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Curcumin inhibits heat-induced oxidative stress by activating the MAPK-Nrf2 / ARE signaling pathway in chicken fibroblasts cells

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ABSTRACT

Curcumin is a natural phenolic component of yellow curry spice, exhibits antioxidant and anti-inflammatory properties. In this study we investigated whether curcumin suppresses heat-induced apoptosis in chicken embryonic fibroblast cells (CEF) and the underlying mechanism.

CEF cells line was divided into 6 groups (4 repetitions per group) including normal temperature group (NC), high temperature control group (H) and 4 experimental groups (H1(5 μmol/L), H2(10 μmol/L), H3(20 μmol/L) and H4(40 μmol/L)). Control groups were cultured in basic medium without Curcumin, while, the experimental groups were supplemented with 5, 10, 20 and 40 μmol/L, respectively. The experimental groups and H control group were cultured at 43 °C (95% air/5% CO_2), whereas NC group cells were cultured at 37 °C. After 6, 12 and 24 h of culture, cells were collected for viability, proliferation, apoptosis, antioxidant status and gene expression analysis.

Results showed that heat stress trigged the ROS production and induced the apoptosis, leading to decrease the cell viability and proliferation. The enzymatic activities of antioxidants (SOD, CAT, and GPX) were down-regulated. The expression of antioxidant enzyme (CAT, SOD1, SOD2, GSTO1, GSTT1 and GSTA3) and MAPK-Nrf2 pathway genes (Nrf2, Jnk, Erk and P38) were down-regulated under heats stress condition. While, the Curcumin treated groups had decreased ROS and MDA content. Down-regulation of the activity and expression of antioxidant enzyme induced by heat were also reversed by curcumin. Furthermore the up-regulation in expression of Nrf2, Jnk, Erk and P38 in supplemented groups revealed the involvement of MAPK-Nrf2 signaling pathway to alleviate oxidative stress induced by heat stress. This study demonstrates that curcumin has the ability to ease the oxidative damage through activating the MAPK-Nrf2 signaling pathway in CEF cells.

1. Introduction

Heat stress is considered as one of the most vital environmental stressors in animal husbandry, especially in poultry sector. It does not only compromise health status and welfare, but also adversely affects the hatchability, growth rate, feed intake, mortality, egg production, and other imperative traits governing the economic success of this industry (Habibian and Sadeghi, 2015). Under heat stress condition, hyperthermia associated biochemical and physiological events can potentially promote the generation of reactive oxygen species (ROSs) (Mujahid et al., 2009). Excessive levels of ROS result in the disturbance of balance between the oxidation and antioxidant defense systems, causing lipid peroxidation and oxidative damages to proteins, DNA, and other biological molecules (Yang et al., 2010), hence the oxidative stress in cells. Generally, cells maintain a balance level between ROSs formation and elimination. Under oxidative stress, the body protects itself against ROS prompted damage with enzymes, including catalases, lactoperoxidases, superoxide dismutases (SODs), glutathione peroxidases and peroxiredoxins (Michiels et al., 1994). Superoxide produced as the key ROS is transformed into hydrogen peroxide (H_2O_2) by SOD, which is then detoxified by glutathione peroxidase and catalase.

The key cause of these ROS is the oxidative phosphorylation at the mitochondria, and numerous enzymes may contribute to the generation of these toxic oxidants, hence the oxidative stress. However, most cells have developed complicated mechanisms to detoxify ROS by activation of a family of antioxidant/detoxification enzymes that boost cellular

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ROS scavenging capability in preserving cellular redox homeostasis and reducing oxidative damage (Copple et al., 2008). Number of studies has proved that oxidative stress triggers transcription of a variety of antioxidant genes directed by key signaling pathways involved in cellular oxidative responses (Sen and Packer, 1996). One of the main oxidative stress-related mechanisms identified in birds is the activation of Nrf2 (nuclear erythroid 2-related factor 2). Nrf2 is a basic region leucine-zipper transcription factor which binds to the antioxidant response element (ARE) and thereby regulates the expression of a large battery of genes involved in the cellular antioxidant and anti-inflammatory defense as well as mitochondrial protection (Nguyen et al., 2009). Nrf2 nuclear translocation needs the activation of numerous signal transduction pathways, including mitogen-activated protein kinases (MAPKs) (Torres and Forman, 2003). Hence, Nrf2 is a main transcriptional regulator of various detoxifying and antioxidant genes (Pi et al., 2008) and MAPKs plays a crucial role in transducing different extracellular signals to the nucleus (Johnson and Lapadat, 2002). MAPKs contain a family of protein kinases that play a crucial role in transmitting extracellular signals from the cell membrane to the nucleus (Boutros et al., 2008). To date, at least six MAPK members have been identified in mammalian cells, but there are three definite subdivisions of MAPKs: the extracellular signal regulated kinases (ERKs) (Gomez and Cohen, 1991), the c-Jun N-terminal kinases (JNKs) (Kyriakis et al., 1994), and the p38 MAPKs (Lee et al., 1994). The activity of Nrf2 to start the transcription of antioxidant genes is also positively regulated ERK2 and p38 MAPK (Zipper and Mulcahy, 2000).

