# Ginger (*Zingiber officinale*) prevents severe damage to the lungs due to hyperoxia and inflammation

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**Background/aim:** Hyperoxia- and inflammation-induced lung injury is an important cause of the development of bronchopulmonary dysplasia (BPD) in premature infants. We aimed to ascertain the beneficial effects of ginger (*Zingiber officinale*) on rat pups exposed to hyperoxia and inflammation.

**Materials and methods:** Thirty-six newborn Wistar rats were randomly divided into 3 groups as the hyperoxia (95% $O_2$) + lipopolysaccharide (LPS) group, the hyperoxia + LPS + ginger-treated group, and the control/no treatment group (21% $O_2$). Pups in the hyperoxia + LPS + ginger group were administered oral ginger at a dose of 1000 mg/kg daily during the study period. Histopathologic, immunochemical (SMA and lamellar body), and biochemical evaluations including total antioxidant status (TAS), total oxidant status (TOS), malondialdehyde (MDA), myeloperoxidase (MPO), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and caspase-3 activities were performed.

**Results:** Better weight gain and survival rates were shown in the hyperoxia + LPS + ginger group (P < 0.05). In the histopathologic and immunochemical evaluation, severity of lung damage was significantly reduced in the hyperoxia + LPS + ginger group, as well as decreased apoptosis (ELISA for caspase-3) (P < 0.05). Tissue TAS levels were significantly protected, and TOS, MDA, and MPO levels were significantly lower in the hyperoxia + LPS + ginger group (P < 0.05). Tissue TNF-α, IL-1β, and IL-6 concentrations were significantly decreased in the ginger-treated group (P < 0.05).

**Conclusion:** Ginger efficiently reduced the lung damage and protected the lungs from severe damage due to hyperoxia and inflammation. Therefore, ginger may be an alternative option for the treatment of BPD.

**Key words:** Bronchopulmonary dysplasia, ginger, inflammation, newborn, oxygen-induced lung injury, rat

## 1. Introduction

Bronchopulmonary dysplasia (BPD), also called neonatal chronic lung disease, is an important cause of significant morbidity and mortality related to lung injury in preterm newborns, leading to frequent hospitalizations, recurrent respiratory exacerbations, exercise intolerance, and adverse neurodevelopmental outcomes (1–4). The exact pathogenic mechanisms underlying the development of BPD are uncertain. However, recent evidence suggests complex roles of pre- and postnatal factors in the development of BPD. Prematurity is believed to be the most important contributing factor, in addition to other factors, including oxidative stress and inflammation-

mediated lung injury (chorioamnionitis), hyperoxia/value-barotrauma, and genetic factors (5,6). Treatment strategies for the prevention of lung injury and appropriate induction of lung growth to avoid the development of BPD are still lacking (3,7). Therefore, new treatment options need to be identified for the prevention of severe injury to the lungs in the course of BPD.

Ginger (*Zingiber officinale*) is one of the most commonly used spices all over the world. It is known to have many health benefits and is also applied to treat respiratory diseases. Ginger contains more than 60 different active ingredients, including volatile (hydrocarbons and sesquiterpenes) and nonvolatile (gingerols, shogaols,

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892

paradols, zingerone) compounds, in addition to various minerals, vitamins, and enzymes (8–12). Some studies have determined that the whole extract and different separated compounds of ginger have antiinflammatory (8,9), antimicrobial (13), and antioxidant properties (13,14), along with immunomodulatory (15), antifibrotic (16), and cytoprotective/regenerative actions on different organ systems with a very low degree of toxicity (17). However, the effects of ginger on lungs with BPD are not yet known. Therefore, we aimed to ascertain the effects of ginger on the lungs of rat pups with BPD induced by hyperoxia and lipopolysaccharide (LPS).

