Ginger constituents ameliorated B(α)P-induced toxicity via modulation of antioxidants and xenobiotic-metabolising enzymes in mice

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Abstract

Accumulating evidence has linked benzo(α)pyrene (BαP) exposure to carcinogenesis with severe damages to reproductive, hematopoietic, hepatic, and renal tissues. Ginger (*Zingiber officinale* Roscoe) rhizome consumed worldwide as a spice and herbal medicine, exhibits a variety of health benefits including antioxidant, anti-inflammatory, and anti-cancer activities. In the present work, the efficacy of three ginger compounds namely 6-gingerol, zingerone, and curcumin against BαP-induced toxicity in mice was investigated. Kunming Swiss albino male mice were orally gavage with curcumin, 6-gingerol, or zingerone (all at a dose of 100 mg/kg body weight) for two weeks before intraperitoneal injection with benzo(α)pyrene (Bα P) at 20 mg/kg body weight. The effect of these ginger compounds on antioxidant and xenobiotic-metabolising enzymes *in vivo* was investigated. Results showed that pre-treatment with curcumin, 6-gingerol, or zingerone significantly ($p < 0.05$) increased catalase (CAT) and glutathione peroxidase (GPx) activities in serum and liver of mice, upregulated activities of phase II enzymes (quinone reductase (QR) and glutathione-S-transferase (GST)), and their protein and mRNA levels in liver of mice; but reduced levels of activities, protein, and mRNA of phase I enzymes (CYP1A1 and CYP1A2) as compared to those of BαP-only treatment. Furthermore, these compounds significantly ($p < 0.05$) stimulated nuclear factor erythroid 2-related factor 2 (Nrf2) protein expression, whilst curcumin suppressed the expression of Kelch-like ECH-associated protein 1 (Keap1) in liver. These results could contribute to our understanding of the potential beneficial effects of consuming ginger as food and/or dietary supplement.

Introduction

Benzo(α)pyrene (BαP), one of the most investigated polycyclic aromatic hydrocarbons (PAHs), is widely present in grilled or smoked meat, workplaces, and the environment (Malik *et al.,* 2018). Exposure to BαP has been linked to genotoxicity in various human cells lines and carcinogenesis with severe damages to reproductive, hematopoietic, hepatic, and renal tissues in experimental animals (Genies *et al.,* 2013). Therefore, it is listed as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) (Dzobo *et al.,* 2018). Bα P undergoes a two-stage metabolic activation to exhibit its carcinogenic effects in biological systems. Firstly, it is oxidised by CYP1A class of cytochrome P450 enzymes to form reactive intermediate metabolites. Secondly, these intermediates are conjugated with glutathione or carbohydrates catalysed by some phase II detoxifying enzymes to form ultimately more polar compounds that are easily

excreted from the body. Mutagenic and carcinogenic effects of BαP are mainly related to some reactive intermediates (*i.e.* BαP-quinones metabolites) produced during CYP450 metabolic process (Akcha *et al.,* 2000). These metabolites are electrophilic, hence able to bind covalently with cellular proteins and nuclear acids (Kim *et al.,* 1998). Besides, some of BαP intermediates are responsible for the generation of reactive oxygen species (ROS), which subsequently cause oxidative damages of DNA, proteins, and antioxidant enzymes (Wong *et al.,* 1992). For example, malondialdehyde (MDA) and carbonyl content, as markers of oxidative damage of lipid and protein, were significantly higher in rabbit erythrocytes exposed to BαP (Lee and Lee, 1997). Both antioxidants and xenobiotic-metabolising enzymes play important Ant roles in carcinogenic metabolism, hence could be important targets for chemoprevention. The induction of phase II enzymes is also proposed as a major mechanism of cellular protection against the toxic and reactive chemical

species, and neoplastic effects of carcinogens (Begleiter *et al.,* 1997).

In the past decades, many classes of plant-derived extracts or phytochemicals are receiving considerable attention as potential chemopreventive agents. Some have been reported to protect experimental animals against BαP exposure (Park *et al.,* 2010). Examples include, but are not limited to, sulphoraphane from broccoli, curcumin from turmeric, and organosulphur compounds from garlic and onion (Surh *et al.,* 2008). The chemopreventive properties of these compounds are attributed to the inhibition of carcinogen formation from procarcinogens, induction of coordinated antioxidant enzyme, and phase II enzyme response of the carcinogens. Ginger (*Zingiber officinale* Roscoe) rhizome is used worldwide, both as culinary spice and medicinal herb. *In vitro* and *in vivo* (animals) studies have shown that ginger or its extracts possess some pharmacological activities such as antioxidative, anti-inflammatory, analgesic, and anti-tumour (Kaur *et al.,* 2016; Ebrahimzadeh Attari *et al.,* 2018). For instance, oral consumption of ginger increased antioxidant levels and decreased oxidative damages in the liver and kidney of rats (Kota *et al.,* 2008). Supplementation with zingerone to ethanol-fed rats decreased the acute alcoholic liver injury by reducing lipid peroxidation, and increasing antioxidant enzymes (Mani *et al.,* 2016). Our earlier study also showed that some constituents isolated from ginger were potent inducers of some phase II metabolising enzymes, which are involved in metabolic transformation of BαP (Li *et al.,* 2011). In view of these, we hypothesise that the pre-treatment of ginger constituents might have protective effects against BαP-induced toxicity in mice. The present work was therefore undertaken to investigate the efficacy of three ginger compounds namely 6-gingerol, zingerone, and curcumin against BαP-induced toxicity in mice.

