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Anti-inflammatory and antioxidant activities of piperine on *t*-BHP-induced in Ac2F cells

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Research

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ABSTRACT

Background: In this study, to investigate whether piperine, an alkaloid from *Pipper longum*, could potentially exerts its effect for the suppression of inflammation and oxidative stress, we examined the modulatory effects of piperine in *tert*-butylhydroperoxide (*t*-BHP)-induced Ac2F rat liver cells.

Methods: Anti-inflammatory mechanism of piperine in Ac2F cells were examined by performing western blotting.

Results: The piperine exhibited remarkable reduction of intracellular reactive species (RS) levels in *t*-BHP- and SIN-1-induced Ac2F cells. In addition, piperine inhibited *t*-BHP-induced activation of nuclear factor kappa B (NF-κB) by suppressing the degradation of inhibitor-κB proteins (IκBα) and translocation of p65 from the cytosol to the nucleus, further indicating piperine's inhibitory effects on nitric oxide synthase (iNOS), cyclooxyganse-2 (COX-2) expressions. As a consequence, piperine modulated through inhibition of ERK, JNK, and p38 MAPKs signal transduction pathway in cells. Moreover, piperine pretreatment also regulated the protein expression of antioxidant enzymes such as manganese-dependent superoxide dismutase (MnSOD) and catalase.

Conclusion: These results indicated that piperine, a major component of black pepper might be a potential anti-inflammatory agent by modulating RS-induced NF-κB activation through the MAPKs signaling pathway and possess the anti-oxidative property. Therefore piperine can be considered as a useful therapeutic and preventive approach for the treatment of inflammation and oxidative stress-related diseases.

Keywords: piperine, anti-inflammatory, reactive species, *t*-BHP, Ac2F cells

1. INTRODUCTION

Inflammation progresses by complex interactions between mediators and inflammatory cells that activate the immune system to remove stimulant, inhibit infection and accelerate healing of tissue damage [1]. Chronic inflammation is associated with the pathogenesis of many diseases, including arthritis, cancer, stroke, and cardiovascular diseases [2]. Additionally, cumulative evidence shows that reactive species (RS) generated from oxidative stress are considered to be important components of inflammation [3, 4].

Nuclear factor-κB (NF-κB) plays a pivotal role in the early stages of the immune and inflammatory responses by regulating expression of inflammatory mediators, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2). In unstimulated cells, NF-κB dimers are bound to inhibitor of κB (IκB), which maintains NF-κB in the cytoplasm, thus preventing its translocation to the nucleus and its transcriptional activity. However, *t*-BHP (*tert*-butyl hydroperoxide)-induced activation of NF-κB involves phosphorylation of IκBα kinase (IKK), which phosphorylates IκBα protein, leading to ubiquitination and degradation of IκBα and translocation of NF-κB into the nucleus [5, 6]. Activation of NF-κB is also regulated by cellular kinases such as mitogen-activated protein kinases (MAPKs) [7]. MAPKs, extracellular signal regulated kinase 1/2 (ERK1/2), c-Jun NH2-terminal kinase (JNK), and p38 MAPK are involved in the transcriptional regulation of pro-inflammatory genes, including iNOS and COX-2, via NF-κB activation [8]. Understanding the underlying molecular mechanisms involved in these pathways is an important step in response to prevent deleterious effects of pro-inflammatory mediators. In contrast, antioxidant mediators play an important role in the cellular defense system against oxidative stress from pro-inflammatory factors such as *t*-BHP [9]. Manganese-dependent superoxide dismutase (MnSOD) is one of the most important antioxidant enzymes essential for reducing mitochondrial oxidative stress [10], whereas, *t*-BHP inhibit the most sensitive antioxidant enzyme in response to inflammation [11].

Piperine, one of the active components of black pepper (*Piper nigrum*) and long pepper (*Pipper longum*) (Figure 1), is commonly used as a spice in human diets, and it is also used as an effective remedy for gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut-related pain and arthritic conditions in several Asian countries and Pacific islands [12]. Piperine has been shown to possess several biological activities, including antioxidant [13, 14], anti-diabetic [15], anti-photoprotective [16], anti-inflammatory [17-24] anti-thyroid [19], anti-platelet aggregation [25], anti-obesity [26], immunomodulatory [27, 28], and anti-tumor [29, 30]. In spite of many previous studies, the mechanism by which piperine inhibits *t*-BHP-induced inflammation and oxidative stress has not been studied so far. Therefore, the objective of this study was to investigate the protective properties of piperine against *t*-BHP-induced inflammation, and to determine the underlying molecular mechanisms of anti-inflammatory action and anti-oxidative stress.