## 2. Materials and methods

### 2.1. Animal model
Ethical approval was obtained from the Experimental Animal Ethics Committee of the Ankara Training and Research Hospital (Ankara, Turkey). The National Institutes of Health Guide for the Care and Use of Laboratory Animals was followed. Five pregnant Wistar rats were housed and cared for in individual cages with 12-h light/dark cycles. Laboratory food and water were available ad libitum. For timed matings, embryonal day 0 was defined as the morning of vaginal plug discovery. Pregnant rats were randomized into two groups: 1) no-treatment group (n = 2); 2) intraamniotic LPS injection group (n = 3). For the establishment of chorioamnionitis in pregnant rats, the rats were anesthetized with sodium pentobarbital injection (50 mg/kg intraperitoneal). The abdominal wall was infiltrated with 0.1–0.2 mL of bupivacaine. A midline abdominal incision allowed externalization of the uterine horns. Direct intraamniotic injection of 1 µg of LPS (*Escherichia coli* serotype 0111:B4, Sigma-Aldrich Chemical, Germany) solubilized in 0.1 mL of normal saline or endotoxin-free saline, was injected into the amniotic sacs of the pregnant rats on embryonal days 16 and 20 (18,19). Pregnant rats delivered spontaneously. Pups were then pooled and randomized, and pups in the LPS group were divided into two groups immediately after birth and returned to nursing dams. One group received only LPS and the other received LPS plus ginger. Each group consisted of 12 pups. The experiment began within 12 h after birth and continued through postnatal day 14. Humidity was maintained at 50%, and environmental temperature was maintained at 24 °C. Pups in the hyperoxia group were exposed to 95% $O_2$, while the pups in the control group were cared for in room air (21% $O_2$). Nursing dams were rotated between hyperoxia and room air-exposed litters every 24 h to prevent oxygen toxicity in the dams. Continuous 95% $O_2$ exposure was achieved in a Plexiglas chamber (70 × 60 × 30 cm) by a flow-through system, and $CO_2$ was removed by soda lime absorption. The oxygen level inside the Plexiglas chamber

was monitored continuously with a Ceramatec (MAXO$_2$) oxygen analyzer to maintain ≥95% $O_2$ saturation. The pups were weighed daily with scale sensitivity of 0.01 g and their weights were recorded.

### 2.2. Preparation and dosage of ginger
Standardized ginger root capsules (SWH025, Swanson Health Products, USA) were used. Capsules combined 250 mg of guaranteed-potency ginger extract (standardized to 5% gingerols and 5% shogaols) with 250 mg of dried ginger powder. Ginger was administered orally with a feeding catheter (2 F) to each rat pup at a dose of 1000 mg/kg through dissolved in 0.2 mL of distilled water (20,21). Pups in the control group (21% $O_2$ for 14 days + placebo) and hyperoxia + LPS group (95% $O_2$ for 14 days + placebo) were administered oral saline (2 mL/kg), but pups in the hyperoxia + LPS + ginger group (95% $O_2$ for 14 days + ginger) were treated once a day from the first day of the study to the end of the study. In the present experiment, we used whole ginger since many different compounds in ginger have been determined to act in a synergic manner that suggests the importance of using whole ginger (8–17).

### 2.3. Preparation of lung tissue for histological and biochemical analysis
At the end of the experiment, all pups were sacrificed on the 14th day of the study under deep anesthesia by ketamine and xylazine (100 and 10 mg/kg) intraperitoneally. The thorax was then opened, and the lungs were resected after perfusion of the heart with normal saline. After a tracheal cannula was placed, the lungs were excised and perfused by normal saline. During perfusion, a constant inflation pressure of 5 cmH$_2$O was maintained via the tracheal catheter. The right main bronchus was then ligated with a surgical suture, excised, and saved for later biochemical analyses. The left lungs were fixed by slowly perfusing 0.1 M phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA). Upon completion of perfusion, the trachea was ligated with a surgical suture, and the lungs were incubated in fresh 4% PFA-PBS solution on ice for 4–5 h. Following this incubation, PFA-PBS solution was replaced with two quick changes of cold PBS to remove exterior debris. The lungs were then transferred to a filtered sterile PBS/30% sucrose solution and stored at 4 °C until fully equilibrated. The right lung tissues were kept in PBS solution and stored at –80 °C until biochemical analysis was performed.