During the past few years, a strong relationship has been proven between dietary supplementation and the reduction of damaging effects of heat stress. Curcumin [1,7-bis (4-hydroxy 3-methoxy phenyl)-1,6-heptadiene-3,5-Dione; Diferulylmethane] is the major active component of turmeric (*Curcuma longa*) and has been used for centuries in traditional medicines. Numerous lines of evidence indicate that curcumin is safe agent with many activities including antioxidant function, anti-inflammatory and antimicrobial properties (Sahin et al., 2012). The ROS scavenging action of curcumin can arise either from CH2 group of the β-diketone moiety or phenolic OH groups (Anand et al., 2008). Curcumin has widely been used in poultry to prevent the oxidative damage induced by heat stress (Zhang et al., 2015), but the mechanisms underlying are poorly understood. Based on the above contemplations, present study was done to explicate the mechanism of that supplemental curcumin prevent the oxidative damage induced by heat stress.

2. Material and method

2.1. Materials

HEPES and dimethyl sulfoxide (DMSO), MTT and Cell Counting Kit (CCK-8) were purchased from Sigma Aldrich Chemicals (St. Louis, MO, USA). Phosphate-Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA and penicillin-streptomycin were purchased from Gibco, Invitrogen (Carlsbad, CA, USA). Catalase (CAT), superoxide dismutase (SOD), Malondialdehyde (MDA) and reactive oxygen (ROS) kits kit were purchased from Nanjing Institute of Bioengineering, Nanjing, China. All other chemicals used were of high purity biochemistry grade.

2.2. Cell culture

Primary CEF cells were prepared using nine-day old Lohmann chicken embryos; the cells were trypsinized and collected by centrifugation. Primary CEF cells and land continuous CEF cell line were cultured in DMEM with 10%FBS at 37 °C in a humidified incubator under 5% CO_2 and 95% air. Upon confluence, the cells were purified via serial passages. Experimental cells were used in exponential phase and from passages 3–6. Cultured CEF cells plates were divided into 6 groups (4 repetitions per group) including normal temperature group (NC), high temperature control group (H) and 4 experimental groups (H1(5 μmol/L), H2(10 μmol/L), H3(20 μmol/L) and H4(40 μmol/L)). Experimental groups and H group were cultured at 43 ℃ (95% air/5% CO_2), whereas NC group plates were cultured at 37 °C. Experimental groups' culture medium was supplemented with 5, 10, 20 and 40 μmol/L of Curcumin, respectively.

2.3. Cell viability and proliferation assay

For measurement of cell viability and proliferation, CEF cells were cultured in 96-well culture plates at a density of 10^4 cells/cm^2, with the temperature experimental plates being transferred to the variable temperature cell culture incubator following 24 h of attachment. After 6, 12 and 24 h of heat stress treatment, cells were separately incubated in medium containing 1.25 mg/mL of the 10ul MTT salt, and 10 μl CCK-8, for 4 h and 2 h, respectively, at 37 °C. After an incubation period of 4 h at 37 °C, formazan was solubilized by adding DMSO. The optical densities were measured at 490 nm.

2.4. Analysis of apoptosis by annexin V-FITC/propidium iodide staining

Samples were labeled with annexin V-fluorescein isothiocyanate (FITC; BD Biosciences, San Diego, CA) and propidium iodide (PI) double staining according to the manufacturer's instructions. Briefly, CEF cells were cultured in 96-well culture plates at a density of 10^4 cells/cm^2, with the temperature experimental plates being transferred to the variable temperature cell culture incubator following 24 h of attachment. After 6, 12 and 24 h of heat stress treatment, cells were incubated in 500 μl of binding buffer with AnnexinV-FITC/PI in dark for 20 min, and following samples were analyzed by fluorescence microscope.

2.5. Antioxidant activity measurement

After heat stress treatment, CEF cells were washed twice with PBS. Following cells were incubated with 0.2%tritonx-100, at 4 ℃ for 30 min. Cells were ruptured with ultrasonic cell oscillators and SOD activity, CAT activity, MAD and ROS content were measured by using respective assay kit obtained from Nanjing Jiancheng Bioengineering Institute (strictly according to kit manufacturer protocol).

2.6. Quantitative real-time PCR

At 6, 12 and 24 h, the total RNA of cultured samples were extracted and the cDNA was synthesized by PrimeScript™ RT reagent kit (Takara) according to the manufacturer's instruction. qPCRs were performed with SYBR® Premix Ex Taq™ II (Takara) according to the manufacturer's instructions on StepOnePlus® RT PCR (applied biosystems). Each reaction was performed in three replicate samples. β- Actin gene was used as the internal control. The relative expression levels were calculated using $2^{-\Delta\Delta Ct}$. List of primers sequences are presented in Table 1.

2.7. Statistical analysis

SPSS (version 20.0) software was used to determine the difference between groups in the same time by T-test. $P < 0.05$ was for significant difference. All the results were expressed as means ± standard error formula for relative.

3. Results

3.1. Effect of different concentration of curcumin on CEF cell viability and proliferation

For measurement of cell viability and proliferation, CEF cells were

Table 1
List of primers.