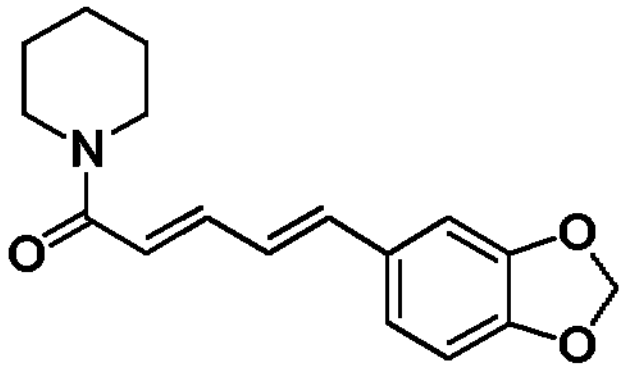

Figure 1. Chemical structure of piperine

2. MATERIALS AND METHODS

2.1. Materials

Piperine (≥ 97%), *t*-BHP, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and 3-morpholinosydnonimine hydrochloride (SIN-1) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2′,7′-Dichlorofluorescein diacetate (DCFH–DA) was obtained from Molecular Probes (Eugene, OR, USA). Polyvinylidene fluoride (PVDF) membrane was obtained from Millipore Corp. (Billelica, MA, Germany) and the enhanced chemiluminescence (ECL) detection system was obtained from Amersham Life Sciences, Inc. (Buckinghamshire, UK).

Antibodies targeted toward p65, p-p65, p-IκBα, IκBα, p-p38, p-ERK1/2, p-JNK, catalase, MnSOD, COX-2, iNOS, TFIIB, and β-actin were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other chemicals were of the highest purity available from either Sigma Chemical Co. (St. Louis, MO) or Junsei Chemical Co. (Tokyo, Japan).

2.2. Cell culture and treatment with piperine

Donryu rat hepatocytes (Ac2F cells) were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). The cells were cultured in Dulbecco's Modified Eagle Media (DMEM, Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone), 100 mg/mL penicillin-streptomycin, and 0.25 mg/mL amphotericin B in an atmosphere of 5% CO_2. Piperine was dissolved in 100% DMSO and added directly to culture media before the addition of *t*-BHP. The final concentration of DMSO did not exceed 0.1%. For all experiments, cells were plated in 100 mm culture dishes and cultures at 70–80% confluence were used for chemical exposure. After a 24 h attachment period, media were replaced with serum free media and cells were preincubated for 2 h with piperine followed by treatment with *t*-BHP. Working solutions of *t*-BHP were made in PBS immediately before use.

2.3. Cell viability assay

Cell viability was determined using an EZ-Cytox assay kit. briefly, Ac2F cells were seeded in 96-wells at (1 X10^ 5cells/well) and allowed to attach at 37°C for 24 h. Media were then replaced with fresh DMEM containing piperine (up to 10 μM) and incubated for 24 h. After incubation, 10 μL of EZ-Cytox solution were added to each well, and cells were incubated for an additional 2–4 h. The absorbance of each well was measured at 450 nm using ELISA reader (Promega, Madison, WI, USA). Cell viabilities were calculated as percentages of the viabilities of untreated controls. All determinations were performed in triplicate then averaged.

2.4. Measurement of intracellular RS accumulation

Intracellular oxidants were evaluated using the fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH–DA). This molecule is cleaved intracellularly by nonspecific esterase to 2′,7′-dichlorofluorescin (DCFH), which then forms the fluorescent compound 2′,7′-dichlorofluorescein (DCF) upon oxidation by RS [31]. To determine the extent of intracellular RS scavenging activity, Ac2F cells (2×10^4 cells/well) were seeded in 96-well black bottom-clear plates. After 24 h, the cells were treated with piperine (1-10 μM) for 1 h and then exposed to *t*-BHP (100 μM) or SIN-1 (10 μM) for 30 min to induce RS production, Cells were subsequently incubated with DCFH-DA (40 μM) for 30 min. The resultant fluorescence intensities were measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm with a fluorescence microplate reader (TECAN, Salzburg, Austria).

