Control+RS
Control+QE
Stress
Stress+RS
Stress+QE
c
Relative quantity of IL-1beta mRNA
IL-1beta / ActB
**
###
###
2.5
2.0
1.5
1.0
0.5
0.0
Control
Control+RS
Control+QE
Stress
Stress+RS
Stress+QE
d
Relative quantity of IL-6 mRNA
IL-6 / ActB
****
###
####
1.5
1.0
0.5
0.0
Control
Control+RS
Control+QE
Stress
Stress+RS
Stress+QE

◀ **Fig. 7** TNF-α (**a**), NF-κB (**b**), IL-1β (**c**), and IL-6 (**d**) mRNA expression in vas deferens tissues obtained from control, control + RS, control + QE, stress, stress + RS, and stress + QE groups. $^{**}p < 0.01$ and $^{****}p < 0.0001$ vs. control group; $^{###}p < 0.001$ and $^{####}p < 0.0001$ vs. stress group. Values represent the mean ± SEM. $n = 8$ for all groups

The contractile response induced by 80 mmol/l KCl was not found to be statistically different among the all experimental groups (data not shown).

## Resveratrol and quercetin reversed changes in oxidative stress parameters in the vas deferens of stressed rats

Effects of RS and QE treatment on lipid peroxidation are depicted in Fig. 6a. MDA levels significantly increased in the VD of stressed rats as compared to the control rats ($p < 0.0001$). However, treatment with RS and QE markedly inhibited the elevation of MDA levels in the VD of stressed rats ($p < 0.01$ and $p < 0.05$, respectively). RS or QE showed no effects on the MDA levels in the VD of control rats.

The changes in CAT activity are presented in Fig. 6b. CAT activity significantly diminished in the VD of stressed rats compared to control rats ($p < 0.01$). Administration of RS and QE to stressed rats caused an increase in CAT activity ($p < 0.01$ and $p < 0.05$, respectively). However, RS or QE did not affect the CAT activity in control rats.

As shown in Fig. 6c, a significant decrease was observed in SOD activity in the VD of stressed rats in comparison to control rats ($p < 0.0001$). This reduction was markedly improved by RS and QE treatment in the VD of stressed rats ($p < 0.01$; Fig. 6c). However, treatment with RS or QE in control rats had no influence on the SOD activity (Fig. 6c).

As indicated in Fig. 6d, the reduced glutathione (GSH) levels markedly diminished in the VD of stressed rats compared with control rats ($p < 0.001$), while application of RS and QE significantly increased these levels in stressed rats ($p < 0.01$ and $p < 0.05$, respectively). Treatment with RS or QE did not alter the GSH levels in the VD of control rats (Fig. 6d).

## Resveratrol and quercetin decreased gene expression of inflammatory mediators in the vas deferens of stressed rats

The mRNA expressions of TNF-α, NF-κB, IL-1β, and IL-6 in VD tissues of all experimental groups are presented in Fig. 8. Chronic stress caused a significant increase in the mRNA expressions of TNF-α and NF-kB compared with controls ($p < 0.0001$; Fig. 7a, b), whereas treatment with RS and QE markedly alleviated these elevations in stressed rats ($p < 0.0001$; Fig. 7a, b). In similar, stress significantly increased the mRNA expression of IL-1β compared to control group ($p < 0.01$; Fig. 7c), while RS and QE treatment remarkably induced mRNA expression reduction of IL-1β in stressed rats ($p < 0.001$; Fig. 7c). Moreover, in stressed group, the mRNA expression of IL-6 was markedly elevated compared to the control group ($p < 0.0001$; Fig. 7d). However, groups which received RS and QE had a dramatic reduction in this expression ($p < 0.0001$ and $p < 0.001$, respectively). The mRNA expressions of aforementioned inflammatory mediators were not significantly changed by the RS or QE treatment in control rats (Fig. 7a–d).

## Resveratrol and quercetin diminished serum corticosterone in stressed rats

As shown in Fig. 8, in response to chronic stress, the circulating concentration of corticosterone significantly increased compared with the control rats ($p < 0.001$). In RS- and QE-treated stressed rats, the corticosterone decreased markedly ($p < 0.001$ and $p < 0.01$, respectively; Fig. 8). However, RS or QE treatment showed no significant effect on the serum corticosterone levels in control rats (Fig. 8).