The lungs were paraffin-embedded and these paraffin blocks were sliced into sections of 4–5 µm. Sections were chosen according to a systematic random sampling procedure and then mounted onto poly-L-lysine-coated slides (Histobond adhesion slides, Marienfeld, Germany). Slides were stained for histopathologic and immunohistochemical evaluations with standard hematoxylin and eosin (H&E) and with the ABC technique

for the lamellar body membrane protein and smooth muscle actin (SMA) expression. Histopathologic examination and immunohistochemical scoring were evaluated in a blinded fashion by an experienced pathologist unaware of the treatment group for each sample.

## 2.4. Histopathological examination

Paraffin blocks of the tissues were sliced into portions of 4 μm and stained with H&E for histologic evaluation. Ten H&E-stained sections of each time point for each rat were randomly selected. Five fields for each section were examined under a light microscope (200×) to observe histological changes, estimate radical alveolar counts (RACs), and calculate the mean value. Histopathologic scoring was graded as follows: grade 1, normal histology; grade 2, moderate leukocytic infiltration; grade 3, leukocytic infiltration, edema, and partial destruction; and grade 4, total destruction of the tissue. Sections were evaluated for RAC through digital images for the assessment of the alveolar development. Briefly, a line superimposed from the center of a respiratory bronchiole to the nearest connective tissue septum at right angles to the epithelium and the number of the alveolar septa crossed by this line were counted on 3 or 4 sections for each animal (22).

## 2.5. Immunohistochemical detection of lamellar body membrane protein

For immunohistochemical detection of lamellar body membrane protein, sections were subjected to rehydration followed by treatment with 3% hydrogen peroxide for 30 min. Subsequently, sections were subjected to nonspecific blocking with goat serum for 30 min, and sections were then incubated with primary antibodies against lamellar body membrane protein (1:100, Chemicon, AB3623-rabbit, USA) overnight at 4 °C, followed by treatment with biotinylated antirabbit secondary antibody (1:200, Vector Laboratories, UK) for 30 min at room temperature. Following the avidin-biotin complex treatment, 3,3'-diaminobenzidine (DAB; Vector Laboratories) was used for color development. For negative control slides, omission of the primary antibody was also done. The tissue sections were examined using a light microscope interfaced with a digital camera. Lamellar body-positive cells (type II cells) in the alveolar area were counted five times on ten sections 25 μm apart for each animal. Data were presented as number of lamellar body-positive cells (type II cells)/mm².

## 2.6. Immunohistochemistry for SMA expression

SMA expression was visualized using the avidin-biotin-peroxidase method. Embedded lung tissues were sectioned on poly-L-lysine-coated slides. The sections were deparaffinized in xylene, rehydrated, and immersed in distillate water. Endogenous peroxidase activity was blocked using a 0.3% solution of hydrogen peroxidase in PBS. The primary antibody against α-SMA (ready-to-use, NeoMarkers, USA) was applied for 30 min at room temperature and washed in PBS. Linking antibody and streptavidin-peroxidase complex (NeoMarkers) were added consecutively for 10 min at room temperature and washed in PBS. The sections were stained using 3-amino-9-ethylcarbazole (AEC) as a chromogen for 7 min for immunohistochemical demonstration of actin. Finally, the sections were counter-stained with Mayer's hematoxylin and the slides were dehydrated and mounted. After that, tissue sections were examined using a light microscope interfaced with a digital camera. Ten nonoverlapping microscopic fields were selected at 10× magnification in a random manner for immunohistochemical scoring. The degree of positive staining was evaluated by a semiquantitative scoring on a scale of 1–4 for intensity (I) and distribution (D). Tissues with I × D less than or equal to 4 were considered weakly positive, and those with I × D greater than 4 were designated as strongly positive (23).

## 2.7. Biochemical analysis

Tissues were homogenized in physiological saline (1 g in 5 mL) using a homogenizer (IKA T18 Basic Ultra-Turrax, Germany) and centrifuged at $4000 \times g$ for 20 min (NF 800 R Nuve). Clear supernatants were removed to be used in the analyses. Protein levels were measured by using Lowry's method (24). Measurements were performed with a spectrophotometer (UV Shimadzu 1700, Japan).