2.5. Preparation of cytosolic and nuclear fractions

Nuclear and cytosolic extracts were prepared according to Deng et al. (2001) [32]. Ac2F cells were plated in 60 mm dishes (2 X 10^5 cells/mL), treated with piperine, stimulated with *t*-BHP (100 μM) for 5 h, washed once with PBS, scraped into 1 mL of cold PBS, and centrifuged at 8,000 g at 4°C for 5 min. The pellets were suspended in 10 mM Tris (pH 8.0) with 1.5 mM $MgCl_2$, 1 mM DTT, 0.1% NP-40, and protease and phosphatase inhibitors and incubated on ice for 15 min. Nuclei were separated from cytosol by centrifugation at 10,000 g at 4°C for 15 min. The cytosolic supernatants were removed and the precipitated pellets were suspended in 10 mM Tris (pH 8.0), with 50 mM KCl, 100 mM NaCl, and protease and phosphatase inhibitors and incubated on ice for 1 h. They were then they were centrifuged at 12,000 rpm at 4°C for another 30 min.

2.6. Measurement of proteins by western blotting

Western blotting was performed as described previously [11]. The cells were harvested, washed twice with ice-cold PBS and lysed for 30 min on ice, vortexing every 5 min. Lysates were centrifuged at 12,000g for 30 min to remove insoluble material. Equal amounts of protein were separated on SDS-PAGE gels. The separated proteins were subsequently transferred onto PVDF by electro blotting. The membranes were blocked in a 5% non-fat milk solution in

TBS with 0.5% Tween-20 and incubated with primary antibodies overnight at 4°C as indicated. Membranes were washed and incubated for 2 h at room temperature with HRP-linked secondary antibodies. Pre-stained blue protein markers (Bio-Rad, Hercules, CA) were used for molecular-weight determination.

2.7. Statistical analysis

All experiments reported in this study were performed independently at least three times and data (expressed as mean ± S.E.M.) from a representative experiment are shown. Statistical significance was assessed by the one-way analysis of variance (ANOVA) for differences within treatments followed by the Bonferroni posttest. $^{*}p < 0.05$ was considered significant.

3. RESULTS

3.1. Protective effects of piperine against *t*-BHP-induced cytotoxicity in Ac2F cells

To investigate the protective effects against *t*-BHP of piperine, Ac2F cells were treated with various concentrations of *t*-BHP (0–300 μM). As shown in Figure 2a, *t*-BHP caused a dose-dependent decrease in Ac2F cell viability. Particularly at 100 mM, cell viability was significantly reduced up to 60.2% compared to untreated cells. Therefore, this concentration of *t*-BHP was selected to induce cell death in subsequent experiments. Before determining whether piperine has anti-inflammatory activity, the cytotoxicity of piperine in Ac2F cells was determined by EZ-Cytox assay. Ac2F cells were incubated for 24 h and then pretreated with piperine (up to 10 μM). As shown in Figure 2b, piperine did not induce cytotoxic effects in Ac2F cells up to 10 mM. To test the extent of the protective action of piperine against *t*-BHP-induced cytotoxicity, Ac2F cells were incubated with different concentrations (2, 5 or 10 μM) of piperine for 24 h *t*-BHP significantly reduced cell viability, whereas pretreatment with piperine dose-dependently inhibited cell death by *t*-BHP (Figure 2c). These concentrations were therefore used in subsequent piperine experiments.

