Effects of resveratrol on intestinal oxidative status and inflammation in heat-stressed rats

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ABSTRACT

Heat stress, experienced by humans and animals under high ambient temperatures, is known to induce oxidative stress and inflammation, which endangers human health as well as animal welfare and production. The gastrointestinal tract is predominantly responsive to heat stress and compromised intestinal functions can contribute to multi-organ injury under heat environment. Resveratrol (RSV) has significant antioxidant and anti-inflammatory activities. The aim of this study was to investigate the potential effects of RSV on intestinal function (digestion and barrier), oxidative stress and inflammation in heat-stressed rats. Male Sprague-Dawley rats were orally fed with 100 mg RSV/kg body weight/day prior to daily heat stress (40 °C per day for 1.5 h) exposure for 3 consecutive days. The results showed that RSV reversed the increased serum cortisol level and diamine oxidase activity, the altered jejunal morphology, the decreased jejunal disaccharidase activities, the elevated malondialdehyde and tumor necrosis factor alpha concentrations and antioxidant enzymes activities in the jejunum, as well as the increased jejunal mRNA expression of toll-like receptor 4, cytokines, antioxidant enzymes and tight junction proteins in heat-stressed rats, to various degrees. In conclusion, RSV could alleviate intestinal injury and dysfunctions by improving oxidative status and suppressing inflammation in heat-stressed rats.

1. Introduction

Humans and animals who experienced considerable heat stress (HS) are susceptible to acute, chronic, and lethal illnesses. In 2003, about 30,000 deaths in Europe are due to the heat-wave event (Kovats and Kristie, 2006). On average, estimates of heat-related deaths range from 170 to 690 in the USA each year (NWS, 2011). Additionally, in 2006, a major heat wave moving across the USA caused the death of 2500 cattle and 700,000 poultry in California (Nienaber and Hahn, 2007). Moreover, the incidence of the deaths related to HS all the over world is increasing at a rapid rate with the increasing global warming and increase in frequency and intensity of HS (Bouchama and Knochel, 2002). The gastrointestinal tract is predominantly responsive to HS, and the dysfunction of intestine is regarded as an important early symptom of thermal stress. Experimental studies showed that HS leads to serious intestinal injury and dysfunctions evidenced by histological changes (He et al., 2015; Yi et al., 2016; Song et al., 2018), abnormal tight junction (TJ) proteins expression and redistribution (Pearce et al., 2013), and digestion and absorption dysfunction (Yi et al., 2016; Song et al., 2018). It is well known that the critical role of intestine is to digest and absorb nutrients, while it simultaneously provides organisms with the first protective barrier (Yu et al., 2013). Once heat-induced intestinal dysfunctions occurs, the toxic luminal substances invade into blood circulation due to the increased intestinal permeability, which may contribute to multiple organs malfunction and even death (Hall et al., 2001). Obviously, a compromised intestinal mucosa is pivotal to the development of HS-related illnesses (Pearce et al., 2013). Oxidative stress (OS) and inflammation have been demonstrated to play a critical role in HS-induced intestinal damage and dysfunctions (Yu et al., 2013; He et al., 2015). Previous studies in animals and cell culture models have reported that phytochemicals from natural products such as ferulic acid (He et al., 2016, 2019) and *schisandra chinensis* (Kim et al., 2012) protect against HS-induced organs/tissues damage partly through suppression of OS.

Resveratrol (RSV), trans-3, 4′, 5 trihydroxystilbene, exhibits numerous biological functions (e.g., anti-aging, hypoglycemia and hypolipidemia) and can be find in many plants (e.g., peanuts, grapes and bilberries) (Charytoniuk et al., 2017; Cheng et al., 2019a; Truong et al., 2018). These preventive and/or therapeutic effects of RSV have been demonstrated in pre-clinical trials such as streptozotoncin-nicotinamide-injected diabetic rats (Palsamy and Subramanian, 2011) and high-fat diet-induced hepatic steatosis of mice (Cheng et al., 2019b), and

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which could partly be attributed to its antioxidant and anti-inflammation properties. In a recent report, Das (2011) found that RSV can prevent HS-induced hepatotoxicity in rats by enhancing antioxidant capacity in the liver. Our previous study also found that RSV supplementation is capable of protecting hepatic dysfunction in heat-stressed rats via sustaining the balance of redox and immune status (Cheng et al., 2019c). Considering the critical role of intestine in HS-related multi-organ injury and the excellent antioxidant and anti-inflammatory activities of RSV, therefore, we hypothesized that oral RSV administration might protect the intestinal functions against the HS-mediated effects on oxidative stress and inflammation. In recent years, the rat has regarded as a highly relevant model for exploring the pathophysiology of hyperthermia stress in humans and for understanding the mechanisms of heat-related intestinal damage (Oliver et al., 2012; Xiao et al., 2015). Meantime, studying the effects of RSV on the HS rat model is of great reference value for its application in livestock production for feed efficiency improvement under HS. Therefore, in this study, the rat model was used to assess the potential protective effects of RSV on intestinal digestion, barrier, OS and inflammation under the high HS condition.