## 2.8. Measurement of TAS and TOS

TAS and TOS were determined using a novel automated colorimetric measurement method developed by Erel (25). The results are expressed as mmol Trolox equivalent/g protein for TAS and μmol $H_2O_2$ equivalent/g protein for TOS in the intestine tissue samples.

## 2.9. Determination of MDA activity

MDA levels were recognized as an index of lipid peroxidation in the intestinal tissues. Tissue MDA levels were determined by the method of Draper and Hadley based on the reaction of MDA with thiobarbituric acid at 95 °C (26). Results were expressed as nmol/g protein.

## 2.10. Detection of MPO activity

This method is based on the principle that homogenate containing MPO activity reduces o-dianisidine dihydrochloride in the presence of $H_2O_2$, and this reduced product gives absorbance at 460 nm. MPO activity was computed using the o-dianisidine extinction quotient. Results were stated as units per gram of wet tissue (U/g) (27).

## 2.11. Determination of TNF-α, IL-1β, and IL-6 concentrations

Lung tissue TNF-α, IL-1β, and IL-6 concentrations were measured in duplicate with a commercially available

enzyme-linked immunosorbent assay kit (BioSource Europe S.A., Belgium) according to the manufacturer's instructions. Results were expressed as pg/mg protein.

## 2.12. Caspase-3 detection

Lung tissue caspase-3 levels were measured with a commercially available enzyme-linked immunosorbent assay kit (rat CASP3, ELISA Kit, BioSource Europe S.A.) (sensitivity: 0.188 ng/mL, detection range: 0.313–20 ng/mL) according to the manufacturer's instructions. Results were expressed as ng/g protein.

## 2.13. Statistical analysis

SPSS 16.0 (SPSS Inc., USA) was used for statistical analysis. The Shapiro–Wilk test was used to examine the normal distribution of values graphically. Descriptive statistics were shown as median (IQR) and mean and standard deviation (SD). ANOVA with the Bonferroni test was performed for intergroup analysis for parametric variables, but the Friedman test was used for intergroup comparisons of nonparametric variables. The Wilcoxon test with Bonferroni adjustment were applied for comparisons of categorical variables for independent groups. ANOVA with Bonferroni adjustment was used to analyze independent data with a normal distribution, and the Mann–Whitney U test was used in the comparison of data without a normal distribution. Survival time analysis was performed using the Kaplan–Meier method, and statistical differences were confirmed by log-rank test. Power analysis was conducted. If 0.80 effect size, 0.05 alpha, and 0.20 beta were taken to obtain 80% power, 12 animals were found to be adequate in each group.

## 3. Results

At the end of the study, 4 (33.3%) rat pups in the hyperoxia + LPS and 1 (8.3%) rat pup in the hyperoxia + LPS + ginger group had died. The survival rate was significantly higher in the hyperoxia + LPS + ginger group than the hyperoxia + LPS group (P < 0.05) (Table 1). The median survival time in the hyperoxia + LPS group was 10.9 days (95% confidence interval: 10.2–12.9 days), and it was 13.9 days (95% confidence interval: 13.8–14.1 days) in the hyperoxia + LPS + ginger group (log rank P = 0.019) (Figure 1). There were no significant differences among the three groups in terms of birth weight (P = 0.53). At the end of the study, the mean body weight of the pups in the hyperoxia + LPS + ginger group was significantly higher than those in the hyperoxia + LPS group (P = 0.02) (Table 1).

In histopathological examination, severity of lung damage was defined as grade 1 to grade 4. Less lung damage was detected in the hyperoxia + LPS + ginger group compared to the hyperoxia + LPS group (P = 0.002) (Figures 2A–2C; Table 1). Thickening of the alveolar septa or cell infiltration was not seen in the control group. However, significant changes in histological grading and improvement were determined in the ginger-treated group compared to the hyperoxia + LPS group (P < 0.05). Lung tissue samples from the hyperoxia + LPS group displayed abnormal alveolar structure, having enlarged alveoli with decreased terminal air spaces and secondary septa (Figures 2A–2C). RACs revealed that the alveolar space count was significantly increased in the ginger-treated group compared with the hyperoxia + LPS group (Figures 2A–2C). These data reflect more intact alveoli in the ginger-treated group (P < 0.05). We also examined lamellar body protein-positive cells (type II cells) for each specimen and a significantly better density of lamellar body membrane protein was observed in the ginger-treated group than the hyperoxia + LPS group (P < 0.05) (Figures 3A–3C; Table 1). In addition, considerably decreased alveolar fibrosis