Materials and methods

Chemicals

Benzo(α)pyrene (BαP, ≥ 96.0%), curcumin (≥ 98.0%), 6-gingerol (≥ 98.0%), and zingerone (≥ 98.0%) were purcahsed from Sigma-Aldrich Co. (St. Louis, MO, USA). Anti-NAD(P)H: quinone oxidoreductase 1 (NQO1), anti-glutathione-S-transferase (GST), anti-Kelch-like ECH-associated protein 1 (Keap1), anti-nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and anti-β actin rabbit monoclonal primary antibodies and secondary antibodies were purchased from CST Inc. (Danvers, MA, USA). SYBR premix EX Tap Kit was purchased from Takara Bio, Japan.

Animal experiment

Laboratory-bred Kunming Swiss albino male mice (3 - 4 w old, 18 - 22 g) with quality-certificated number SCXK 2013-0006 were purchased from Taibang Biological Products Co. Ltd. (Taian, China). The animal procedures were approved by the Institutional Animal Care and Use Committee, Shandong Agricultural University. The animals were housed in polycarbonate cages with filter tops. An ambient temperature of $22 \pm 2°C$, relative humidity of $50 \pm 5\%$, and 12-h day/night cycles were maintained throughout the experiment. Mice had free access to food and deionised water.

All mice were randomly assigned into six groups of 10 mice per group: (1) the control group (vehicle only); (2) BαP-treated group (20 mg/kg body weight); (3 - 5) BαP + one of the three ginger compounds (curcumin, 6-gingerol, or zingerone) at a dose of 100 mg/kg body weight. The doses of three ginger compounds and BαP were selected based on the pharmacological data from previously published studies (Ajayi *et al.,* 2015; 2019). BαP and ginger compounds were dissolved in corn oil (0.02 mL/g body weight). Mice were orally gavage with corn oil or ginger compounds dissolved in corn oil once a day for 14 consecutive days before intraperitoneal injection with BαP at 20 mg/kg body weight according to established procedures (Ajayi *et al.,* 2019). The mice that were orally administrated with corn oil alone served as the control. Body weight of all animals was weighed weekly throughout the experiment. At the end of experimental period, blood samples were collected from the orbital plexus into heparinised polypropylene tubes. Animals were sacrificed by cervical dislocation, and the liver was collected and frozen under liquid nitrogen for further analysis.

Preparation of serum and liver tissue homogenates

The serum and liver tissue homogenates were prepared following the procedures described by Delgado-Roche *et al.* (2019). Blood sample was centrifuged at 3,000 *g* for 20 min at 4°C, and the supernatant was obtained and used for the determination of antioxidant enzymes. The liver tissue was minced and homogenised in ice-cold physiological saline solution using a polytron homogeniser to yield a 10% homogenate. After centrifugation at 2,000 *g* for 10 min, an aliquot of the supernatant (0.5 mL) was used for antioxidant

enzyme assay, while the remainder was centrifuged at 10,000 *g* for 15 min at 4°C. The resultant supernatant (cytosolic fraction) was collected for measuring activities of phase II enzymes, including glutathione-S-transferase (GST) and quinone reductase (QR).

Determination of antioxidant and phase I and II enzymes

The activities of the antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were measured using commercial assay kits following the manufacturer's instructions (Jiancheng Bioengineering Co. Ltd., Nanjing, China). The activities of QR and GST were measured using Bangyi's commercial assay kits following the manufacturer's instructions (Shanghai Bangyi Co. Ltd., Shanghai, China). The activities of CYP1A1 and CYP1A2 were measured by the spectrofluorometric method as reported previously (Perepechaeva *et al.,* 2017). 7-Ethoxyresorufin and 7-methoxyresorufin were used as substrates for isoforms of CYP1A enzyme: CYP1A1 and CYP1A2, respectively. A mixture containing mice microsomes, 5 μM of 7-ethoxyresorufin or 7-methoxyresorufin in PBS (pH 7.6) was incubated at 37°C for 3 min in the presence and absence of inhibitors (naringenin and dihydroxybergamottin). Then, the reaction was initiated by the addition of 0.5 mM NADPH. The reaction mixture was incubated at 37°C and fluorescence readings were recorded every 60 s for 10 min at an excitation wavelength of 530 nm, and an emission wavelength of 590 nm using a microplate reader (SPETRA max M2 Fluorescent, MD, USA). The protein concentration in each sample was determined by the bicinchoninic acid assay kit (Bangyi Co. Ltd., Shanghai, China). EROD activity was calculated from a standard curve, and expressed in pmoles resorufin/min/mg protein.

Western blot analysis

Western blot analysis was performed according to Li *et al.* (2012). Briefly, the cell lysate was prepared by solubilising the tissue in 100 μL of RIPA buffer (50 mM Tris-base, 150 mM NaCl, 0.1% Triton X-100, and 0.1% SDS) with protease inhibitor (10×; Calbiochem, San Diego, CA) and subsequent centrifugation. Total proteins of liver (50 μg) were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% polyacrylamide gel. The proteins were then transferred onto a polyvinylidene fluoride (PVDF) membrane. After incubation with 5% skim milk for 1 h, the membrane was probed with a primary antibody (1:1000) in TBST buffer overnight at 4°C, and then incubated with corresponding secondary antibody (1:2000) for 1 h. Finally, the signals were visualised using the ECL™ Western blotting detection reagents (Amersham Biosciences Corp., Piscataway, NJ).