Gene	Forward	Reverse	Length (bp)
GSTA3	TTGGATAAGGCCGCAAACAGATA	TTTCCAGTAAATGCACGTCTGCTC	115
GSTO1	CATGATGTGGCCCTGGTTTG	CAGTGCTGGAGCTTTGGAGTATGA	81
GSTT1	GACGGAGACTTCACCCTAGCAGA	TGATGGGTACCAGTGGTCAGGA	87
SOD1	TTGTCTGATGGAGATCATGGCTTC	TGCTTGCCTTCAGGATTAAAGTGAG	98
SOD2	CAGATAGCAGCCTGTGCAAATCA	GCATGTTCCCATACATCGATTCC	86
CAT1	ACCAAGTACTGCAAGGCGAAAGT	ACCCAGATTCTCCAGCAACAGTG	91
Nrf2	ATC ACG AGC CCT GAA ACC AA	GGC TGC AAA ATG CTG GAA AA	143
ERK	AGC AAG CTT TAG CCC ATC CA	CCT TCG GCA AGT CAT CCA AT	108
JNK	AGC AGC CTC GAT GCC TTG AC	CAA GCA ATT CAG GCC CAA TG	110
p38	TGT GTT CAC CCC TGC CAA GT	GCC CCC GAA GAA TCT GGT AT	149
β-actin	ATCCGGACCCTCCATTGTC	AGCCATGCCAATCTCGTCTT	120

Table 2
Effect of curcumin on the viability of chicken fibroblasts.

Parameter	Duration (h)	NC	H	H1 (5 μmol/L)	H2(10 μmol/L)	H3(20 μmol/L)	H4(40 μmol/L)
MTT (OD)	6	0.205 ± 0.023	0.176 ± 0.023 *	0.179 ± 0.007	0.204 ± 0.001	0.211 ± 0.031 *	0.204 ± 0.023
	12	0.231 ± 0.051	0.168 ± 0.036 *	0.165 ± 0.031 *	0.193 ± 0.027	0.209 ± 0.087	0.185 ± 0.020 *
	24	0.282 ± 0.006	0.216 ± 0.014 *	0.203 ± 0.005 *	0.224 ± 0.025	0.260 ± 0.062	0.226 ± 0.022

Values with * , within the row the numbers with * sign indicated significant difference at the 5% level. ($P < 0.05$).

treated MTT salt, and CCK-8. Table 2 lists the effect of different concentration of Curcumin on CEF cell viability. Results showed that Curcumin increased the cell viability compared to the H group. After 6 h of treatment the viability of group H2(10 μmol/L), H3(20 μmol/L), and H4(40 μmol/L) CEF was significantly higher compared to the H group. At 12 h the H2(10 μmol/L), H3(20 μmol/L), and H4(40 μmol/L) had improved CEF viability, but the difference was not significant. While, at 24 h, only H3(20 μmol/L) group had significantly improved cell viability compared to H group.

Table 3 lists the effect of different concentration of Curcumin on CEF cell proliferation. Results showed that Curcumin supplemented groups had improved cell proliferation rate compared to H group. The cell proliferation was significantly improved in H4(40 μmol/L) group compared to H group at 24 h, which was about near to the N control group.

3.2. Effect of different concentration of curcumin on CEF cell apoptosis

In order to examine the effect of different concentration of Curcumin on CEF Cell Apoptosis in vitro, Annexin V-FITC/Propidium Iodide Staining was performed on CEF cells exposed to the different of concentration of Curcumin at 6, 12 and 24 h. The results showed that the curcumin could reduce the cell apoptosis rate at 6, 12 and 24 h of treatment when more than 20 μmol/L Curcumin was added in culture media (Fig. 1A, B, C).

3.3. Effect of different concentration of curcumin on ROS production and MDA content

To examine heat stress-induced cell toxicity, intracellular ROS and MDA generation using ROS and MDA (Nanjing Jiancheng Bioengineering Institute, China) kit was analyzed. Table 4 lists the effect of different concentration of Curcumin on ROS production. Result showed that after 12 h in heat stress, the ROS content increased markedly in the cells. The ROS content in cells treated with 20 μmol/L and 40 μmol/L significantly decreased 13.34% and 19.14%.

Table 5 list the effect of different concentration of Curcumin on MDA content. Result showed that the content of MDA increased with the high temperature. Compared to H group, the supplemented groups had decreased MDA content, while the H4(40 μmol/L) had significantly decreased content, which was near to N group.

3.4. Effects of different concentrations of curcumin on antioxidant enzyme activity and genes expression

Table 6 list the effect of different concentration of Curcumin on antioxidant enzyme activity. Result showed that the activity of CAT was higher in supplemented groups compared to H group. At 12 h, the activity of CAT in H4(40 μmol/L) was significantly higher, while, at 24 h that difference was not significant. The H4(40 μmol/L) group had significantly higher SOD activity compared to H group at 12 h, while, at 24 h, the H3(20 μmol/L), H4 had significantly higher activity, compared to H and N group. Result showed that the H4(40 μmol/L) group, at 6, 12 and 24 h had significantly higher GSH-Px activity compared to H group.

The qPCR analysis showed that Curcumin up regulated the expression of CAT, SOD1, SOD2, GSTO1, GSTT1 and GSTA3 (Fig. 2). The expression of SOD1was significantly higher in H2(10 μmol/L) and H4(40 μmol/L) at 24 h, while, H4(40 μmol/L) group had significant higher SOD2 expression compared to H at 24 h. The expression of GSTO1 was significantly up regulated in H2(10 μmol/L), H3(20 μmol/L) and H4(40 μmol/L) group compared to H group at 6 h. The GSTA3 expression was significantly higher of H1(5 μmol/L) at 6 h and H4(40 μmol/L) group at 24 h of treatment compared to H.

Table 3
Effect of curcumin on the proliferation of chicken fibroblasts.