**Table 1.** Comparison of weight changes, histopathological evaluation, radial alveolar count, lamellar body protein levels, and mortality of the study groups.

| Variables | Control (n = 12), mean ± SD, median (IQR) | Hyperoxia (n = 12/8), mean ± SD, median (IQR) | Hyperoxia + ginger (n = 12/11), mean ± SD, median (IQR) | P |
|---|---|---|---|---|
| Birth weight (g) | 5.3 ± 0.4 | 5.23 ± 0.5 | 5.25 ± 0.35 | 0.53 |
| Weight on day 14 (g) | 18.8 ± 3.5 | 13.7 ± 1.4 | 17.1 ± 1.3 | 0.02* |
| Death, n (%) | 0 | 4 (33.3%) | 1 (8.3%) | 0.03* |
| Histopathologic examination | 0 | 3.1 (1) | 2 (1) | 0.02* |
| Radial alveolar count | 12 ± 3.5 | 6.2 ± 2.1 | 8.9 ± 1.5 | 0.03* |
| Lamellar body protein-positive cells/unit area | 8 ± 2.2 | 2 ± 0.3 | 6 ± 1.5 | <0.01* |

*Significant differences among all groups (P < 0.05).
SD: Standard deviation; IQR: interquartile range.
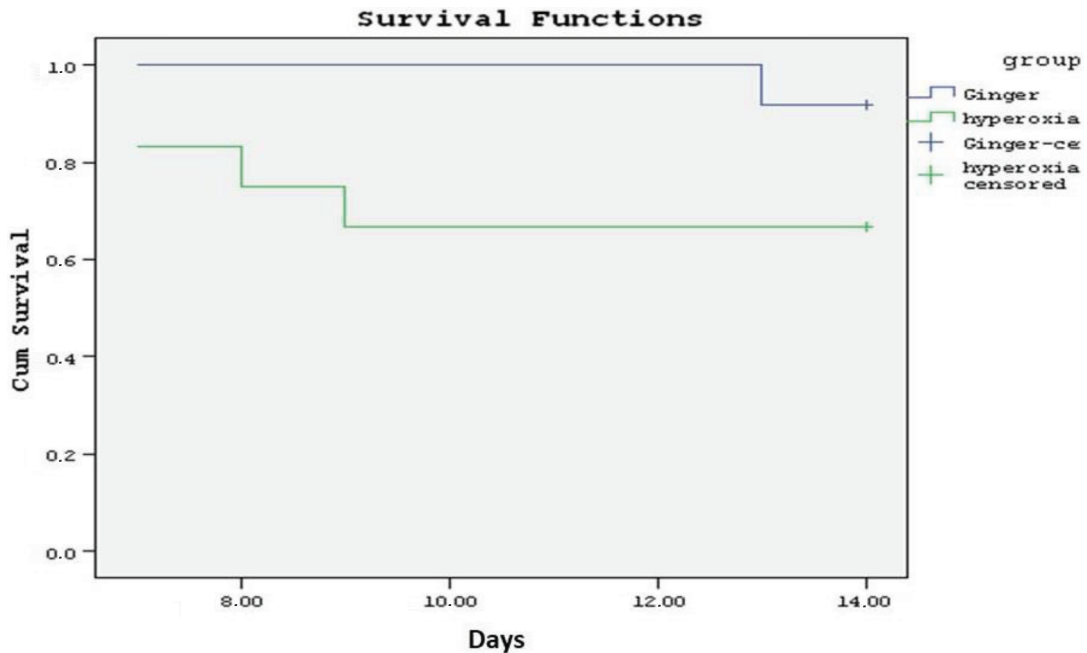
**Figure 1.** Kaplan–Meier survival curve: median survival times of pups in the study groups.
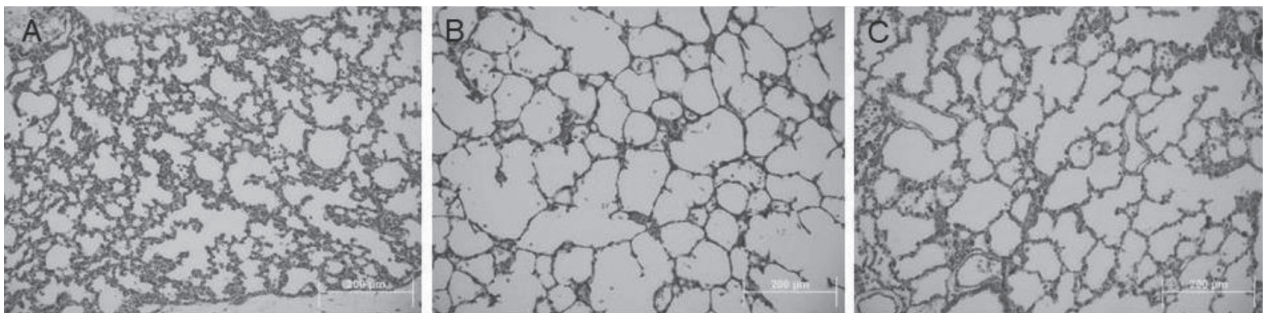