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

RNA extraction was performed according to Li *et al.* (2019). Total RNA was extracted using an RNAsimple Total RNA Isolation Kit (Tiangen Biotech Co. Ltd., Beijing, China) following the manufacturer's instructions. Chromosomal DNA contamination was removed by incubating the RNA samples with gDNA Eraser (Takara Company, Dalian, China) at 42°C for 3 min, and 2 μg of RNA was reverse-transcribed to complimentary DNA using a PrimeScript RT reagent kit (Takara). The primer sequences used for real-time PCR are listed in Table 1 (Chen *et al.,* 2021). The reactions were conducted on a Bio-Rad IQ5 Real-time PCR System (Bio-Rad Laboratories Inc., Hercules, CA, USA) using Takara SYBR Premix Ex Taq in a total volume of 20 μL containing: 10 μL of SYBR Premix Ex Taq, 8 μL of sterile deionised water, 1 μL of diluted cDNA, and 1 μL of each primer. Real time cycles were same for all primers: 95°C for 30 s, followed by 40 cycles at 95°C for 5 s, 60°C for 15 s, and finally 72°C for 20 s. Expression levels of genes were calculated using the C_T method with the

Table 1. List of primers used for RT-PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Nrf2	AAAGCACAGCCAGCACATTC	GGGATTCACGCATAGGAGCA
Keap1	GATATGAGCCAGAGCGGGAC	CATACAGCAAGCGGTTGAGC
GST	ATCGTTCCCTTTCTCGGCAT	GCAGCCTGTAAGCCATTGAC
NQO1	GTCTGGAAACCGTCTGGGAG	GAATGGACTTGCCCAGGTGA
β-actin	AGTGTGACGTTGACATCCGT	GCAGCTCAGTAACAGTCCGC

transcript activity of the β-actin gene as the normaliser (Livak and Schmittgen, 2001).

Statistical analysis

All data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to calculate significant differences among treatment means, and multiple comparisons of means were done by the Duncan's multiple range test using the statistical software SPSS 13.0 (SPSS Inc., Chicago, IL). A probability value of $p < 0.05$ was considered statistically significant.

Results

Body weight and liver-to-body weight ratio

All the mice survived well and none died throughout the experiment. The body weight of mice continuously increased throughout the experiment, with gains ranging from 6.92 ± 1.57 to 8.87 ± 2.52 g. The liver-to-body weight ratios of mice changed from 4.84 ± 0.32 to 5.31 ± 0.31 mg/g. However, no significant ($p > 0.05$) differences were observed between the control and any of other treatments in body weight and liver-to-body weight ratios.

Antioxidant enzymes

The CAT activities of serum and liver in BαP-only treated mice were not significantly ($p > 0.05$) different from those of the control (Table 2). However, pre-treatment of curcumin, 6-gingerol, or zingerone led to a significant increase of 74.5, 49.8, and 23.8%, respectively, in serum CAT activity; and 13.8, 27.1, and 29.0%, respectively, in liver CAT activity, as compared to the BαP-only treatment. Curcumin pre-treatment was more effective in stimulating the serum CAT activity (13.21 ± 0.03 U/mL) among all the pre-treatments, while zingerone and 6-gingerol were more potent than curcumin in the case of hepatic CAT activity (99.28 ± 0.30 and 97.76 ± 1.60 U/mg protein, respectively).

Exposure of mice to BαP led to a significant ($p < 0.05$) increase in liver SOD activity from the basal level of 338.19 ± 15.29 to 390.88 ± 26.79 U/mg proteins, but no effects on serum SOD activity. The pre-treatment of the ginger compounds led to significantly ($p < 0.05$) higher SOD activities in mice serum ranging from 225.86 ± 4.12 to 257.16 ± 1.50 U/mL, as well as in liver (422.01 ± 19.91 U/mg protein for curcumin) against the BαP-only group.

Neither serum GPx nor hepatic GPx activities in mice were affected by the treatment of 20 mg/kg body weight of BαP as compared to the control. As compared to the BαP-treated group, significant ($p < 0.05$) increases of GPx activities were observed in the ginger compounds-fed groups with the exception of 6-gingerol for serum GPx activity. The extent of stimulation in GPx activity in liver crystal for curcumin, 6-gingerol, and zingerone reached 35.6, 20.4, and 17.7%, respectively. GPx activity in serum was also significantly improved by the pre-treatment of curcumin and zingerone, with the percentages of stimulation being 30.2 and 24.5%, respectively.

Phase I enzymes

Treatment of mice with BαP led to a significant ($p < 0.05$) 41.1 and 60.7% increase in CYP1A1 and CYP1A2 activity, respectively, as compared to the control (Figure 1A). Similar results were also reported by Villa-Cruz *et al.* (2009) who found that CYP450 levels measured as CYP1A1 activity showed a significant increase 12 h after BαP injection. However, the BαP-stimulated upward trend of CYP1A1 and CYP1A2 in liver homogenates of mice was significantly ($p < 0.05$) suppressed following the pre-treatment with the ginger compounds. As compared to the BαP-only group, the percentages of decrease in CYP1A1 observed for curcumin, 6-gingerol, and zingerone were 48.2, 12.0, and 47.0%, respectively, while they were 60.0, 33.3, and 26.6% in the case of CYP1A2.