2. Methods and materials

2.1. Animals and experimental design

All animals' procedures in the experiment were allowed by the Institutional Animal Care and Use Committee of Nanjing Agricultural University (Nanjing, China). Male Sprague-Dawley rats (ages, 8 weeks; initial body weight, 200 ± 20 g) were purchased from Qinglongshan Animal Breeding Farm (Nanjing, China). Rats were received with standard chow diet and tap water *ad libitum*, and maintained under the condition of controlled temperature (22 ± 2 °C), humidity (50 ± 10%), and light (12-h light/12-h dark cycle, lights on at 7 a.m.). The acclimation period of rats was 1 week prior to the experiment. Then, rats with similar weight were assigned into 3 groups (n = 8, 4 rats per cage (40 × 30 × 18 cm)): (1) the control (CON) group, (2) the HS group, and (3) the HS-RSV group. The HS treatment was set up with slight modification according to the previous studies (Lu et al., 2011; Yu et al., 2011). Briefly, rats in the HS and HS-RSV group were exposed to an incubator at 40 °C for 1.5 h from 11:30 to 13:00 daily for 3 consecutive days. Rats in the CON group were kept in the controlled temperature (22 ± 2 °C). Two hours before daily HS exposure, rats in the HS-RSV group were received with 100 mg RSV/kg body weight/day (purity 99%; TCI Co., Ltd., Tokyo, Japan; diluted in 0.5% carboxymethylcellulose sodium (CMC-Na)) by oral administration, while the CON and HS group were orally fed with same volume of 0.5% CMC-Na (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China; diluted in 0.86% saline). The dose of RSV used in the experiment was chosen on the basis of previous studies in which RSV at the same dose was effective against oxidative stress and/or inflammation in different mice (Cheng et al., 2019a, Cheng et al., 2019b) and rats (Zheng et al., 2012) models.

2.2. Sample collection

On the third day of this experiment, all rats were anesthetized and sacrificed immediately after the termination of heat treatment. Blood of each rat was collected through eyeballs and centrifuged at 2000 g (4 °C, 15 min) to obtain the serum. The serum was stored at −80 °C until analysis. In a previous study, the most serious damage in the jejunum of heat-stressed rats was observed on the third day (Lu et al., 2011). Therefore, this study focused on the beneficial effects of RSV on HS-induced jejunal injury of rats. The jejunum sample collection was conducted according to the method of Lu et al. (2011). Each jejunal section was divided into two parts: a 2 cm-segment was fixed in 4% buffered paraformaldehyde for histological measurement; the rest was

immediately snap frozen in liquid nitrogen for subsequent analysis.

2.3. Serum cortisol level and diamine oxidase (DAO) activity assay

For the serum cortisol level, the competitive enzyme-linked immunosorbent assay (ELISA; Multisciences Biotech Co., Ltd, Hangzhou, China) was performed according to the instructions of the manufacturer. The detection limit was 66.43 pg/mL for cortisol, and the inter- and intra-assay coefficients of variation were less than 5% and 8%, respectively. The DAO activity in the serum, an intestinal integrity indicator (Song et al., 2017), was quantified according to the method as described by Song et al. (2017) using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Histological analysis

The fixed intestinal sample (jejunum) was dehydrated and embedded in paraffin. Five-μm section was cut and then stained with hematoxylin and eosin. Ten well-oriented, intact villi and their associated crypts per rat were selected, and images were collected using an optical binocular microscope (Olympus BX5; Olympus Optical Co. Ltd, Tokyo, Japan) equipped with a digital camera (Nikon H550L; Nikon, Tokyo, Japan). Measurement of histological parameters including villus length, crypt depth, and villus width was detected using the Image-Pro Plus software (version 6.0, Media Cybernetics, Inc., Rockville, MD, USA). Villus: crypt ratio and villous surface area were calculated according to Dong et al. (2014). The same investigator was blinded to perform intestinal morphology analysis in the experiment.

2.5. Disaccharidase activities determination

Following the manufacturer's protocol, the jejunum mucosal lactase, sucrose and maltase activities were measured using assay kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All results were normalized to the total protein concentration in each sample for inter-sample comparison. The jejunal protein concentration was detected according to the Bradford (1976) method.

2.6. Redox status assay

The jejunal glutathione peroxidase (GPX) and total superoxide dismutase (T-SOD) activities, malondialdehyde (MDA) concentration, and total antioxidant capacity (T-AOC) level were analyzed using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. All results were normalized to the total protein concentration in each sample for inter-sample comparison. The jejunal protein concentration was detected according to the Bradford (1976) method.

2.7. Cytokine analysis

The tumor necrosis factor alpha (TNF-α) concentration in the jejunum was analyzed by an ELISA kit (Beijing 4A Biotech Co., Ltd, Beijing, China), as described by the manufacturer's instructions. The minimum detectable concentration of TNF-α was 15 pg/mL, the intra- and inter-assay coefficients of variation were less than 10%. All results were normalized to the total protein concentration in each sample for inter-sample comparison. The jejunal protein concentration was detected according to the Bradford (1976) method.