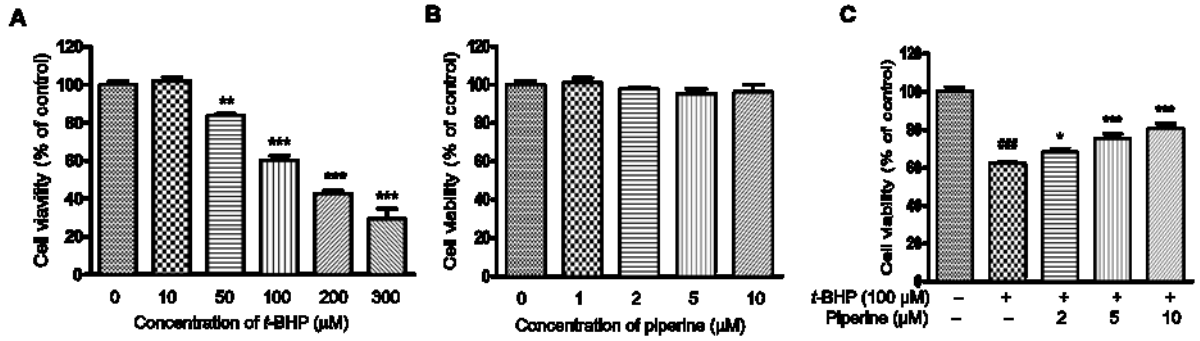

Figure 2. Effect of piperine on cell viability and *t*-BHP-induced cytotoxicity in Ac2F cells. Cells (1X10^5 cells/well) were incubated with different concentrations of *t*-BHP for 5 h (A). Cells were preincubated using various concentrations (up to 10 μM) of piperine for 24 h (B) and then incubated with 100 mM *t*-BHP for another 5 h (C). Cell viability was determined using the EZ-Cytox assay and expressed as the percentage of absorbance values relative to the control group. Data shown represent mean ± S.E.M. of triplicate experiments. One-factor ANOVA: $^{###}p < 0.001$ versus vehicle treated controls; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus 100 μM *t*-BHP-induced cells.

3.2. Inhibitory effect of piperine against oxidative stress induced RS production

t-BHP stimulates oxidative stress in cells to produce ROS [33, 34]. To determine whether piperine has a protective effect on *t*-BHP-induced Ac2F cells, cells were pretreated with non-toxic doses for 24 h. As shown in Figure 3a, the level of increased intracellular ROS as a result of *t*-BHP treatment was significantly reduced by treatment with piperine in a dose-dependent manner. SIN-1, a metabolite of the vasodilator molsidomine, is used as RNS inducer [8]. The effect of piperine on the production of RS in SIN-1-induced Ac2F cells is shown in Figure 3b. Cells treated with SIN-1 increased fluorescence intensity compared to unstimulated cells. Pretreatment with different concentrations of piperine significantly inhibited RS production in a dose-dependent manner in SIN-1-

induced Ac2F cells. Thus, piperine strongly scavenges RS production in *t*-BHP and SIN-1-induced Ac2F cells, indicating that piperine possessed anti-oxidative potential by suppressing RS production.

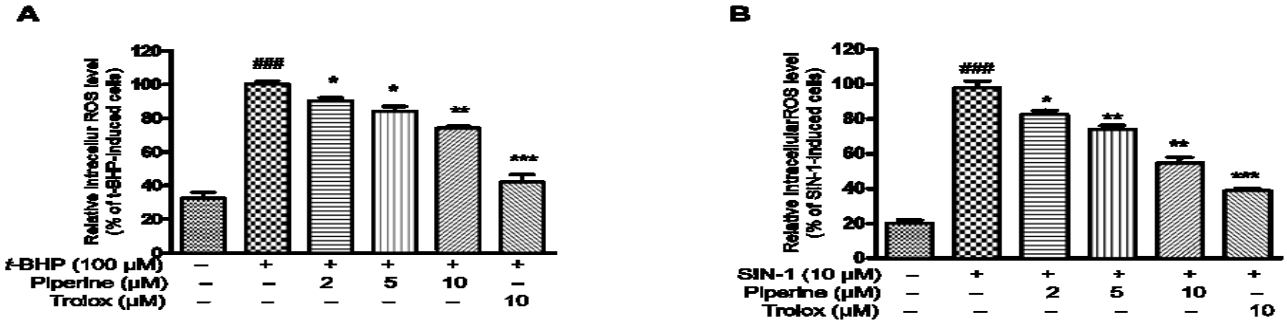

Figure 3. Effect of piperine against oxidative stress. Cells were pretreated with the indicated concentrations (2, 5, or 10 μM) of piperine for 2 h and further treated with *t*-BHP (100 μM) for 30 min (A). Cells were pretreated with the indicated concentrations (2, 5, or 10 μM) of piperine for 2 h and further treated with SIN-1 (10 μM) for 30 min (B). RS production was evaluated using a DCFH-DA (40 μM) assay to detect RS. Data are represented as mean ± S.E.M. of triplicate experiments. One-factor ANOVA: $^{###}p < 0.001$ versus vehicle treated controls; $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ versus 100 μM *t*-BHP-treated cells.