Parameter	Duration (h)	NC	H	H1 (5 μmol/L)	H2(10 μmol/L)	H3(20 μmol/L)	H4(40 μmol/L)
CCK8 (OD)	6	0.410 ± 0.051	0.335 ± 0.026 *	0.347 ± 0.016 *	0.349 ± 0.015 *	0.370 ± 0.014	0.367 ± 0.049
	12	0.288 ± 0.060	0.225 ± 0.032 *	0.226 ± 0.034 *	0.234 ± 0.013	0.235 ± 0.023	0.259 ± 0.067
	24	0.330 ± 0.007	0.299 ± 0.011 *	0.302 ± 0.008	0.300 ± 0.011	0.300 ± 0.027	0.324 ± 0.005 *

Values with *, within the row the numbers with * sign indicated significant difference at the 5% level. ($P < 0.05$).

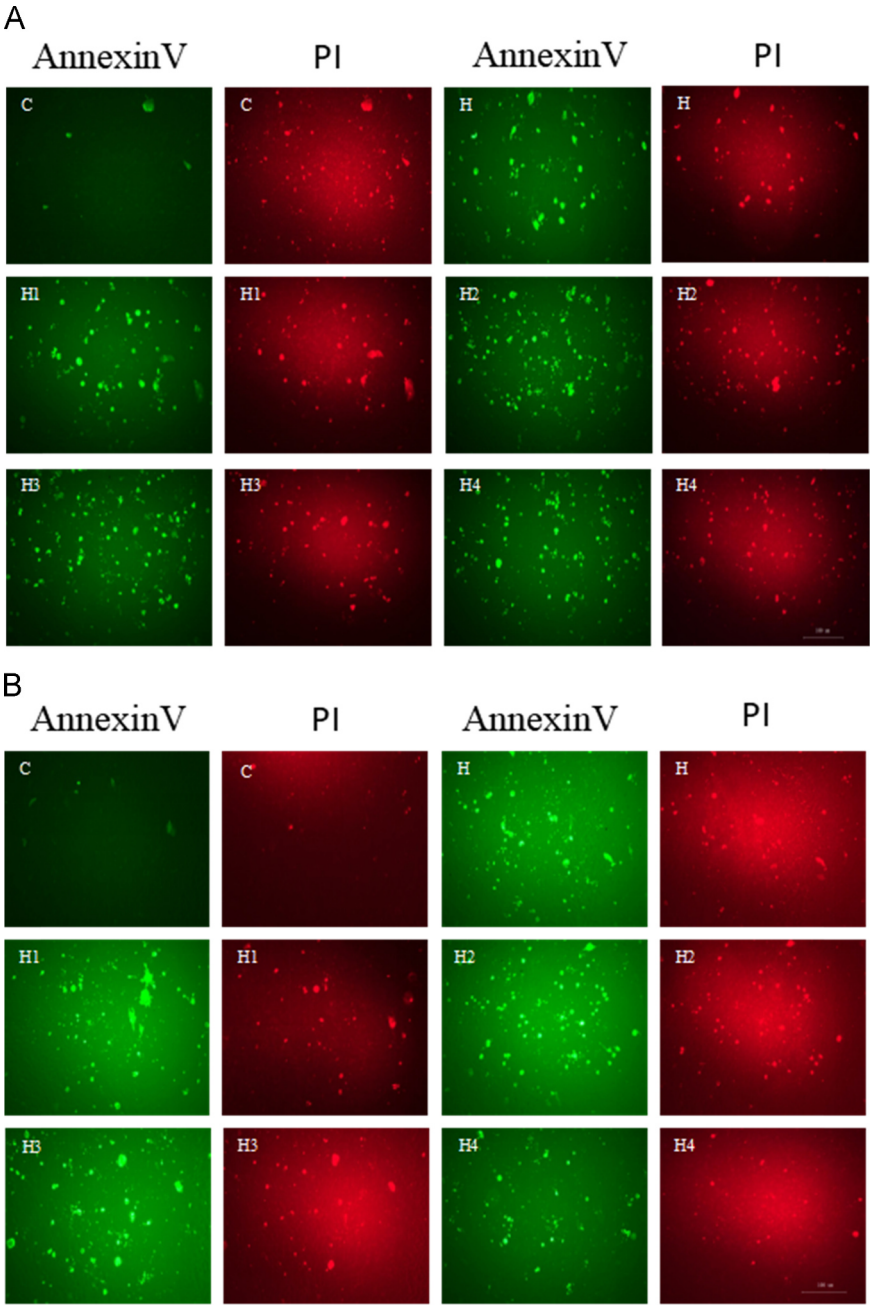

Fig. 1. -A; **Effects of Different Concentrations of Curcumin on the Heat induced Apoptosis After 6 h of Treatment**. Annexin V-FITC(green)/Propidium Iodide (red) Staining showed that H3(20 μmol/L) and H4(40 μmol/L) groups had lower number of apoptotic cells compared to H1(5 μmol/L) and H2(10 μmol/L) group after 6 h of treatment. C, control group; H, heats stressed control group; 1, H1(5 μmol/L) group; 2, H2(10 μmol/L) group; 3, H3(20 μmol/L) group;4, H4(40 μmol/L) group. bar = 100 μm **B; Effects of Different Concentrations of Curcumin on the Heat induced Apoptosis After 12 h of Treatment.** Annexin V-FITC(green)/Propidium Iodide(red) Staining showed that H3(20 μmol/L) and H4(40 μmol/L) groups had lower number of apoptotic cells compared to H1(5 μmol/L) and H2(10 μmol/L) group after 12 h of treatment. C, control group; H, heats stressed control group; 1, H1(5 μmol/L) group; 2, H2(10 μmol/L) group; 3, H3(20 μmol/L) group; 4, H4(40 μmol/L) group. bar = 100 μm **C; Effects of Different Concentrations of Curcumin on the Heat induced Apoptosis After 24 h of Treatment.** Annexin V-FITC(green)/Propidium Iodide(red) Staining showed that H3(20 μmol/L) and H4(40 μmol/L) groups had lower number of apoptotic cells compared to H1(5 μmol/L) and H2(10 μmol/L) group after 24 h of treatment. C, control group; H, heats stressed control group; 1, H1(5 μmol/L) group; 2, H2(10 μmol/L) group; 3, H3(20 μmol/L) group; 4, H4(40 μmol/L) group. bar = 100 μm.