2.8. mRNA expression analysis

The mRNA expression of jejunum was detected according to the method previously described (Cheng et al., 2016). In brief, total RNA isolated from the jejunum using TRIzol Reagent (TaKaRa, Dalian, China) according to the protocols of the manufacturer. The RNA

Table 1
Primer sequences used for qRT-PCR.

Gene	Gene bank ID	Primer sequence, sense/antisense	Length (bp)
HSP70	NM_153629.1	TCAGAGCTGCTATGTCGCTG	73
		GCAGCGGTCGCTATACTCAT	
Ccl2	NM_031530.1	CAGGTCTCTGTCACGCTTCT	87
		GGCATTAACTGCATCTGGCTG	
TNF-α	NM_012675.3	AACACACGAGACGCTGAAGT	93
		TCCAGTGAGTTCCGAAAGCC	
IL6	NM_012589.2	ACAAGTCCGGAGAGGAGACT	172
		TTCTGACAGTGCATCATCGC	
IL10	NM_012854.2	TGCGACGCTGTCATCGATTT	186
		GTAGATGCCGGGTGGTTCAA	
TLR4	NM_019178.1	TCCACAAGAGCCGGAAAGTT	126
		TGAAGATGATGCCAGAGCGG	
ZO1	NM_001106266.1	GCCAGCTTTAAGCCTCCAGA	144
		TGGCTTCGCTTGAGGTTTCT	
CLDN1	NM_031699.2	GCTGTCATCGGGGGCATAAT	136
		CCTGGCCAAATTCATACCTGG	
CLDN2	NM_001106846.2	CGAGAAAGAACAGCTCCGTTT	100
		GTGTCTCTGGCAAGCTGACT	
CLDN3	NM_031700.2	TGGGAACTGGGTTGTACGTG	106
		TTGGTGGGTGCGTACTTCTC	
OCLN	NM_031329.2	GATCTAGAGCCTGGAGCAACG	166
		ATTGGGTTTGAATTCATCCGGC	
SOD1	NM_017050.1	GCATGGGTTCCATGTCCATC	127
		CAGGTCTCCAACATGCCTCTC	
GPX1	NM_030826.4	GCTCACCCGCTCTTTACCTT	162
		TGGAACACCGTCTGGACCTA	
Nrf2	NM_031789.2	TTTGTAGATGACCATGAGTCGC	142
		TGTCCTGCTGTATGCTGCTT	
Keap1	NM_057152.2	TGTGCTGCATGTGATGAACG	198
		AAGAACTCCTCCTCCCCGAA	
β-actin	NM_031144.3	GCAGGAGTACGATGAGTCCG	74
		ACGCAGCTCAGTAACAGTCC	

Ccl2, C–C motif chemokine ligand 2; CLDN1, claudin 1; CLDN 2, claudin 2; CLDN 3, claudin 3; GPX1, glutathione peroxidase 1; HSP70, heat shock protein 70; IL6, interleukin 6; IL10, interleukin 10; Nrf2, nuclear factor, erythroid 2-like 2; SOD1, superoxide dismutase 1; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor alpha; OCLN, occludin; ZO1, zonula occludens 1; Keap1, Kelch-like ECH-associated protein 1; β-actin, beta actin.

integrity was checked on 1% agarose gel with ethidium bromide staining. The RNA concentration and purity were determined from OD260/280 readings (ratio > 1.8) using a spectrophotometer (Nano-Drop 2000c; Thermo Scientific, USA). After then, total RNA (1 μg) was reverse-transcribed into cDNA using the PrimeScriptTM RT Reagent Kit (TaKaRa, Dalian, China) according to the guidelines of the manufacturer.

The primer of C–C motif chemokine ligand 2 (Ccl2), claudin 1 (CLDN1), CLDN2, CLDN3, GPX1, heat shock protein 70 (HSP70), interleukin 6 (IL6), IL10, nuclear factor, erythroid 2-like 2 (Nrf2), SOD1, toll-like receptor 4 (TLR4), TNF-α, occludin (OCLN), zonula occludens 1 (ZO1), Kelch-like ECH-associated protein 1 (Keap1), and beta actin (β-actin) are given in Table 1. The qRT-PCR was performed with a SYBR Green qPCR Kit (Vazyme, Nanjing, China) on an Applied Biosystems 7500 Real-Time PCR System (Life Technologies). The PCR reaction mixture contained 2 μL of cDNA, 0.4 μL of forward primer, 0.4 μL of reverse primer, 10 μL of SYBR Premix Ex Taqe (Vazyme, Nanjing, China), 0.4 μL of ROX Reference Dye (Vazyme, Nanjing, China), and 6.8 μL of double-distilled water. The PCR consisted of a pre-run at 95 °C for 30 s and forty cycles of denaturation at 95 °C for 5 s, followed by a 60 °C annealing step for 30 s. The condition of the melting curve analysis were as follows: one cycle of denaturation at 95 °C for 10 s, followed by an increase in temperature from 65 to 95 °C at a rate of 0.5 °C/s. Each sample was run in duplicate and melt curve analysis was performed to validate the specificity of the PCR-amplified product. After normalization against the housekeeping gene β-actin, the relative levels of mRNA expression of target genes were calculated via the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The values of the CON group were used as a calibrator.