3.3. Modulatory effects of piperine against *t*-BHP-induced NF-κB transcriptional activation via inhibition of IκB-α degradation

NF-κB is one of the major transcription factors that expresses and regulates the expression of iNOS, COX-2, and inflammatory mediators [35]. We next investigated the effects of piperine on the translocation of NF-κB by Western blot. Our results showed that *t*-BHP induced nuclear phosphorylation of NF-κB p65, and pretreatment with piperine significantly down-regulated phosphorylated of p65 (Figure 4a). We also confirmed that the phosphorylation of IκBα was suppressed by pretreatment with 2 or 10 μM piperine in a dose-dependent manner (Figure 4b). Correspondingly, total level of IκBα was reduced by *t*-BHP and restored by piperine. Although the concentration of p65 was decreased in the cytoplasm and increased in nucleus after *t*-BHP-induction, pretreatment with piperine reversed these trends in a dose-dependent manner. Thus, piperine potently modulates *t*-BHP -induced NF-κB activation in Ac2F cells.

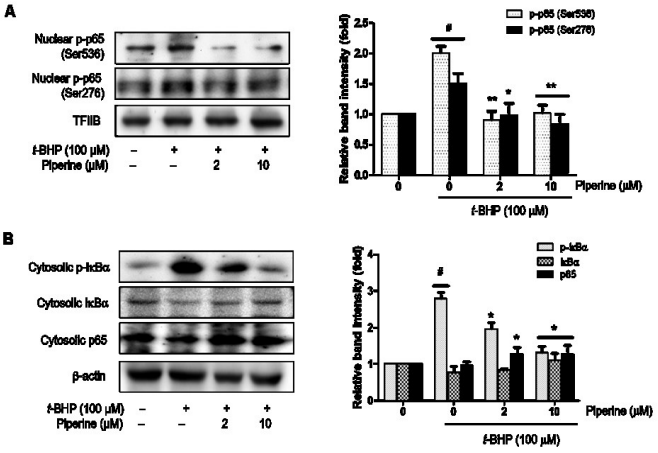

Figure 4. Effects of piperine on *t*-BHP-induced NF-κB activation in Ac2F cells. Cells were grown to 80% confluence in DMEM and changed to serum-free media. Pre-treatment with piperine (2 or 10 μM) for 2 h and treatment with of 100 μM *t*-BHP for 5 h. (A) Western blot was performed to detect p-p65 protein levels in the nuclear fraction. Levels were normalized to transcription factor IIB (TFIIB). (B) Western blot was performed to detect p-IκBα, IκBα, and p-65 protein in the cytosol fraction. Levels were normalized to β-actin. Values are the relative optical intensity of each band normalized as a percentage of the untreated control. One-factor ANOVA: $^{#}p < 0.05$ versus vehicle treated controls; $^{*}p < 0.05$ and $^{**}p < 0.01$ versus 100 μM *t*-BHP-treated cells.

3.4. Modulatory effects of piperine against *t*-BHP-induced phosphorylation of ERK1/2, JNK, and p38 MAPKs

MAPKs, including ERK1/2, p38, and JNK regulate the expression of iNOS, COX-2, and proinflammatory enzymes. Phosphorylation of MAPKs is known to be modulated by *t*-BHP-induced oxidative stress [36]. Thus, we investigated the effects of piperine on the activation of intracellular signaling kinases, including the family of MAPKs, in *t*-BHP-induced Ac2F cells. As shown in Figure 5, *t*-BHP treatment induced increased MAPK phosphorylation however, pre-treatment with piperine (2 or 10 μM) decreased *t*-BHP-induced phosphorylation of ERK1/2, JNK and p38 in dose-dependent manner. These results indicate that piperine attenuates *t*-BHP activation in the MAPK signaling pathway including ERK, JNK, and p38.