The expression of CYP1A1/2 protein and mRNA in liver of mice was consistent with these enzymatic activities. Exposure of mice to BαP led to a significant increase of CYP1A1 and CYP1A2 proteins as compared to the control (Figures 1B and 1C). However, pre-treatment of the ginger compounds exhibited inhibitory effects on the expression of these two CYP1 proteins, with percentage inhibition of CYP1A1 protein reaching 27.7, 13.8, and 22.3% by curcumin, 6-gingerol, and zingerone, respectively; and 44.6, 48.2, and 33.0% in the case of CYP1A2 protein, respectively. Similar results were also observed for mRNA levels of CYP1A1 and CYP1A2 (Figures 1D and 1E). The mice fed with curcumin had significantly ($p < 0.05$) lower level of CYP1A1, while those with 6-gingerol presented the lowest CYP1A2 mRNA among the treatments.

Phase II enzymes

Figure 2A depicts the effects of ginger compounds on the QR activity in mice exposed to BαP. In the control, the QR activity was 101.93 ± 2.39 U/mg proteins. Upon the treatment with 20 mg/kg body weight of BαP, the QR activities increased to

Table 2. Effects of ginger compounds on the activities of antioxidant enzymes in mice exposed to benzo(α)pyrene.

Treatment	Catalase activity		Superoxide dismutase activity		Glutathione peroxidase activity	
	Serum (U/mL)	Liver (U/mg protein)	Serum (U/mL)	Liver (U/mg protein)	Serum (U/mL)	Liver (U/mg protein)
Control	7.32 ± 0.57^{de}	68.85 ± 1.84^{d}	198.99 ± 14.55^{c}	338.19 ± 15.29^{c}	382.06 ± 7.61^{c}	403.02 ± 15.01^{d}
BαP	7.57 ± 0.16^{d}	76.94 ± 7.85^{d}	190.12 ± 12.04^{cd}	390.88 ± 26.79^{b}	379.37 ± 3.81^{c}	448.62 ± 41.46^{cd}
BαP + curcumin	13.21 ± 0.03^{a}	87.53 ± 8.96^{c}	257.16 ± 1.50^{a}	422.01 ± 19.91^{a}	493.99 ± 38.05^{a}	608.42 ± 36.38^{a}
BαP + 6-gingerol	11.34 ± 0.06^{b}	97.76 ± 1.60^{ab}	237.65 ± 5.02^{b}	388.10 ± 23.15^{b}	403.59 ± 7.61^{c}	540.19 ± 35.59^{b}
BαP + zingerone	9.37 ± 0.48^{c}	99.28 ± 0.30^{a}	225.86 ± 4.12^{b}	392.06 ± 19.99^{b}	472.20 ± 9.51^{ab}	527.84 ± 55.56^{b}

Values are mean ± standard deviation of ten mice per group. Means in the same column followed by different lowercase superscripts are significantly different at $p < 0.05$ by Duncan's multiple range test.
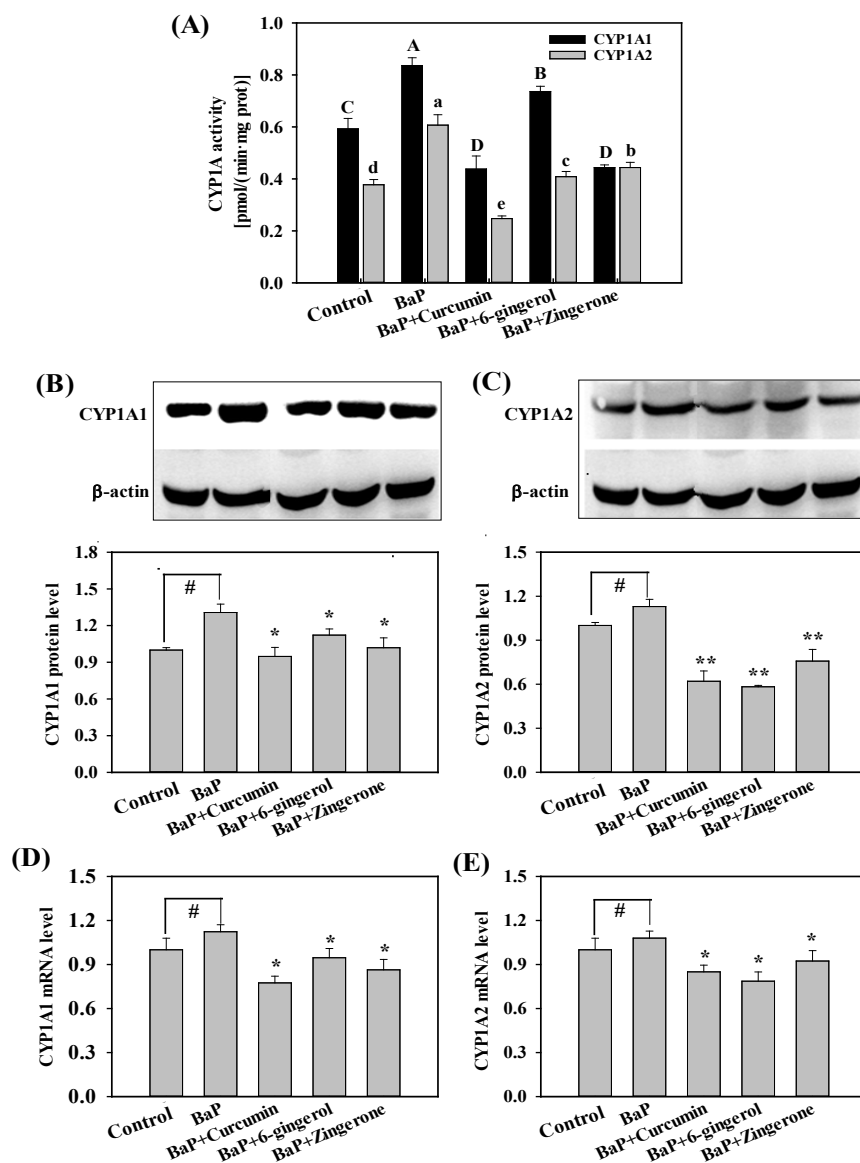
Figure 1. Effect of curcumin, 6-gingerol, and zingerone on CY1A1 and CY1A2 enzymes in liver of mice exposed to BαP. (A) CY1A1 and CY1A2 activity; (B) CYP1A1 protein; (C) CYP1A2 protein; (D) CYP1A1 mRNA; and (E) CYP1A2 mRNA. Data are means of ten mice per group with error bars indicating standard deviations. Histograms marked with different uppercase or lowercase letters indicate significant different at $p < 0.05$. # = $p < 0.05$ *vs.* control; * and ** = $p < 0.05$ and $p < 0.01$ *vs.* the BαP-treated group.