2.9. Statistical analysis

Data were analyzed by using SPSS statistical software (version 17.0, SPSS Inc., Chicago, IL). The rat was used as the experimental unit. Statistical differences between different groups were determined via one-way analysis of variance and Tukey's post hoc test for multiple comparisons when F was significant. Difference were considered significant at $P < 0.05$, and P values between 0.05 and 0.1 were considered a trend. Data are expressed as means and standard error.

3. Results

3.1. Serum cortisol level and DAO activity

In Fig. 1A and B, heat exposure in the HS group significantly increased ($P < 0.05$) the cortisol level and the DAO activity in the serum of rats compared with the CON group. However, administration of RSV to heat-stressed rats inhibited ($P < 0.05$) the serum cortisol level and DAO activity in the HS-RSV group compared with the HS group.
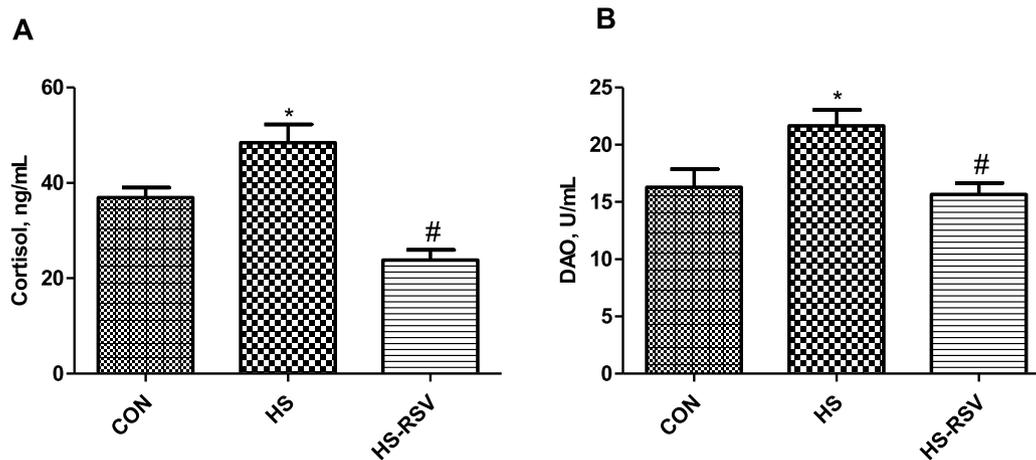

Fig. 1. The serum (A) cortisol level and (B) diamine oxidase (DAO) activity in rats. CON, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol (RSV)/kg body weight/day and exposed to heat treatment. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as *$P < 0.05$ when compared with the CON group, #$P < 0.05$ when compared with the HS group.
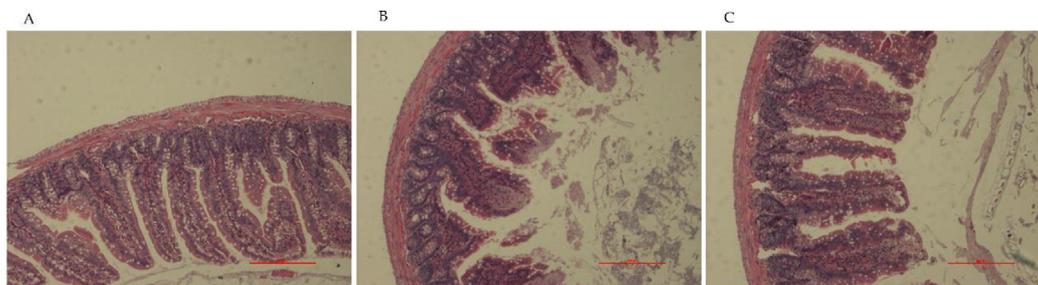
Fig. 2. The jejunum histological appearance of rats (hematoxylin and eosin). (A) CON, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to normal condition; (B) HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to heat treatment; (C) HS-RSV, rats were orally fed with 100 mg resveratrol (RSV)/kg body weight/day and exposed to heat treatment. Original magnification 100 × , Scale bars = 100 μm.

3.2. Morphology

As showed in Fig. 2, the CON group had normal jejunal morphology, while jejunal damage (i.e., villous atrophy and shedding) was observed in the HS group. Interestingly, RSV treatment partly improved the histological morphology of jejunum in the HS-RSV group. Rats exposed to HS have lower ($P < 0.05$, Table 2) villus height, villus height/crypt depth and villous surface area in the jejunum than that of the CON group. Supplementation of RSV to heat-stressed rats led to the increased villus height/crypt depth in the HS-RSV group compared with the HS group ($P = 0.09$). No differences were observed in the crypt depth and villus width in the jejunum of rats among 3 groups ($P > 0.05$).