3.5. Effect of curcumin on expression of genes related to MAPK-Nrf2 / ARE signaling pathway

Different doses of Curcumin had improved the expression of Jnk, Erk, P38 and Nrf2 gene (Fig. 3). At 6, 12 and 24, the expression of Nrf2 was higher compared to control H, but only at 6 and 24 h the expression was significantly higher in H3(20 μmol/L). At 12 h, H1(5 μmol/L), H3(20 μmol/L) and H4(40 μmol/L), while, at 24 h, H3(20 μmol/L) and H4(40 μmol/L) had significantly higher expression of Jnk compared to H group. The expression of Erk was improved in treatment group, at 24 h, the expression of H3(20 μmol/L), and H4(40 μmol/L) was significantly higher compared to H group. The expression of P38 was significantly higher than the H group in H3(20 μmol/L) at 6 h, while, at 12 h the H1(5 μmol/L), H3(20 μmol/L) and H4(40 μmol/L) had significantly higher expression.

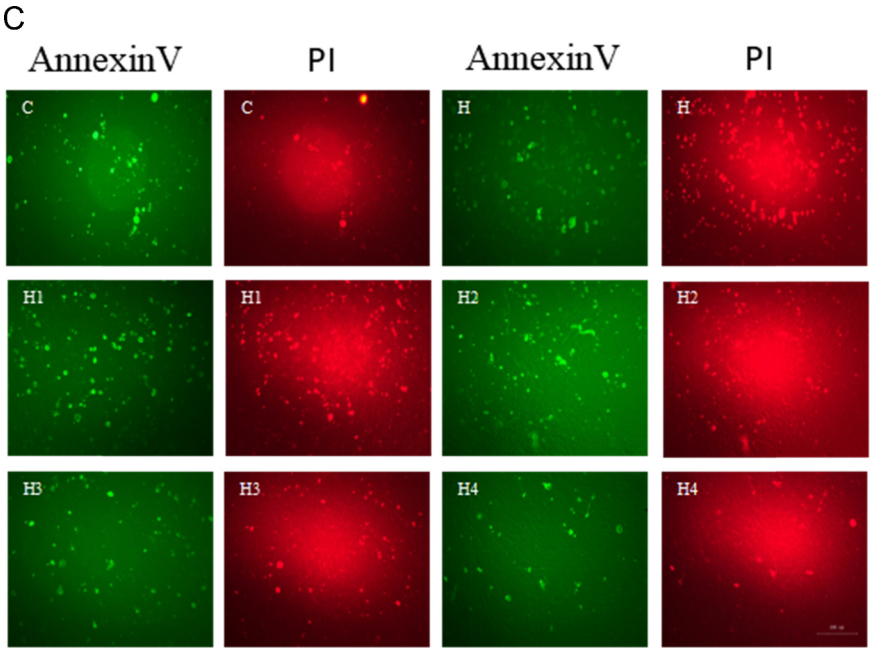

Fig. 1. (*continued*)

Table 4
Effects of different concentrations of curcumin on ROS content in chicken fibroblasts.

Parameter	Duration (h)	NC	H	H1 (5 μmol/L)	H2(10 μmol/L)	H3(20 μmol/L)	H4(40 μmol/L)
ROS	6	63.91 ± 10.64	70.91 ± 12.50 *	62.91 ± 18.50	67.00 ± 15.05	60.91 ± 13.50	63.48 ± 18.36
	12	85.22 ± 16.28	118.84 ± 22.54 *	120.59 ± 22.40 *	115.54 ± 13.12	112.84 ± 12.54	105.59 ± 22.40
	24	100.91 ± 14.22	135.02 ± 23.14 *	128.99 ± 21.64	126.48 ± 13.36	117.01 ± 15.64 *	109.18 ± 34.06 *

Values with *, within the row the numbers with * sign indicated significant difference at the 5% level. ($P < 0.05$).

Table 5
Effects of different concentrations of curcumin on MDA content in chicken fibroblasts.

Parameter	Duration (h)	NC	H	H1 (5 μmol/L)	H2(10 μmol/L)	H3(20 μmol/L)	H4(40 μmol/L)
MDA (nmol/mL)	6	0.65 ± 0.16	0.69 ± 0.10	0.68 ± 0.19	0.66 ± 0.13	0.66 ± 0.16	0.63 ± 0.15
	12	0.75 ± 0.21	0.68 ± 0.14	0.70 ± 0.19	0.68 ± 0.15	0.70 ± 0.19	0.62 ± 0.22
	24	1.25 ± 0.21	1.36 ± 0.14	1.28 ± 0.19	1.28 ± 0.15	1.30 ± 0.19	1.24 ± 0.19

Values with * , within the row the numbers with * sign indicated significant difference at the 5% level. ($P < 0.05$).