134.52 ± 3.67 U/mg protein. Pre-treatment of curcumin, 6-gingerol, and zingerone for 14 consecutive days significantly ($p < 0.05$) elevated the QR activities as compared to the BαP group, with the percentage stimulation being 9.0, 17.8, and 22.5%, respectively. The mice exposed to BαP had significantly ($p < 0.05$) increased GST activity as compared to the control (Figure 2B). Pre-treatment of curcumin, 6-gingerol, and zingerone for 14 consecutive days caused a significantly (13.9, 32.4, and 15.1%, respectively) increase in hepatic GST activity as compared to BαP-only group.

Treatment with BαP led to a 209.0%

increase in the expression of NQO1 protein against the control ($p < 0.05$) (Figure 2C). Pre-treatment of ginger compounds significantly ($p < 0.05$) further improved the NQO1 protein expression as compared to the BαP-treated group. The mice fed with curcumin, 6-gingerol, and zingerone presented an increase of 26.8, 14.9, and 94.5%, respectively, of hepatic NQO1 protein as compared to those treated with BαP alone. In the case of GST, the protein expression significantly ($p < 0.05$) increased by 125.3, 100.0, and 83.1% in the mice fed with curcumin, 6-gingerol, and zingerone, respectively, as compared to BαP-treated group (Figure 2D).
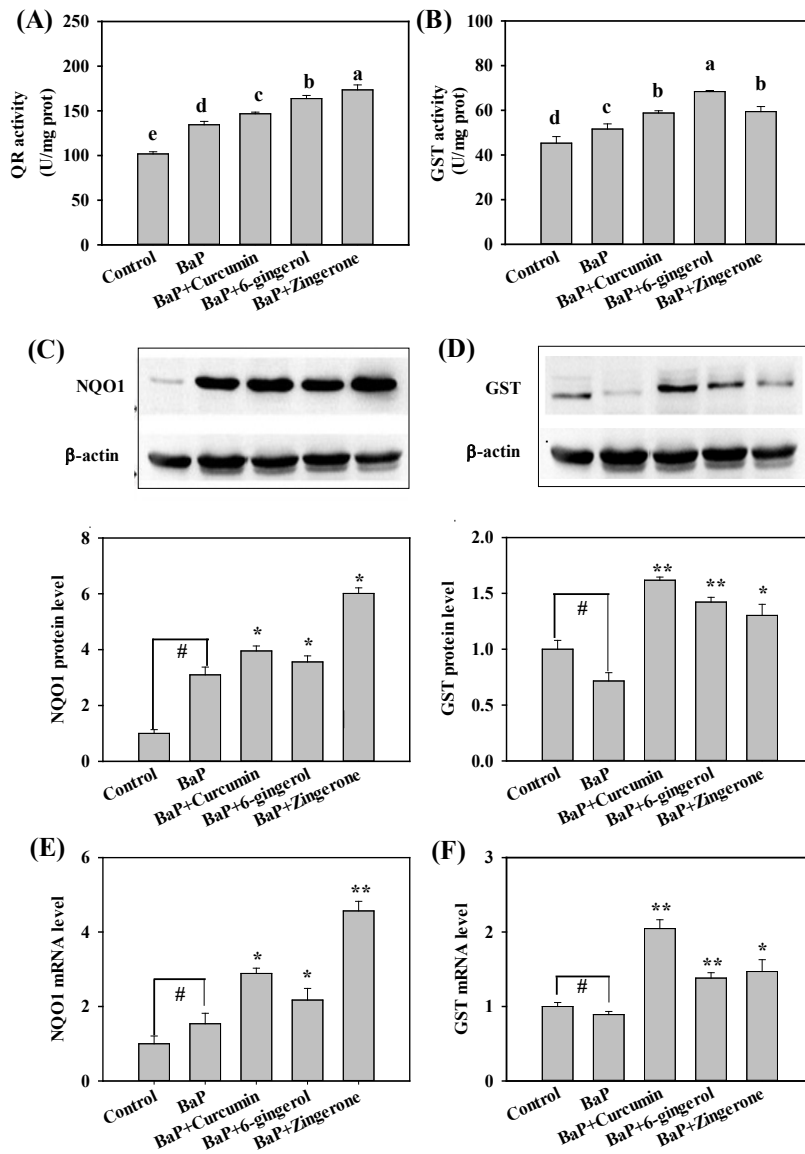
Figure 2. Effect of curcumin, 6-gingerol, and zingerone on quinone reductase (QR) and glutathione-S-transferase (GST) in liver of mice exposed to BαP. (A) QR activity; (B) GST activity; (C) QR protein; (D) GST protein; (E) QR mRNA; and (F) GST mRNA. Data are means of ten mice per group with error bars indicating standard deviations. Histograms marked with different lowercase letters indicate significant different at $p < 0.05$. # = $p < 0.05$ vs. control; * and ** = $p < 0.05$ and $p < 0.01$ vs. the BαP-treated group.