3.3. Disaccharidase activities

The maltase activity was lower ($P < 0.05$, Fig. 3C) and the lactase activity tended to be lower ($P = 0.054$, Fig. 3B) in the jejunum of the HS group compared with the CON group. The activity of jejunal sucrose was higher ($P < 0.05$, Fig. 3A) in the HS-RSV group compared with the HS group.

3.4. Redox status

Compared with the CON group, in the jejunum, the activities of GPX (Fig. 4B) and T-SOD (Fig. 4C), and the MDA (Fig. 4A) concentration were greater ($P < 0.05$) in the HS group. In contrast, the T-SOD activity and the MDA content were decreased ($P < 0.05$) by RSV administration in the HS-RSV group compared with the HS group, while the GPX activity was not significantly decreased ($P = 0.087$). There was no difference in the jejunal T-AOC level among 3 groups ($P > 0.05$, Fig. 4D).

Table 2
The jejunal morphology in rats.

Item	CON	HS	HS-RSV
Villus height (μm)	183.29 ± 9.29	142.66 ± 9.68*	160.66 ± 9.24
Crypt depth (μm)	71.67 ± 4.45	80.27 ± 5.73	76.69 ± 4.39
Villus height/crypt depth (μm/μm)	2.58 ± 0.13	1.79 ± 0.08*	2.10 ± 0.08
Villus width (μm)	68.64 ± 4.91	57.60 ± 3.12	58.27 ± 3.24
Villous surface area (× 10³μm²)	20.37 ± 2.34	13.29 ± 1.43*	15.05 ± 1.38

CON, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to heat treatment; HS-RSV, rats were orally fed with 100 mg RSV/kg body weight/day and exposed to heat treatment. Results are expressed as mean and standard error, n = 6. Significant difference is depicted as *$P < 0.05$ when compared with the CON group.

3.5. Cytokine level

As exhibited in Fig. 5, administration of RSV to the HS-RSV group inhibited ($P < 0.05$) HS-induced enlargement of TNF-α production in the jejunum.

3.6. mRNA expression

In the jejunum, the mRNA expression of HSP70, Ccl2, IL10, TLR4, CLDN3, OCLN, SOD1, GPX1, and Nrf2 were markedly higher in the HS group compared with the CON group ($P < 0.05$, Fig. 6). The jejunal HSP70, Ccl2, TNF-α, IL10, TLR4, OCLN, SOD1, GPX1 and Nrf2 gene transcriptional expression were lower ($P < 0.05$) in the HS-RSV group compared with the HS group. No differences ($P > 0.05$) were observed between the experimental groups with regard to the mRNA abundance of IL6, ZO1, CLDN1, CLDN2 and Keap1 in the jejunum.

4. Discussion

HS, experienced by humans and animals under high ambient temperatures, is known to induce oxidative stress (Yu et al., 2013) and inflammation (He et al., 2015), which plays an important role in compromised intestinal functions. In this study, we found for the first time that RSV reduced intestinal heat stress response and improved intestinal digestion and barrier function in heat-stressed rats evidenced by the reduced serum cortisol level and intestinal HSP70 gene expression, the increased sucrose activity and villus height/crypt depth, and the decreased serum DAO activity. In the present study, RSV protected HS-induced intestinal dysfunctions by suppressing OS and inflammation, which indicated by the reduced MDA and TNF-α concentrations, and the down-regulated mRNA expression of TLR4 and cytokines.

In response to HS, there are a wide range of physiological events, which includes endocrine changes such as the increased circulating cortisol concentration (Wang et al., 2015), and molecular chaperones induction such as HSPs overexpression (Song et al., 2017). Therefore, these changes are always considered as the most important indicators in studying HS (Serra et al., 2005; Song et al., 2017). In the present study, rats subjected to HS showed higher serum cortisol level and jejunal HSP70 mRNA expression. The current results fit well with the changes in the serum cortisol and jejunal HSP70 production in the previous study (Hou et al., 2017), indicating that the HS rat model was effectively established in this study. The cells in intestinal epithelium not only secrete disaccharidase to exert digestion, but also form the mechanism barrier with TJ proteins to prevent the penetration of luminal harmful substances (Xiao et al., 2015). In the present study, histological morphology, disaccharidase activities and TJ proteins expression in the jejunum of heat-stressed rats were irregularly altered. In addition, we also found that HS increased the serum DAO level in rats, an indicator of intestinal permeability and mucosal injury (Song et al., 2017). These results suggested that the HS-induced abnormal intestinal functions and increased permeability occurred. We can conclude that HS jeopardizes
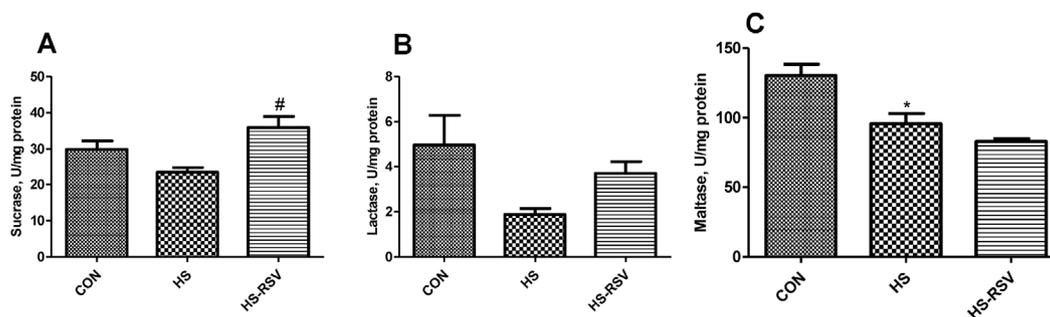
Fig. 3. The jejunal disaccharidase activities in rats. CON, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol (RSV)/kg body weight/day and exposed to heat treatment. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as *$P < 0.05$ when compared with the CON group, #$P < 0.05$ when compared with the HS group.