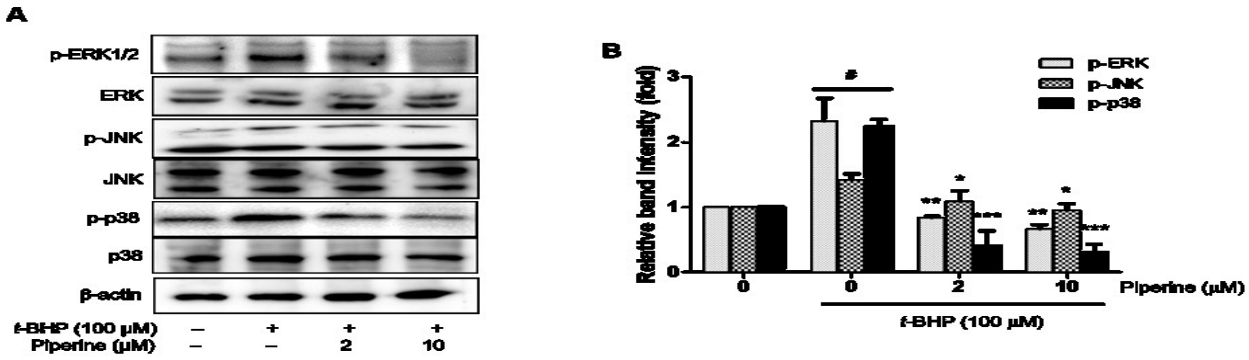

Figure 5. Effects of piperine on *t*-BHP-induced phosphorylation of MAPKs in Ac2F cells. Cells were grown to 80% confluence in DMEM and changed to serum-free media. Pre-treatment with piperine (2 or 10 μM) for 2 h and treatment with 100 μM *t*-BHP for 5 h. Western blot was performed to detect p-ERK1/2, p-JNK, p-p38, and the total form of each phosphor-form levels in whole cell lysate. Levels were normalized to β-actin. Values are the relative optical intensity of each band normalized as a percentage of the untreated control. One-factor ANOVA: $^{\#}p < 0.05$ versus vehicle-treated controls; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus 100 μM *t*-BHP-treated cells.

3.5. Modulation of pro-inflammatory genes by piperine

In order to determine whether the expression of *t*-BHP-induced NF-κB-dependent pro-inflammatory genes was suppressed by piperine, we analyzed expression of COX-2 and iNOS by Western blot. As shown in Figure 6, exposure of Ac2F cells to *t*-BHP induced significant induction of iNOS and COX-2 proteins, while piperine down-regulated *t*-BHP-induced iNOS and COX-2 expression in a dose-dependent manner. These results indicate that piperine modulates iNOS and COX-2 expression in *t*-BHP-induced Ac2F cells by suppressing the NF-κB signaling pathway.

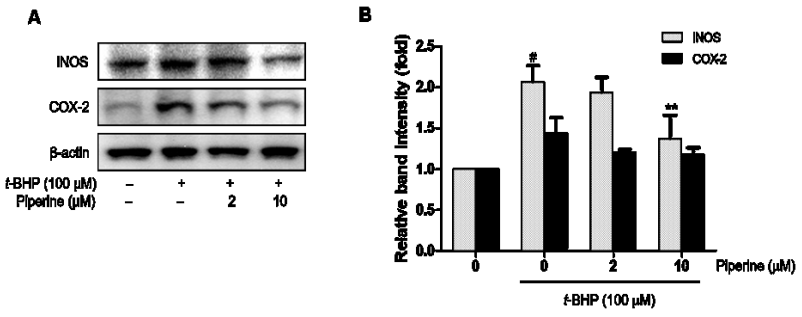

Figure 6. Effects of piperine on *t*-BHP-induced NF-κB-dependent pro-inflammatory gene expression in Ac2F cells. Cells were grown to 80% confluence in DMEM and changed to serum-free media. Cells were pre-treated with piperine (2 or 10 μM) for 2 h and sequentially treated with 100 μM *t*-BHP for 5 h. Western blot was performed to detect iNOS and COX-2 protein levels in whole cell lysate. Levels were normalized to β-actin. Values are the relative optical intensity of each band normalized as a percentage of the untreated control. One-factor ANOVA: $^{\#}p < 0.05$ versus vehicle-treated controls; $^{*}p < 0.05$ and $^{**}p < 0.01$ versus 100 μM *t*-BHP-treated cells.

3.6. Modulation of antioxidant enzyme expression by piperine

Antioxidant mediators also play an important role in regulating the inflammatory reaction [37]. MnSOD and catalase are two major antioxidant enzymes that protect against oxidative stress by metabolizing RS [11]. Therefore, in order to investigate whether piperine has the ability to upregulate the expression of antioxidant enzymes such as catalase and MnSOD, Ac2F cells were pretreated with piperine for 2 h and subsequently co-incubated with *t*-BHP for an additional 5 h. As shown in Figure 7, the expression of MnSOD and catalase was decreased by oxidative stress; conversely, piperine increased expression of both catalase and MnSOD. These results suggest that piperine exhibits antioxidant activity through increasing of the protein expression levels of antioxidant enzymes in hepatocytes.