Pre-treatment with curcumin, 6-gingerol, and zingerone before BαP exposure also caused significant increases of NQO1 mRNA levels as compared to the BαP treatment, and percentages increase for curcumin, 6-gingerol, and zingerone were 88.2, 41.8, and 198.0%, respectively (Figure 2E). Similar effects were also observed for GST mRNA, with the curcumin group showing the strongest effect (Figure 2F).

Expression of Nrf2, Keap1 protein, and mRNA
As shown in Figure 3, it is obvious that the expression of Nrf2 protein and mRNA in liver of

mice exposed to BαP was significantly affected by the pre-treatment of the ginger compounds, with zingerone being the most potent in stimulating the expression of hepatic Nrf2 protein and mRNA. A significant 194.3% increase in hepatic Nrf2 protein was observed in the zingerone-fed group against the BαP-treated group, followed by 172.2% for gingerol-fed and 105.3% for 6-curcumin-fed group. By contrast, the expression of Keap1 protein and mRNA in the mice exposed to BαP was significantly ($p < 0.05$) reduced as compared to the control (Figures 3B and 3D). However, the pre-treatment of curcumin or zingerone significantly ($p < 0.05$)
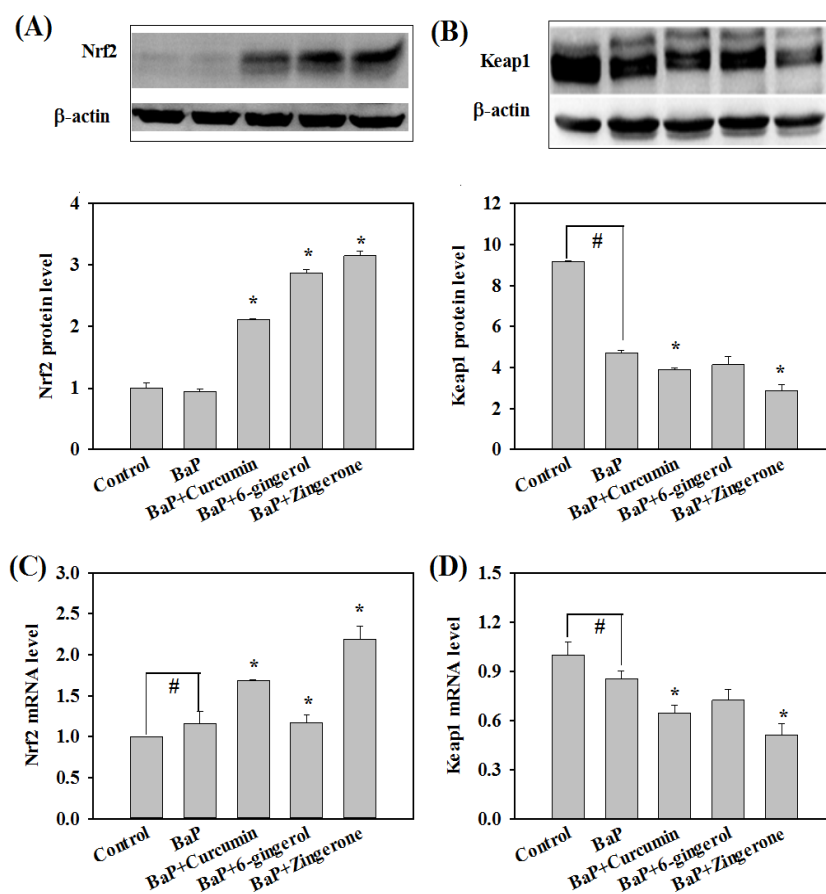
Figure 3. Effect of ginger compounds on the expression of Nrf2, Keap1 protein, and mRNA in mice exposed to BαP. (A) Nrf2 protein; (B) Keap1 protein; (C) Nrf2 mRNA; and (D) Keap1 mRNA. Data are means of ten mice per group with error bars indicating standard deviations. # = $p < 0.05$ vs. control; * and ** = $p < 0.05$ and $p < 0.01$ vs. the BαP-treated group.

inhibited this decreasing trend of Keap1 protein and mRNA, with zingerone exhibiting a stronger effect in suppressing the expression of Keap1 than any of other two ginger compounds when compared to the BαP treatment alone.