the health and functions of intestine and results in intestinal damage which matched with enormous studies have mainly focused on the effects of HS on intestine (Hall et al., 2001; Pearce et al., 2013; Yu et al., 2013; Yin et al., 2015; He et al., 2016; Song et al., 2017, 2018). Expectedly, administration of RSV reduced the heat stress response and relieved the HS-mediated jejunal injury and malfunctions of rats. At present, little is known about whether RSV could reduce the impacts of HS on intestine. Only a recent report in heat-stressed broilers found that diet supplemented with RSV improves the impairment of intestinal morphology, microflora, and barrier integrity (Zhang et al., 2017b). One possible explanation for the RSV's beneficial role in intestinal damage and dysfunctions of heat-stressed rats in the present study is the inhibited OS and inflammation.

The stimulation of OS by the disequilibrium between reactive oxygen species (ROS) production and antioxidants levels, which damages biomacromolecules (i.e., DNA, proteins and lipids) and eventually results in cell dysfunction and even death (Cheng et al., 2017). The previous study showed that the occurrence of OS was characterized by the higher levels of MDA and antioxidants (Kim et al., 2012). In the

present study, we found that the jejunal MDA content and antioxidant enzymes (i.e., T-SOD and GPX) activities were higher in the HS group compared with the CON group. Thus, in this study, OS had been demonstrated to happen in the jejunum of rats exposed to HS, which could lead to intestinal injury and dysfunctions. Similar studies in mice (Oliver et al., 2012) and IEC-6 cells (He et al., 2019) confirm that hyperthermia exposure causes the intestinal epithelial barrier dysfunction that be partly attributed to OS. In addition, Nrf2, a prime molecular target against oxidative stress, plays a critical role in protecting against cell damage through the enhancement of Nrf2-related antioxidants levels. We also observed that Nrf2 and its target genes (SOD1 and GPX) were increased in the jejunum of rats by HS exposure, which may explain the results of antioxidant enzymes activities. It is reasonable to speculate that the increased Nrf2 and its related antioxidants genes expression could be associated with a response of adaptation in the body triggered by HS, which has been confirmed extensively in the previous studies (Zhang et al., 2002; Bhusari et al., 2008). Interestingly, RSV treatment decreased the MDA concentration and the T-SOD activity, and downregulated the mRNA expression of Nrf2, SOD1 and
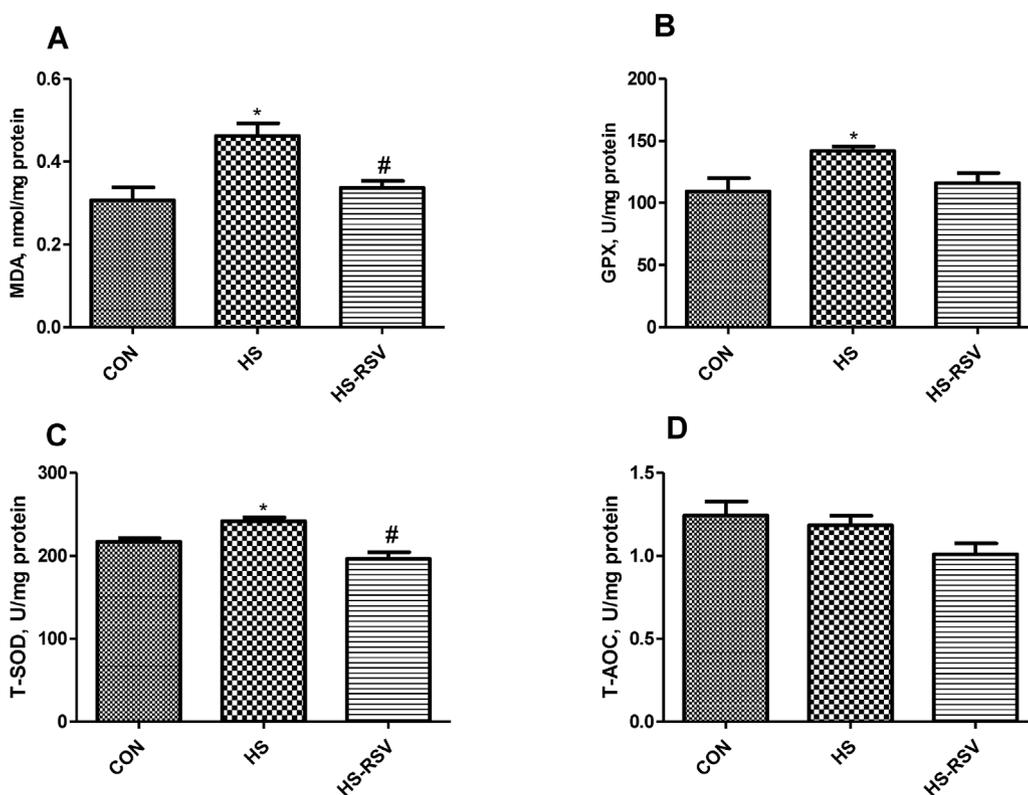