**Figure 2.** Lung sections of rat pups stained with H&E. A) Control group exposed to room air; B) after exposure to 95% oxygen and LPS; C) exposed to 95% oxygen + LPS and treated with ginger. Microphotographs are representative and were obtained at the same magnification (200×). Hyperoxia and LPS caused distal air space enlargement, and the alveolar architecture was simplified with reduced RACs.
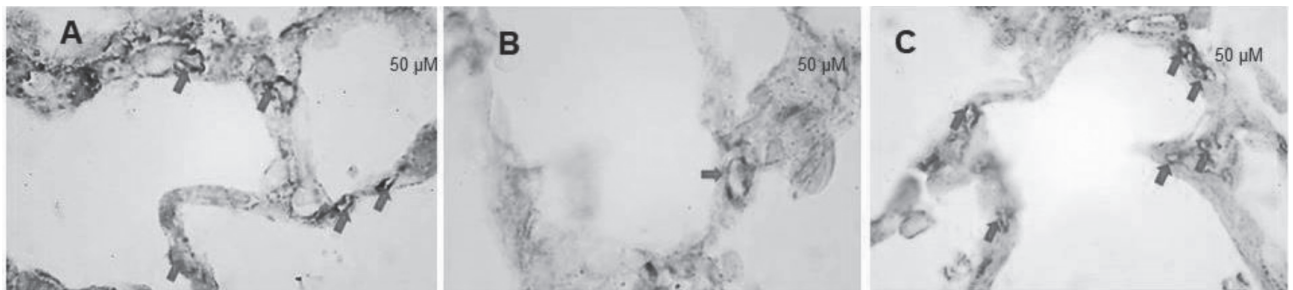


**Figure 3.** Representing the immunohistochemistry for the lamellar body membrane protein in each group (50 μm): A) control group exposed to room air (21% oxygen); significantly worse density of the lamellar body membrane protein in the hyperoxia + LPS group (B) compared to the hyperoxia + LPS + ginger group (C).

and decreased SMA immunostaining in smooth muscle content were seen in the ginger-treated group compared to the hyperoxia + LPS group (P < 0.05) (Table 2; Figures 4A–4C). In the harvested lung tissues, caspase-3 levels were evaluated to determine apoptotic changes in all groups. Caspase-3 levels were significantly lower in the ginger-treated group compared to the hyperoxia + LPS group (P < 0.05) (Table 3).