Discussion

BαP is an environmental and foodborne pollutant often generated during industrial processes and anthropogenic activities, including cigarette smoke, automobile emission, and culinary processes (Shiizaki et al., 2017; Reed et al., 2020). It is listed as a Group 1 carcinogen by International Agency for Research on Cancer (IARC) due to its roles as a potent mutagenic, carcinogenic, and pro-oxidative agent in experimental animals (Wester et al., 2012). BαP undergoes a metabolic activation to exhibit its carcinogenic effects in biological systems. Firstly, it is oxidised by the multi-enzymatic system CYP450 to generate intermediate metabolites (Shi et al., 2017). Secondly, these intermediates are conjugated with glutathione or carbohydrates catalysed by some

phase II drug metabolising enzymes (sulphotransferase, glutathione-S-transferase, and UDP-glucorosyl transferase) to form ultimately more polar compounds, and excreted from the body (Strobel et al., 2015). Carcinogenic effect of BαP is mainly related to some intermediates (i.e. BαP-quinones metabolites) produced during CYP450 metabolic process (Akcha et al., 2000). These quinones are electrophilic, hence covalently bind to cellular proteins and nuclear acids (Kim et al., 1998).

In the past decades, many classes of plant extracts or phytochemicals have been reported to protect experimental animals against BαP exposure (Park et al., 2010). Ginger rhizome is used worldwide both as a culinary spice and as a medicinal herb (Alsherbiny et al., 2019). Ginger extracts, especially gingerol and shogaol, have some degree of pharmacological effects including antioxidant, anti-inflammatory, anti-tumour, and analgesic (Kou et al., 2018; Mao et al., 2019; Ma et al., 2021). Our earlier results from cellular assay also indicated that some ginger compounds were potent inducers towards some phase II metabolising

enzymes involved during metabolic transformation of BαP (Li *et al.,* 2011). However, the effects of ginger compounds on xenobiotic toxicity in BαP-exposed mice are rarely reported.

In the present work, the mice treated with BαP alone or both BαP and ginger compounds did not show signs of general toxicity or acute adverse effects. No significant differences in body weight and liver-to-body weight ratio were observed between the control and any of these treatment groups, thus suggesting that neither BαP nor ginger compound at the dose used in the present work has negative effect on the status of mice. A dose of 20 mg/kg of BαP was far lower than its half-lethal dose of 500 mg/kg. Our results are in agreement with previous literature, showing that a dose of BαP less than 250 mg/kg body weight did not significantly affect the body weight gains in the treated mice (Ajayi *et al.,* 2016).

The toxicity of BαP metabolic intermediates (BαP-quinones) are known to be related to ROS such as superoxide anion radicals, hydrogen peroxides, as well as semiquinone radicals (Lesko and Lorentzen, 1985). Antioxidant enzymes involved in the elimination of ROS include SOD, CAT, and GPx. Therefore, these antioxidant enzyme activities were investigated in the liver and serum of mice exposed to BαP. The levels of three antioxidant enzymes were significantly stimulated in liver tissue and blood of mice fed with ginger compounds. Our results are in agreement with previous reports. Curcumin was shown to be a potent agent in the prevention of chemical-induced toxicity through enhancing antioxidative enzymes and lowering lipid peroxidation (Rong *et al.,* 2012). It was also able to inhibit the lipid oxidation in different animal models (Sreejayan and Rao, 1994). Physicochemical and antioxidant properties of curcumin were determined by the keto-enol-enolate equilibrium of the heptadienone moiety of curcumin (Ak and Gülçin, 2008). The antioxidant effect of gingerol and other ginger constituents has been proposed as one of major mechanisms for its pharmacological activities (Khan, 2019). The 6-gingerol-rich ginger extract could reduce the levels of H_2O_2 and MDA, enhance antioxidant enzyme activity, and increase glutathione in rats with oxidative damage induced by chlorpyrifos (Abolaji *et al.,* 2017). Studies on structure-activity relationship (SAR) of ginger constituents showed that the phenyl moieties and substituents on the alkyl chain of ginger phenolics might play important roles in exerting their antioxidant potency (Peng *et al.,* 2012). *In vivo* antioxidant activity of zingerone was reported by Kabuto *et al.* (2005), showing that zingerone supplementation to ethanol-fed rats increased the activities of enzymatic antioxidants in both the plasma and tissues.

Cytochrome P450 isoforms are known to metabolise BαP and other polycyclic aromatic hydrocarbons into their mutagenic metabolites (Zhang *et al.,* 2018). Therefore, the blockage of activity of cytochrome P450 was associated with a decreased metabolism of BαP and fewer BαP/DNA adducts. Our results showed that BαP exposure led to a significant increase in CYP1A1/2 activity in mice liver, and pre-treatment of ginger compounds for 14 consecutive days was able to significantly prevent the BαP-stimulated increase of both enzymatic activities. The expression of CYP1A1/2 proteins and mRNA in liver of mice was consistent with these enzymatic activities. Similar results were also observed by other researchers (Sidorova *et al.,* 2016) who found a significant stimulation of CYP1A1/2 activities in mice liver due to BαP treatment. Gan *et al.* (2019) found that there were no significant ($p < 0.05$) changes in the rats supplemented with zingerone alone, even for a period of 16 weeks, as compared to normal rats. However, the pre-treatment of zingerone effectively prevented the 7,12-dimethylbenz(a)anthracene-induced increase of CYP450 in rats. Mukkavilli *et al.* (2014) evaluated the effects of ginger extract and its major constituents on cytochrome P450 enzyme activity in human liver microsomes. Results showed that 6-gingerol and 6-shogaol were inhibitors of CYP1A2 with an IC_{50} of 5.6 and 0.70 μg/mL, respectively.