Table 6
Effects of different concentrations of curcumin on the antioxidant properties of chicken fibroblasts.

Parameter	Duration (h)	NC	H	H1 (5 μmol/L)	H2(10 μmol/L)	H3(20 μmol/L)	H4(40 μmol/L)
CAT (U/mL)	6	32.91 ± 4.24	28.48 ± 3.36	27.91 ± 6.50	29.00 ± 4.05	29.91 ± 5.50	33.48 ± 6.36
	12	43.22 ± 3.24	38.59 ± 4.40 *	37.84 ± 3.54	35.54 ± 4.92	39.14 ± 2.14	42.50 ± 3.40 *
	24	53.91 ± 4.22	50.18 ± 4.36	49.99 ± 3.54	50.28 ± 10.36	54.01 ± 5.64	54.08 ± 4.26
SOD (U/mL)	6	65.91 ± 8.64	60.48 ± 7.36	62.91 ± 4.50	63.11 ± 4.05	67.91 ± 5.50	70.48 ± 10.36
	12	99.22 ± 5.28	95.59 ± 7.40	90.84 ± 6.54	100.54 ± 10.92	102.84 ± 6.54	110.12 ± 5.40 *
	24	90.91 ± 4.22	89.88 ± 6.16	94.69 ± 6.64	96.28 ± 10.36	97.01 ± 8.64 *	109.18 ± 10.24 *
GSH-Px (U/mL)	6	68.35 ± 4.11	64.33 ± 5.10	65.30 ± 5.29	68.36 ± 7.34	70.49 ± 8.96	75.57 ± 6.15 *
	12	79.75 ± 7.01	74.68 ± 6.04	76.70 ± 6.89	78.78 ± 9.15	80.90 ± 7.19	85.92 ± 10.12 *
	24	110.35 ± 14.34	99.36 ± 10.14 *	104.28 ± 15.19	107.14 ± 13.25	110.10 ± 10.19 *	117.14 ± 15.12 *

Values with * , within the row the numbers with * sign indicated significant difference at the 5% level. ($P < 0.05$).

4. Discussion

Heat stress is one of the most key abiotic elements that impact the physiology of organisms. High ambient temperature is a major problem of poultry production in tropical region. Heat stress extremely affected the body weight gain, feed consumption and production performance of layers. The reduction of animal's feed intake under the heat stress condition was usually believed to be the prime reason for low production performances. However, it has lately been shown that high temperate change the sturdy state concentrations of free radicals, subsequent in both mitochondrial and cellular oxidative damage. Heat stress induces ROS. ROS continuously generated internal cells by several oxidase enzymes and by dismutation of the superoxide anion formed by electron leakage during mitochondrial respiration