The behavioral, functional, biochemical, and molecular results gathered from all experiments are summarized in Table 2.

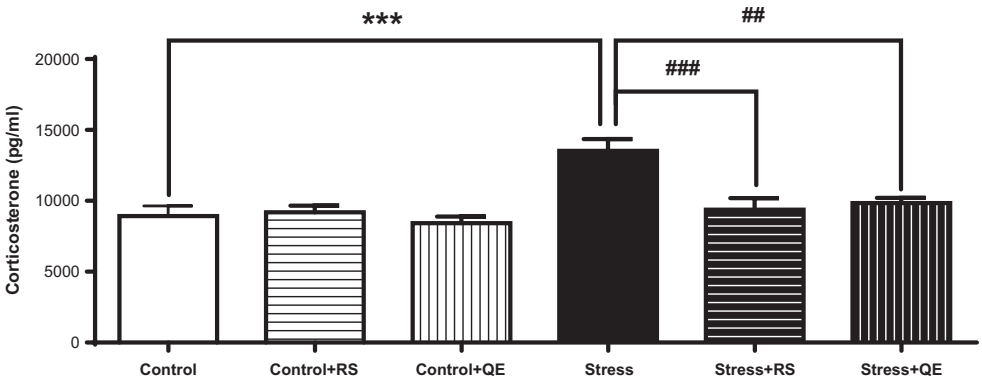



**Fig. 8** Serum levels of corticosterone in control, control + RS, control + QE, stress, stress + RS, and stress + QE groups. $^{***}p < 0.001$ vs. control group; $^{##}p < 0.01$ and $^{###}p < 0.001$ vs. stress group. Values represent the mean ± SEM. $n = 8$ for all groups

**Table 2** Summary of behavioral, functional, biochemical, and molecular results gathered from all experiments

| | Control + RS vs. control | Control + QE vs. control | Stress vs. control | Stress + RS vs. stress | Stress + QE vs. stress |
|---|---|---|---|---|---|
| Locomotor activity | – | – | – | – | – |
| Depressive-like behavior in FST | – | – | ↑ | ↓ | ↓ |
| Depressive-like behavior in SCT/SPT | – | – | ↑ | ↓ | ↓ |
| Serum corticosterone | – | – | ↑ | ↓ | ↓ |
| VD contractility | | | | | |
| NA-induced | – | – | ↑ | ↓ | ↓ |
| ATP-induced | – | – | ↑ | ↓ | ↓ |
| EFS-induced | – | – | ↓ | ↑ | ↑ |
| Oxidative stress in VD | | | | | |
| MDA | – | – | ↑ | ↓ | ↓ |
| Catalase | – | – | ↓ | ↑ | ↑ |
| SOD | – | – | ↓ | ↑ | ↑ |
| GSH | – | – | ↓ | ↑ | ↑ |
| Inflammation in VD | | | | | |
| TNF-α | – | – | ↑ | ↓ | ↓ |
| NF-κB | – | – | ↑ | ↓ | ↓ |
| IL-1β | – | – | ↑ | ↓ | ↓ |
| IL-6 | – | – | ↑ | ↓ | ↓ |

"–" denotes no difference, "↑" denotes significant increase, "↓" denotes significant decrease

*FST* forced swimming test, *SCT* sucrose consumption test, *SPT* sucrose preference test, *VD* vas deferens

## Discussion

Chronic stress is a crucial risk factor for emotional disorders such as major depression. Experimental evidence have shown that chronic stress induces behavioral alterations that are representative of depressive behavior, such as diminished immobility time in the forced swimming test and reduced sucrose consumption/preference (Demirtaş Şahin et al. 2018). Accordingly, our behavioral findings revealed a marked increase in the immobility behavior and, reduction in the sucrose intake and the preference with no change in locomotor activity in stressed rats. The effect of chronic stress on locomotor activity remains controversial with some reports showing impaired locomotor activity (Gawali et al. 2017), whereas others studies demonstrated no change (Demirtaş Şahin et al. 2018). This discrepancy may be attributed to differences in stress schedule, stressors applied to the animals, animal strain, and/or stimuli intensity. We report here that RS and QE displayed antidepressant-like effects in stressed rats, as evidenced by the reduction of immobility time and the increment of sucrose consumption and preference. On the other hand, neither RS nor QE caused no behavioral change in control animals, suggesting that these polyphenols exhibit antidepressant-like effect in only stress conditions. These findings were also consistent with previous studies (Mehta et al. 2017; Fahim et al. 2019).