Fig. 4. The jejunal redox status in rats. (A) MDA, malondialdehyde; (B) GPX, glutathione peroxidase; (C) T-SOD, total superoxide dismutase; (D) T-AOC, total antioxidant capacity; CON, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol (RSV)/kg body weight/day and exposed to heat treatment. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as *$P < 0.05$ when compared with the CON group, #$P < 0.05$ when compared with the HS group.
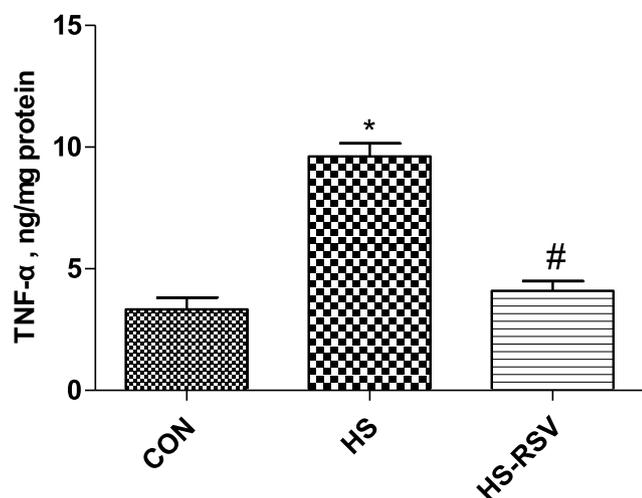
Fig. 5. The jejunal tumor necrosis factor alpha (TNF-α) concentration in rats. CON, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol (RSV)/kg body weight/day and exposed to heat treatment. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as *$P < 0.05$ when compared with the CON group, #$P < 0.05$ when compared with the HS group.

GPX1 in the HS-RSV group compared with the HS group. It is not hard to find in this study that RSV improved HS-induced OS in the jejunum but not through increasing the antioxidant capacity. However, Das (2011) found that administration of RSV reduced the hepatic MDA content and improved the liver function in heat-stressed rats by elevating the activities of SOD, GPX and catalase. Zhang et al.(2017a, 2018), 2018) also found that dietary supplementation of RSV reversed the impaired antioxidant capacity in muscle and spleen of heat-stressed broilers. The dosage and duration of RSV applied in studies and tissues specificity may explain the different modulation mechanism of RSV in reducing HS-mediated OS in different tissues. Additionally, the time of heat exposure and animal species may contribute to different responses of oxidative status in tissues. In the present study, we reckon that the beneficial role of RSV in intestinal inflammation may be responsible for decreased OS because the overproduction of pro-inflammatory cytokines can drive a rise in ROS under HS exposure (Yun et al., 2012).