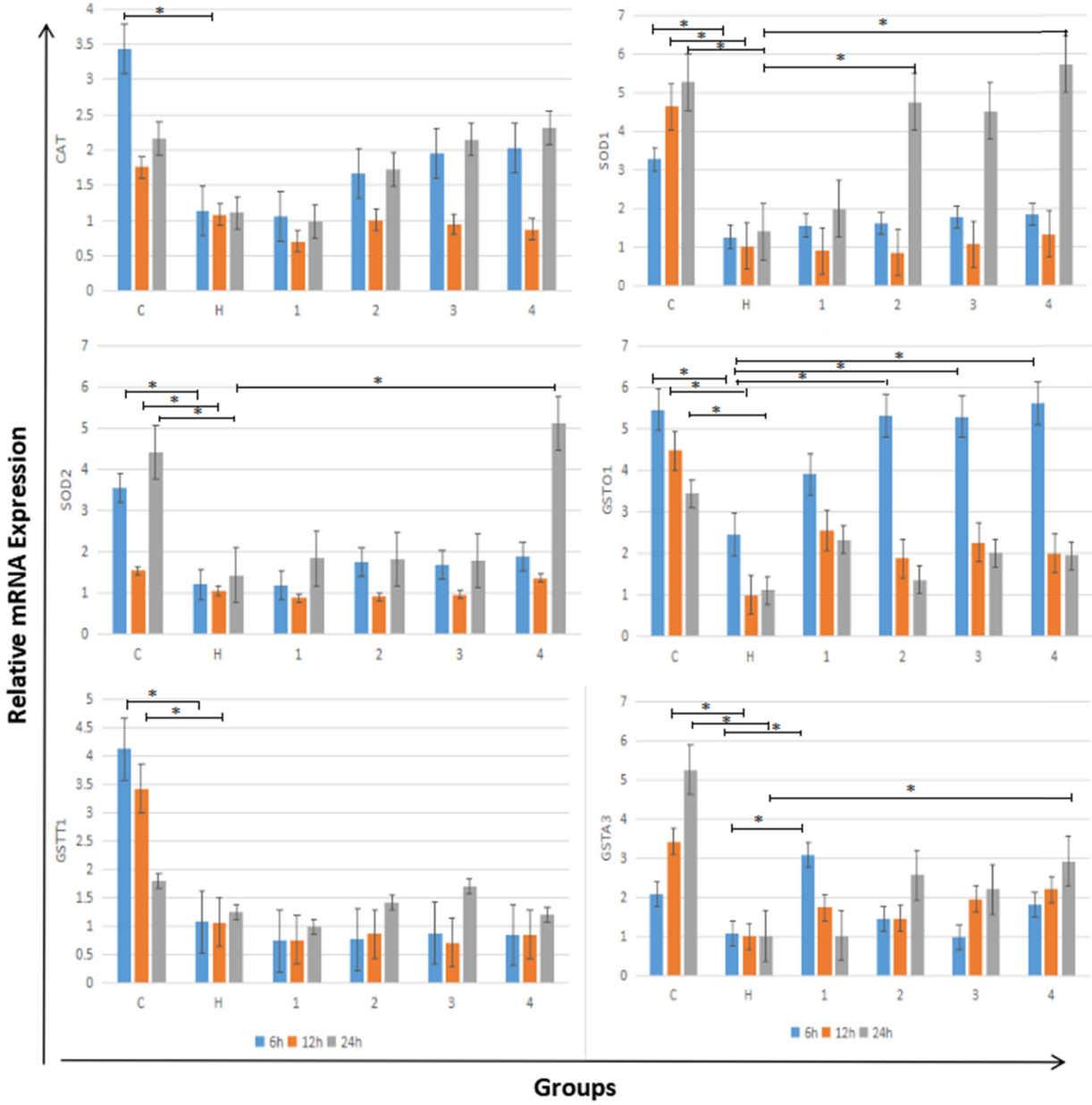

Fig. 2. Effects of different concentrations of curcumin on the relative expression of anti-oxidant enzyme mRNA of CEF cells. C, control group; H, heats stressed control group; 1, H1(5 μmol/L) group; 2, H2(10 μmol/L) group; 3, H3(20 μmol/L) group; 4, H4(40 μmol/L) group. (* sign indicate significant difference ($P < 0.05$).

(Fridovich, 1978). Higher level of free radical than the antioxidant leads to oxidative stress. In current study we found that oxidative stress induced by heat stress has significantly increased the ROS production, which induced the apoptosis and decreased the cell viability. So, such damaging effect of heat stress and associated generation of ROS can be protected by antioxidants, which act by different mechanisms (Kim et al., 2010). Our result showed that the ROS content and apoptotic rate were in down trend in Curcumin supplemented groups and cell viability and proliferation were in up trend. The results suggest that Curcumin, is a potent antioxidant, and may help inhibit the development of a range of conditions related to oxidative and free-radical damage (Cai et al., 2012).

SOD and GSH-Px activity and MDA concentration are the main parameter to assess oxidative status (Calabrese et al., 2008). In present study, MDA concentration was reduced, while, SOD and GSH-Px activity were up-regulated in supplemented groups compared to H. Lipid peroxidation is an autocatalytic free radical facilitated damaging process whereby polyunsaturated fatty acids in the cell membranes experience degradation to form lipid hydroperoxides. These latter compounds spontaneously rearrange to produce multiple degradation products, including MDA (Chance et al., 1979). In current study we found heat stress increased the production of MDA, while the Curcumin supplemented group had lower level of it. Result suggested that Curcumin prevent the cell from oxidative damage. These data are in agreement with previous studies done by various antioxidants supplementation (Kang et al., 2011; Wang et al., 2010).

As a primary antioxidant enzyme, SOD plays an important role cell fate. High level of SOD increase cells resistance to ROS and reduce oxidative stress. In the current study, the SOD level were reduced in heat stressed groups, while the Curcumin supplemented groups had high level of SOD, suggesting that curcumin improves SOD levels to remit heat-induced oxidative damage in the Cells. Glutathione (GSH) is impotent for normal cell growth and proliferation; it reduces the oxides which could hinder the regulation of cell. GSH also prevent the DNA from oxidative damage, it is also known as body's master antioxidant. In antioxidation systems GSH acts as a hydrogen donor, which neutralizes