It is well established that chronic stress induces hyperactivity of the hypothalamo-pituitary-adrenal (HPA) axis, which has been indicated as one of the main pathophysiological mechanisms underlying the development of depression (Dean and Keshavan 2017). In animals, chronic stress activates the HPA axis as demonstrated by enhanced circulating levels of corticosterone, the hormone resembling cortisol in human (Ayensu et al. 1995). Consistently, the findings of the current report showed a significant increase in circulating corticosterone concentration in chronically stressed rats, which confirm the stress status. However, in accordance with earlier studies (Fahim et al. 2019), chronic treatment with RS and QE ameliorated the corticosterone levels elevated by UCMS. It has been previously noted that total glucocorticoid receptor (GR) expression diminished in UCMS-exposed rats (Li et al. 2017). Moreover, decreased GR sensitivity has been reported in depressed patients (Pariante 2004). Therefore, the restoration of GR function by decreasing the resistance of receptors and/or upregulating receptor expression could be an explanation for the effects of RS and QE on normalizing corticosterone levels. Also, the antidepressant-like effects of these polyphenols may be the result of normalization of the HPA axis.

Oxidative stress, an imbalance between the production of ROS and the antioxidant defense system, has been suggested to be involved in the development of diverse pathologies due to chronic stress (Matchkov et al. 2015; Sakr et al. 2015; Guo et al. 2017). Various stressors provoke ROS generation

including hydrogen peroxide, superoxide anion, and hydroxyl radical ions in the organism, which in turn leads to cell lipid membrane peroxidation and widespread cellular injury. Enzymatic antioxidants such as SOD, which converts the reactive superoxide anion into either molecular oxygen or less reactive species hydrogen peroxide ($H_2O_2$) (Fukai and Ushio-Fukai 2011) and CAT, which scavenges $H_2O_2$ to water and molecular oxygen (Zamocky et al. 2008) are important in the protection of biological systems from oxidative stress. GSH is also one of the main antioxidant molecules in neutralizing free radicals and preserving cell integrity (Circu and Aw 2012). In the current study, the level of lipid peroxidation product (MDA) and antioxidants (GSH level, CAT, and SOD activity) was evaluated in the VD of rats exposed to UCMS-induced depression. Our results demonstrated that a marked increase in MDA level and reduction in antioxidants (GSH, CAT, and SOD) in stressed rats indicated the inability of the VD to eliminate ROS and/or inactivation of the antioxidant mechanisms caused by excess production of ROS in the VD. Earlier reports demonstrated that different types of chronic stress have been resulted in oxidative stress in various tissues including brain, heart, lungs, kidney, liver, pancreas, and testis (Sahin and Gümüşlü 2007; Nirmal et al. 2008; Sakr et al. 2015; López-López et al. 2016). This is the first report showing alterations in the major markers of oxidative stress in the VD in response to chronic stress. Treatment with RS and QE significantly decreased MDA and enhanced antioxidants (GSH, CAT, and SOD) in the VD of rats exposed to UCMS. These findings were unsurprising because of the potent antioxidant activity of RS and QE, which have natural phenolic hydroxyl structure that can neutralize ROS, thereby protecting tissues from oxidative stress damage.

Chronic stress not only results in oxidative damage, but also induces a low-grade inflammatory response (Kemeny and Schedlowski 2007). A recent study reported a marked increase in the testicular expression levels of NF-κB, TNF-α, and IL-1β in UCMS-exposed rats (Fahim et al. 2019). Similarly, our findings showed enhanced expression levels of NF-κB and proinflammatory cytokines (TNF-α, IL-1β, and IL-6) in the VD of rats exposed to UCMS. This may be dependent on chronic stress-induced oxidative stress, which favors the activation of transcription factors such as NF-κB, and in turn stimulates the production of proinflammatory cytokines. Moreover, our results revealed that RS and QE treatment caused a significant decrease in the expression levels of NF-κB and aforementioned proinflammatory cytokines in VD tissues of UCMS-exposed rats, indicating the ability of RS and QE to suppress chronic stress-induced inflammation in VD tissues. These findings are in agreement with other studies which RS and QE reduced inflammation markers in other tissues of UCMS-exposed rats (Mehta et al. 2017; Yazir et al. 2018; Fahim et al. 2019).