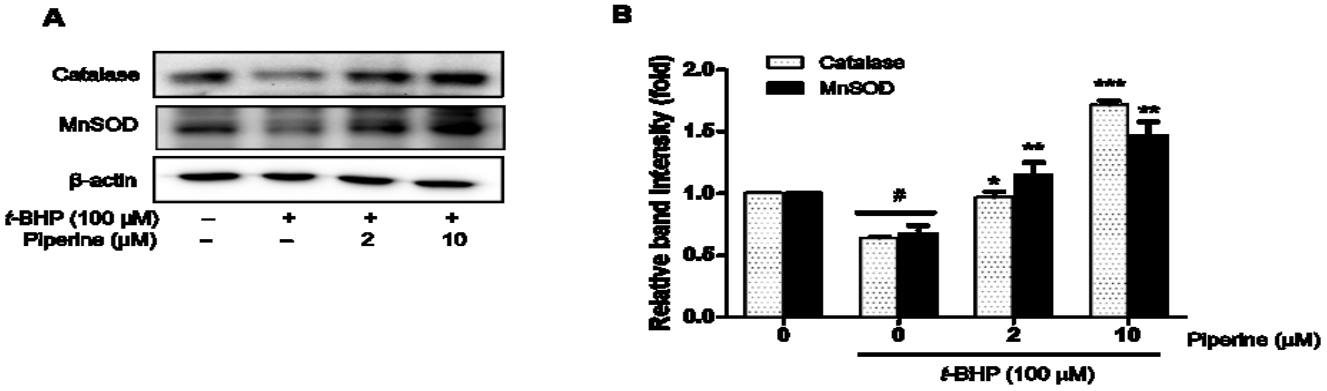

Figure 7. Effects of piperine on *t*-BHP-induced antioxidant enzyme gene expression in Ac2F cells. Cells were grown to 80% confluence in DMEM and changed to serum-free media. Western blot was performed to detect catalase and MnSOD protein levels in whole cell lysate. Levels were normalized to β-actin. Values are the relative optical intensity of each band normalized as a percentage of the untreated control. One-factor ANOVA: $^{\#}p < 0.05$ versus vehicle-treated controls; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus 100 μM *t*-BHP-treated cells.

4. DISCUSSION

In this study, we investigated antioxidant and anti-inflammatory potential of piperine, one of the active constituents of black pepper [12, 38], in *t*-BHP-induced inflammation. To further comprehend the molecular mechanisms of piperine-mediated anti-inflammation and antioxidant effects, we demonstrated the effects of piperine on NF-κB and MAPK signaling via RS radical scavenging in Ac2F cells. Our results indicate that piperine effectively inhibits pro-inflammatory genes, NF-κB and MAPKs, as well as upregulating radical scavenging activity by increasing antioxidant enzymes including, catalase and MnSOD. These results indicate that piperine can be further studied to develop therapeutics for the prevention of inflammatory by significantly preventing oxidative stress.

Piper species have been shown to be effective as anti-inflammatory reagent [39, 40]. In particular, anti-inflammatory activity of piperine has been reported in rats, with several experimental models, such as carrageenan-induced rat paw edema, cotton pellet granuloma and cotton-oil-induced granuloma pouch [17], but the mechanism of action remains unknown in *t*-BHP-induced rat liver cells. Though several of beneficial effects of piperine have been reported, to the best of our knowledge, this is the first report establishing that piperine exerts an anti-inflammatory and antioxidant effects.

Oxidative stress is characterized by overwhelming cellular antioxidant defenses in the increased production of RS [41]. Increased RS levels result in the oxidation of many biomolecules including lipids, carbohydrates, proteins and DNA [42]. These evidences support the involvement of oxidative stress in the initiation and progres-

sion of various inflammatory diseases. According to our study, piperine significantly reduced RS generation in an oxidative stress-induced condition. Therefore, inhibition of RS generated oxidative stress by piperine is likely attributed to down regulation of pro -inflammatory responses and induction of antioxidant enzymes, including MnSOD and catalase. To confirm the mechanisms by which piperine inhibits NF-κB activity, we tested the effect of piperine on NF-κB signaling. Inactive NF-κB is retained in the cytoplasm with IκBα, and *t*-BHP activates NF-kB via triggering IκBα degradation. Once activated, NF-κB subunit p65 dissociates from its inhibitory protein IkBa and may trigger the transcription of specific target genes such as iNOS and COX-2 [43]. In the present study, we demonstrated that piperine significantly inhibits *t*-BHP-induced phosphorylation of IκBα, IκBα degradation and the subsequent reduction of nuclear p65 in a dose-dependent manner. These results suggest that piperine inhibits NF-κB activation by inhibiting IκBα phosphorylation and the translocation of the p65 subunit of NF-κB from the cytosol to the nucleus in *t*-BHP-induced Ac2F cells.