Xenobiotic transformation and antioxidant defence systems are widely viewed as affording protection from cancers through the detoxification of potential carcinogens and mitigation of oxidative stress. The induction of phase II enzymes was proposed as a major mechanism of cellular protection against the toxic and reactive chemical species (Begleiter *et al.,* 1997). NAD(P)H quinone oxidoreductase, one of the important components of phase II detoxification enzyme systems, catalyses the two-electron reductions of a variety of quinones, thereby protecting cells against mutagenicity and carcinogenicity (Cuendet *et al.,* 2006). Many classes of plant extracts or phytochemicals, which are identified as potential chemopreventive agents, are related to their potency in inducing quinone reductase (Surh *et al.,* 2008). The GST is another critical detoxification enzyme that primarily catalyses the conjugation of electrophilic compound with reduced glutathione, thus favouring their

elimination from the body (Yang, 2006). The elevation in the activities of QR and GSTs are known to provide protection against the onset of redox cycling, oxidative stress, and other toxic effects of many carcinogens (Rushmore and Pickett, 1993). Herein, pre-treatment of ginger compounds to mice showed a significant elevation in the activities, protein expression, and mRNA levels of NQO1 and GST in the liver of mice. Similar results were reported by other researchers (Garg *et al.,* 2008) showing that dietary curcumin (0.05%) led to significant induction of GST and NQO1 activity, protein, and mRNA expressions in the liver of Bα P-treated mice. These findings are in agreement with our previous report that some compounds isolated from ginger rhizome are potent quinone reductase inducers (Li *et al.,* 2011). Previous studies have shown that the activation of Nrf2/ARE-Keap1 pathway forms the major node of cellular defence against oxidative and electrophilic stresses of both exogenous and endogenous origins, such as BαP exposure (Yamamoto *et al.,* 2018). Therefore, we next addressed whether these compounds-mediated enhancement of phase II enzymes *in vivo* paralleled the increase in Nrf2 expression. The pre-treatment with the ginger compounds led to significantly ($p <$ 0.05) increased hepatic Nrf2 protein and mRNA expression (Figure 3A and 3C), and decreased Keap1 protein and mRNA levels as compared to the BαP-only treatment (Figures 3B and 3D). These findings show the parallel increase in activities of antioxidant enzymes and phase II enzymes like GSTs and NQO1, and increased nuclear accumulation of Nrf2 in the liver of BαP-treated mice, thus suggesting that the alleviation of xenobiotic toxicity in BαP-treated mice might be related to Nrf2/ARE-Keap1 signalling pathway. Under physiological conditions, Keap1 acts as an adaptor component in the Cul3-based E3 ligase to mediate the ubiquitination of Nrf2, which maintains the cellular content of Nrf2 at low level (Zhang *et al.,* 2019). Under stressed conditions (such as BαP exposure), excessive ROS insults can covalently modify the sensitive cysteine residues in Keap1 protein, thus leading to the elevation and nuclear translocation of Nrf2 and the activation of Nrf2-regulated antioxidant genes (Yamamoto *et al.,* 2018). Our data showed that these ginger compounds inhibited the expression of Keap1, which caused a depression of Nrf2, thereby elevating the expression of Nrf2 as well as subsequent downstream antioxidant enzymes. Previous studies have shown that Michael reaction acceptor and unsaturated carbonyl structures are important for the

ability of the various active compounds to activate the Nrf2/Keap1 pathway (Imm *et al.,* 2010). In the present work, curcumin possesses two Michael reaction groups, whereas 6-gingerol and zingerone contain an unsaturated carbonyl moiety. It is noteworthy that as compared to the BαP-only treatment, the expression of Keap1 protein in 6-gingerol pre-treatment group did not exhibit significant difference, thus suggesting that the position of the carbonyl moiety may influence the inhibitory effect of ginger compounds on the expression of Keap1 protein. Our results are in agreement with earlier report that dietary curcumin showed significant inhibition of BαP-induced phase I enzyme and concomitant induction of phase II enzymes, as well as a paralleled activation of Nrf2 (Garg *et al.,* 2008). Liu *et al.* (2007) also found that phase II genes (GCLC, GST-P1, and NQO1) were induced by *Ginkgo biloba* extract through the Nrf2-Keap1-ARE signalling pathway. However, more efforts are needed to investigate whether the enhanced Nrf2 is due to the dissociation of the Nrf2-Keap1 complex or the stimulation of Nrf2 synthesis in mice.

Conclusion

The present work evaluated the effect of three ginger compounds (curcumin, 6-gingerol, and zingerone) on antioxidant and xenobiotic-metabolis-ing enzymes in mice exposed to B(α)P. Results suggested that these ginger compounds inhibited carcinogen-induced CYP450 isozymes by modulating the transcriptional regulator of CYP1A, and inducing phase II enzymes through Nrf2-Keap1 pathway. These findings contribute to our theoretical understanding of the potential beneficial effects of consuming ginger as food and/or dietary supplement.

Acknowledgement

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