The contractile activity of the VD is mainly mediated through endogenous noradrenaline and ATP released from sympathetic nerves which activate post synaptic $\alpha_1$-adrenoceptors and P2X-purinoceptors, respectively (Koslov and Andersson 2013). It is well known that EFS of the sympathetic nerves in the rat VD results in biphasic contractions, the first phase to ATP and the second phase is attributed to noradrenaline (Koslov and Andersson 2013). In the current study, we showed that EFS-induced contractile responses of the VD markedly decreased in chronic stress-exposed rats. We also evaluated the contraction responses of the VD tissues to EFS in the presence of prazosin and suramin to elucidate how chronic stress affects purinergic or adrenergic components independently and we observed similar impairment on the responses to purinergic and adrenergic neurotransmission in stressed rats. This result indicated that these two separate systems were similarly affected by stress. Additionally, chronic stress caused increased noradrenalin- and ATP-induced contractility in VD of chronically stressed rats. These enhanced α-adrenergic and purinergic responsiveness of VD may be dependent on new receptor synthesis or activation of post-receptor mechanisms rather than an increment in the affinity of α-adrenoceptors and P2X-purinoceptors, since pD2 values for the contractile effects of noradrenaline and ATP were found to be unaltered. However, contractile responses induced by KCl in VD tissues did not differ between the groups, indicating that the documented changes in VD contractility of chronically stressed rats in this study cannot be associated with the alterations in calcium movements.

We have previously reported impaired reactivity of vascular and penile cavernosal smooth muscle in UCMS-exposed rats (Demirtaş et al. 2014; Demirtaş Şahin et al. 2018), but this is the first study showing abnormal function of VD in response to chronic stress. It has been suggested that ROS were responsible for impaired adrenergic neurotransmission and abnormal function of VD (Güneş et al. 2005). An earlier report also showed a significant correlation between oxidative stress level and VD contractility (Tsounapi et al. 2018). In the current study, we also demonstrated that UCMS lead to oxidative stress which may be the main reason for the impairment of the contractility of VD. Moreover, RS and QE reversed the inhibitor effect of chronic stress on EFS-induced contractile responses of the VD in the absence or presence of prazosin or suramin, indicating that these agents protected peripheral adrenergic nerves in the VD against chronic stress-induced oxidative stress. Also, treatment with RS or QE significantly restored both noradrenalin- and ATP-induced contractile responses in VD of chronically stressed rats. These protective effects of RS and QE on the nerve-evoked, noradrenaline-, or ATP-induced contractile responses of the VD could be attributed to their scavenging capacity of free radicals and ability to induce the production of intracellular antioxidants as we reported here. Besides, protective effects of RS and QE on VD

function may be due to their anti-inflammatory effects on VD in chronically stressed rats. It is well known that RS and QE possess both antioxidant and anti-inflammatory properties. Therefore, they participate in a number of biological processes providing protection to various tissues and organs against oxidative stress and inflammation induced by different pathologies, including sexual and reproductive dysfunctions (Adedara et al. 2017; Adefegha et al. 2018; Yazir et al. 2018; Fahim et al. 2019). Thus, considering the role of VD in ejaculatory process, we suggest here that RS and QE may have beneficial effects on ejaculatory dysfunctions associated with chronic stress.

In summary, present study demonstrated the impaired VD contractility in response to EFS, adrenergic, or purinergic agonist in chronically stressed rats. Treatment with RS and QE protected against chronic stress-induced VD dysfunction by their antioxidant and anti-inflammatory effects on VD, suggesting that oxidative stress and inflammation may be synergistic parts in the development of VD dysfunction associated with chronic stress. Antioxidant and/or anti-inflammatory therapy may be considered to be a therapeutic choice in ejaculatory disorders related with chronic stress-induced depression.

**Authors' contribution** TDŞ conceived and designed the study. TDŞ, SSG, and GD performed experiments. TDŞ, SSG, GD, YY, and TU analyzed data. TDŞ wrote the manuscript. All authors read and approved the manuscript.

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## Compliance with ethical standards

Kocaeli University Animal Research Ethics Committee approved all experiments, and all procedures and processes in this study were conducted as set out by the National Institutes of Health Guidelines on the use of laboratory animals.

**Conflict of interest** The authors declare that they have no conflict of interest.

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