The initiation of inflammation is activated due to the imbalance between pro- and anti-inflammatory cytokines. Studies have demonstrated that the excessive production of pro-inflammatory cytokines such as TNF-α under heat exposure can contribute to hemorrhage and necrosis in organs including intestine (Bouchama and Knochel, 2002).
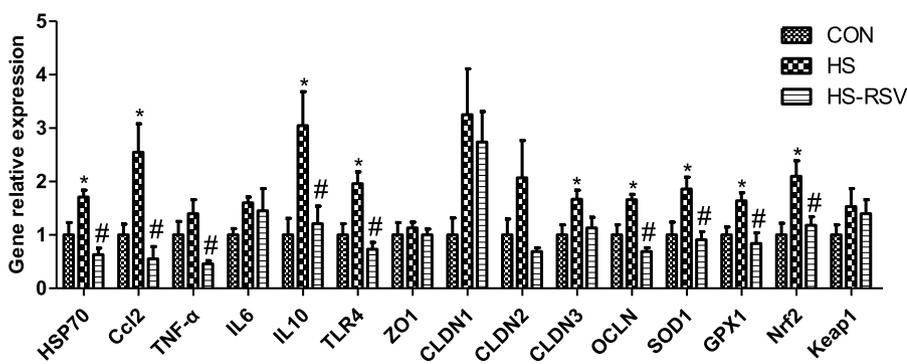
In the present study, the jejunal TNF-α protein level, and TLR4, IL-10 and Ccl2 mRNA abundant were higher in the HS group compared with the CON group, indicating that HS led to inflammation in the jejunum of rats. TLR4, a stress-related biosensor in initial injury response (Mollen et al., 2006), stimulation of which activates pro-inflammatory pathways and induces cytokines (e.g., TNF-α) and chemokines (e.g., Ccl2) productions in various cell types (Shi et al., 2006). The increased TLR4 mRNA expression in the present study may account for the jejunal inflammation induced by HS. Expectedly, RSV administration inhibited the TLR4 and cytokines expression in the HS-RSV group compared with the HS group. Similar results were noted in high-fat diet-induced renal injury (Cheng et al., 2019a) and osteoarthritis (Jiang et al., 2017) of mice, in which oral RSV administration inhibited inflammation in these tissues partly through the down-regulation of TLR4 expression at transcriptional level. Previous studies also reported that RSV inhibited intestinal inflammation in dextran sodium sulfate-induced colitis (Larrosa et al., 2009; Yao et al., 2010) and ischemia/repefusion injury (Petrat and de Groot, 2011) rat model. Using post-septic mice model, TLR4 deficiency improves immune paralysis by regulating the regulatory T cells activity and restoring a pro-inflammatory cytokine balance (Cao et al., 2018), suggesting that modulation of TLR4 may be an effective approach to prevent immune dysfunctions. Therefore, in this study, RSV may also suppress the HS-induced jejunal inflammation via TLR4 inactivation, which may be beneficial to the reduction of OS.

In addition, in the present study, it is worth noting the effects of RSV and HS on TJ proteins expression in the jejunum. TJ proteins are composed of multiple proteins which includes transmembrane proteins (CLDN and OCLN) and peripheral membrane proteins (ZO) (Aijaz et al., 2006; He et al., 2016). These proteins play an important role in the integrity of the intestinal barrier. Increasing evidence have demonstrated that HS disrupts the TJ and increases the permeability of intestine (Xiao et al., 2015; He et al., 2016, 2019). However, in this study, the mRNA expression of TJs including CLDN3 and OCLN in the jejunum were increased by HS exposure, which may be a self-adaptive protective mechanism in the organism. Interestingly, RSV reduced the HS-mediated upregulation of the OCLN gene. Although the corresponding proteins expression levels were not determined in the present study, the results of the serum DAO measurement confirmed the beneficial effects of RSV on the jejunal integrity under HS exposure. In this study, we can infer that the RSV-induced alteration of the TJ protein is due to the alleviation of the HS response. A recent report showed that RSV augmented the deoxynivalenol (DON)-induced upregulation of CLDN4 at transcriptional level, while it had no protective effects on the reduced CLDN4 protein expression (Ling et al., 2016). Although the protein level of CLDN4 was not increased, they found that RSV protects against the DON-induced intestinal barrier dysfunction in the IPEC-J2 cell line by promoting the assembly of CLDN4 in the TJ complex through the modulation of IL6 and IL8 secretion. Evidence has also showed that, in addition to its' expression levels, the post-translational modification of

Fig. 6. Resveratrol affects gene expression involved in inflammation, oxidative stress and tight junction proteins in the jejunum of heat-stressed rats. Ccl2, C–C motif chemokine ligand 2; CLDN1, cloudin 1; CLDN2, cloudin 2; CLDN3, cloudin 3; GPX1, glutathione peroxidase 1; HSP70, heat shock protein 70; IL6, interleukin 6; IL10, interleukin 10; Keap1, Kelch-like ECH-associated protein 1; Nrf2, nuclear factor, erythroid 2-like 2; SOD1, superoxide dismutase 1; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor alpha; OCLN, occluding; ZO1, zonula occludens 1; CON, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and exposed to heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol (RSV)/kg body weight/day and exposed to heat treatment. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as *$P < 0.05$ when compared with the CON group, #$P < 0.05$ when compared with the HS group.

TJ proteins at the site of cell-cell contacts is also important for the role of it in the intestinal barrier function (Lui and Lee, 2006). Pro-inflammatory cytokines (Ling et al., 2016) and ROS (Carrasco-Pozo et al., 2013) have been reported to disrupt the TJ barrier under pathological conditions. Therefore, in the present study, the beneficial effects of RSV on the intestinal barrier function are associated with the suppression of inflammation and OS. Of course, the molecular mechanism of RSV in the TJ proteins interaction (redistribution and disassembly) between adjacent enterocytes in the HS rat model need to be further investigated in the future.

In summary, the present study demonstrated that RSV could alleviate intestinal injury and dysfunctions in heat-stressed rats through the suppression of inflammation and the improvement of oxidative status, suggesting that oral RSV may be a new therapeutic strategy to reduce the adverse effects of HS. Of course, deeper understandings of the action mechanism of RSV in the heat-stressed animal model need to be explored in the future.

Acknowledgments

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