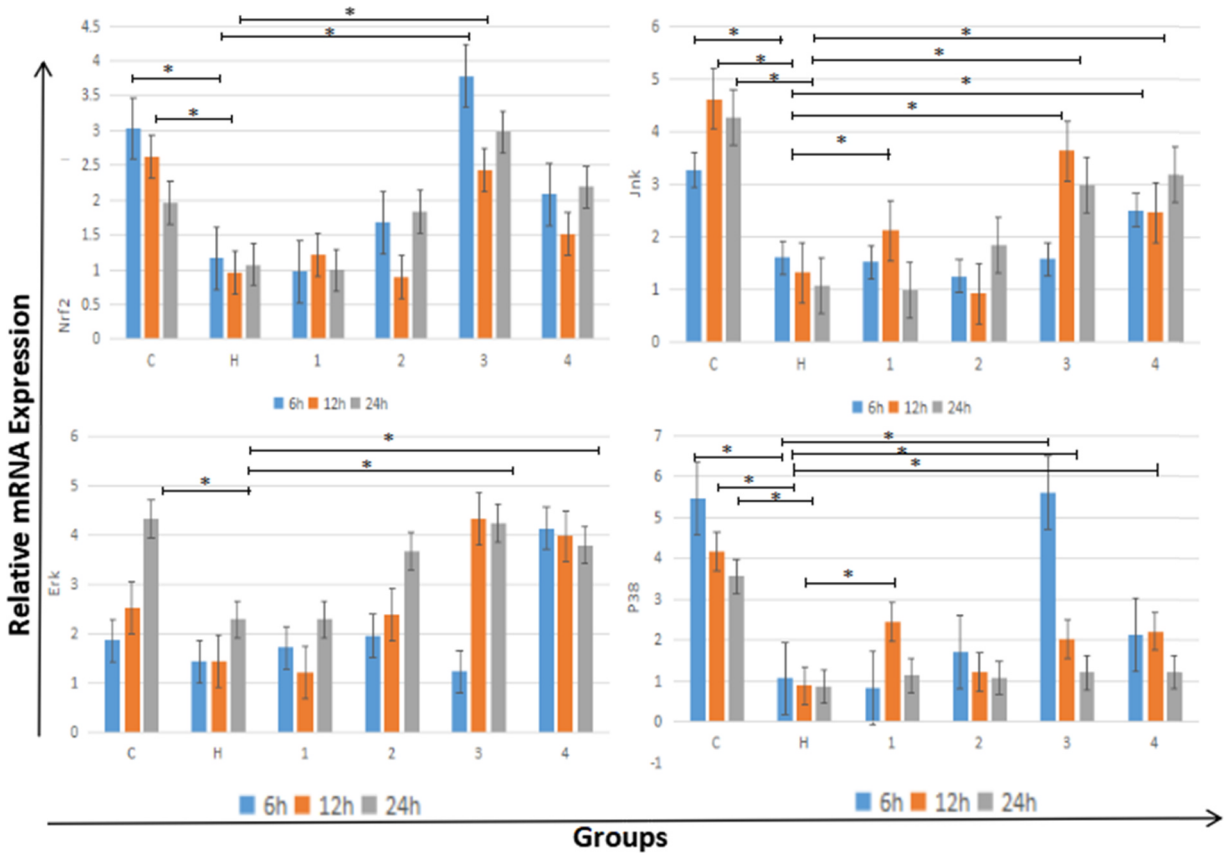

Fig. 3. Effects of different concentrations of curcumin on the relative expression of MAPK-Nrf2 pathway genes. C, control group; H, heats stressed control group; 1, H1(5 μmol/L) group; 2, H2(10 μmol/L) group; 3, H3(20 μmol/L) group; 4, H4(40 μmol/L) group. (* sign indicate significant difference ($P < 0.05$).

the free radicals. Since the GSH level increased in supplemented groups it appears that Curcumin was stimulating the action of glutathione to scavenge free radicals. CAT can remove the hydrogen peroxide radical (HOOH) by catalyzing two HOOH molecules to form two molecules of water and one molecule of O_2. Experimental results showed that diet supplementation raised the CAT activity compared to the control. This means that Curcumin can increase scavenging of HOOH radicals. Previous studies on heat stress show that its negative effects are associated to diverse mechanisms, comprising antioxidant defense system, ROS generation and oxidative stress. On the other hand, in vitro and in vivo studies have shown that curcumin activates expression of some intracellular antioxidative defense systems for free radicals (Mancuso and Barone, 2009). The analysis of antioxidant enzyme mRNA expression also showed the same pattern. In the Curcumin supplemented groups the expressions level CAT, SOD1, SOD2, GSTO1, GSTT1 and GSTA3 were up-regulated. The increase in the expression levels of genes involved in detoxification and/or oxidative stress response and enzymatic activities of antioxidants (SOD, CAT, GPX) led to an increased defense of cell membranes against oxygen and free radicals. These cooperative antioxidant defense mechanisms minimized oxidative damage. As a whole, these results suggest that CEF supplemented with Curcumin had better antioxidant status compared to the control. Several studies have been reported that chicken fed with turmeric (curcumin, one of the most active ingredients of turmeric) had increased antioxidative activity (Ahmadi, 2010).

A major mechanism in the cellular defense against oxidative or electrophilic stress is activation of the Nrf2-antioxidant response element signaling pathway, which controls the expression of genes whose protein products are involved in the detoxication and elimination of reactive oxidants and electrophilic agents through conjugative reactions and by enhancing cellular antioxidant capacity. In present study, the expression of Nrf2 was down-regulated in the heat stress. While, the curcumin supplemented groups had increased expression. Nrf2 nuclear translocation needs the activation of numerous signal transduction pathways, including MAPKs. MAPKs plays a crucial role in transducing different extracellular signals to the nucleus (Johnson and Lapadat, 2002). So, the activation of MAPKs pathways is known to have important roles in regulating cellular responses to oxidative stresses (Martindale and Holbrook, 2002). In the present study, we found that the expression of Jnk, Erk, and P38 were down-regulated in the heat stress condition, while, up-regulation was observed in supplemented group. In general, ERK signaling pathways are related to cell survival and proliferation during oxidant injury, whereas activation of JNK and p38 are more commonly linked to apoptosis (Matsuzawa and Ichijo, 2008). The transcription factors status in CEF cells suggests that supplementation with curcumin stimulated the transcription in the CEF cells to counteract the oxidative damage caused by heat stress.

Collectively, this study demonstrated that Curcumin inhibited the heat stress induced oxidative stress and underlying mechanism is the activation of MAPKs-Nrf2/ARE pathway. Therefore, Curcumin could be valuable therapeutic agent for the prevention of oxidative stress and damage.

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Competing Interests

The authors declare that they have no competing interests

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