MAPKs, including ERK1/2, p38, and JNK, are activated by extracellular stimuli and control a variety cellular responses, such as inflammatory cytokines, mitosis, differentiation and cell survival/apoptosis. In addition, the MAPK pathway is associated with NF-κB activation, where inhibition of MAPKs inhibits NF-κB expression [44]. We found that piperine suppresses phosphorylation of ERK1/2, JNK and p38 in *t*-BHP-induced Ac2F cells. Within the MAPKs pathway, phosphorylation of ERK1/2 was the most significantly decreased signal by concentration-dependent manner. Based on our findings, piperine suppresses MAPKs activation, resulting in NF-κB inactivation in *t*-BHP-induced Ac2F cells. In our study, the anti-inflammatory properties of piperine were mediated by the down-regulation of NF-κB activation in Ac2F cells. Thus, modulation of NF-κB activation is an effective approach to treat inflammation-related diseases. Our results indicate that piperine inhibits *t*-BHP-induced phosphorylation of NF-κB and IκBα in a dose-dependent manner, and these findings suggest that piperine negatively regulates protein expression of iNOS and COX-2 through inactivation of NF-κB in *t*-BHP-induced Ac2F cells. Nevertheless, the precise mechanism involved in the regulation of inflammation by piperine in Ac2F cells is still unclear.

MnSOD and catalase are major antioxidant enzymes that play an important role in the antioxidant protection mechanisms to protect the cells from radical-mediated damage. Downregulation of antioxidant enzyme has been reported in relation to chemical/oxidative stress, where the antioxidant system stabilizes the generated free radicals [45, 46]. Accordingly, in the present investigation, we observed a decrease in antioxidant levels in *t*-BHP-induced Ac2F cells that was recovered with piperine pretreatment. These results demonstrate that the antioxidant activity of piperine is due to induction of the antioxidant enzymes, including MnSOD and catalase. In summary, piperine has been shown to reduce RS through induction of antioxidant enzymes including catalase and MnSOD, and expression of inflammatory-promoting enzymes, such as iNOS and COX-2, as well as inhibition of MAPKs, IκBα phosphorylation and p65 nuclear translocation in *t*-BHP-induced Ac2F cells (Figure 8).

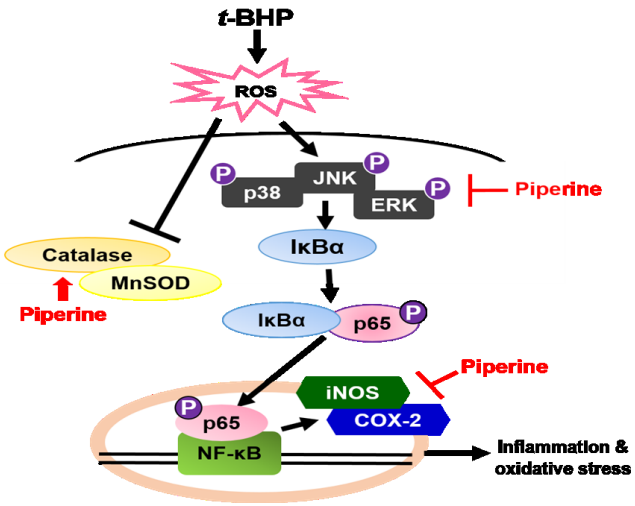

Figure 8. Possible mechanism of piperine on anti-inflammation and antioxidant. COX-2, cyclooxygenase-2; ERK, extracellular-signal-regulated kinase; iNOS, inducible nitric oxide synthase; IκBα, inhibitor κ B-alpha; JNK, c-Jun N-terminal kinase; MnSOD, manganese-dependent superoxide dismutase.

5. CONCLUSION

The results of our study show that piperine protects *t*-BHP-induced Ac2F cells against oxidative damage, which is due to the regulation of RS production via inactivation of the NF-κB and MAPK signaling pathways. With our findings, we expect that piperine possesses great potential as a therapeutic agent for prevention of inflammatory diseases caused by oxidative stress.

ACKNOWLEDGMEMTS

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