In biochemical analysis, lung tissue TOS and MDA levels were significantly lower in the ginger-treated group than in the hyperoxia + LPS group (P < 0.05). However, significantly lower TAS and higher MDA levels in the hyperoxia + LPS group were seen, indicating decreased oxidative stress and lower lipid peroxidation in the ginger-treated group (P < 0.05) (Table 3). In the ginger-treated group, there was significantly reduced MPO activity in the lungs (P < 0.05). Additionally, lung tissue TNF-α, IL-1β, and IL-6 levels were significantly decreased in the ginger-treated group (P < 0.05) in contrast to the hyperoxia + LPS group. These data suggest that ginger treatment decreased lipid peroxidation due to ROS as well as reduced neutrophil infiltration/inflammation in rat lungs subjected to hyperoxia + LPS (P < 0.05) (Table 3).

## 4. Discussion

Through this study, the beneficial effects of ginger on the lungs were investigated in a model of experimental lung injury that mirrors the findings of BPD. Bronchopulmonary dysplasia was created by hyperoxia and intrauterine LPS-induced inflammation as a model of chorioamnionitis. We determined that use of whole ginger protected the lungs from histopathological damage (parenchymal tissue destruction, fibrosis, abnormal alveolar structure, and cell apoptosis) as well as reduced ongoing tissue damage in the lungs. Biochemical analysis of harvested lung tissues revealed that factors playing important roles in the pathogenesis of BPD such as neutrophil infiltration, inflammation, oxidant stress, and lipid peroxidation were reduced, in addition to increased antioxidant activities in the injured lung tissues through the antiinflammatory, antioxidant, antifibrotic, and antiapoptotic effects of ginger on the lungs during the course of experimental BPD.

One of the most important risk factors in the development of BPD is believed to be preterm birth (1–6). Once a premature baby is born, he or she does not have fully developed lungs to adapt to external life. They

**Table 2.** Fibrosis and SMA immunostaining scores for all groups.

| Groups | Fibrosis | | | | SMA immunostaining | | | |
|---|---|---|---|---|---|---|---|---|
| | None | Weak | Moderate | Severe | None | Weak | Moderate | Severe |
| 1: Control (n = 12) | 12 | 0 | 0 | 0 | 10 | 2 | 0 | 0 |
| 2: Hyperoxia (n = 8) | 0 | 0 | 2 | 6 | 0 | 0 | 2 | 6 |
| 3: Hyperoxia + ginger (n = 11) | 6 | 3 | 1 | 0 | 6 | 3 | 1 | 0 |
| P-values<br>1 vs. 2<br>1 vs. 3<br>2 vs. 3 | 0.002*<br>0.021*<br><0.001* | | | | 0.001*<br>0.12<br>0.002* | | | |

SMA: Smooth muscle actin. *Significant differences among all groups (P < 0.05).
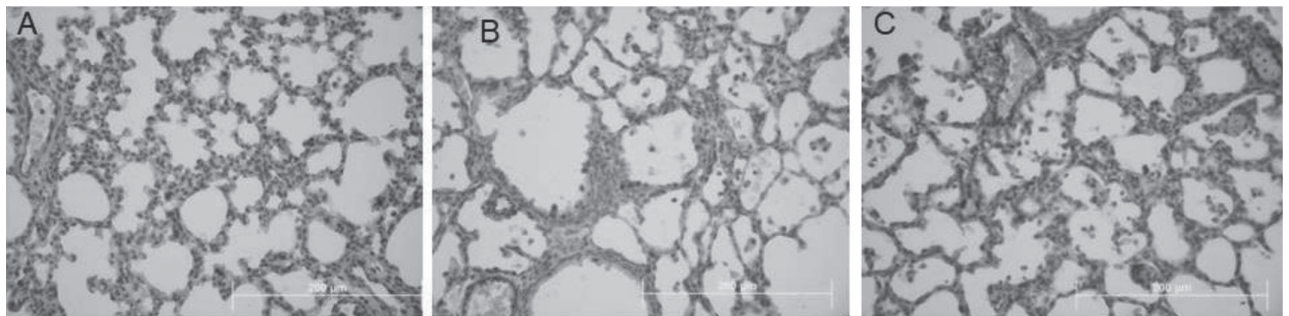


**Figure 4.** Immunohistochemical α-SMA staining (α-SMA, original magnification 200×): A) minimal fibrosis could be seen in the group exposed to room air; B) hyperoxia exposure increased local pulmonary interstitial fibrosis; C) the ginger-treated group had reduced fibrosis.

**Table 3.** Biochemical and apoptosis analysis of groups, including TAS, TOS, MDA, MPO, TNF-α, IL-1β, IL-6, and caspase-3 activities of the studied groups.

| Variables | Control (n = 12), mean ± SD, median (IQR) | Hyperoxia (n = 8), mean ± SD, median (IQR) | Hyperoxia + ginger (n = 11), mean ± SD, median (IQR) | P |
|---|---|---|---|---|
| TAS (mmol Trolox equivalent/g protein) | 9.43 ± 2.12 | 5.23 ± 0.34 | 7.13 ± 0.78 | <0.05* |
| TOS (µmol $H_2O_2$ equivalent/g protein) | 5.89 ± 1.5 | 63.34 ± 21.42 | 18.56 ± 7.41 | <0.05* |
| MDA (nmol/g protein) | 5.78 (0.64) | 52.33 (15.34) | 14.57 (4.12) | <0.05* |
| MPO (U/g protein) | 49.34 (8.58) | 174.61 (37.15) | 78.59 (15.61) | <0.05* |
| TNF-α (pg/mg protein) | 3.8 ± 2.2 | 12.2 ± 2.9 | 5.3 ± 2.4 | <0.05* |
| IL-1β (pg/mg protein) | 5.1 ± 1.8 | 12.9 | 6.3 ± 1.3 | <0.05* |
| IL-6 (pg/mg protein) | 48. ± 14.8 | 303.6 ± 24.3 | 157.3 ± 18.4 | <0.05* |
| Caspase-3 (ng/g protein) | 4.6 ± 0.3 | 24.3 ± 0.5 | 10.5 ± 0.6 | <0.05* |

*Significant differences among all groups (P < 0.05).
TAS: Total antioxidant status; TOS: total oxidant status; MDA: malondialdehyde; MPO: myeloperoxidase; TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin-1 beta, IL-6: interleukin-6.

experience a pause in the development of the alveolar epithelial, mesenchymal, and endothelial cell structures (5). Furthermore, inflammation has a considerable role in the pathogenesis of BPD. Particularly, premature infants have a high prevalence of inflammatory conditions such as chorioamnionitis during the perinatal period. Chorioamnionitis seems to be another important risk factor for the development of BPD by inducing inflammation in the lungs (5,6,28). During the inflammation process, several different proinflammatory cytokines are synthesized and released from alveolar macrophages, fibroblasts, type II pneumocytes, and endothelial cells through stimulation of hyperoxia, endotoxins, and other bacterial products (5,6). Additionally, there is an imbalance between pro- and antiinflammatory cytokines in the pulmonary tissue of preterm infants due to an inability to regulate inflammation in the lung (29,30). Increased levels of some proinflammatory cytokines such as IL-1β, IL-6, and TNF-α have been determined in the tracheal aspirate and serum of premature infants with BPD, and those with respiratory distress syndrome who subsequently developed BPD (31,32). In the present experiment, tissue levels of IL-1β, IL-6, and TNF-α were found to be reduced in the lungs of ginger-treated pups. Some experimental and clinical studies have reported that ginger extract diminishes acute and chronic inflammation through decreasing overexpression of proinflammatory cytokines in the lungs (8–11). After proinflammatory cytokines increase, inflammatory cells are attracted and influx into the lung. These cells are dominated by alveolar macrophages and neutrophils, leading to the persistence of the inflammation

by the production of further proinflammatory cytokines (5,6). During the inflammation process, neutrophil migration and infiltration into the lungs can be determined by measuring MPO activity in the lung tissues (33). MPO, a peroxidase enzyme, is specific for neutrophils and is expressed in neutrophil lysosomes (34). However, our results showed that MPO activity in the lungs was reduced with ginger treatment. Therefore, we suggest that ginger has antiinflammatory properties by decreasing neutrophil activation and migration into the lungs with evidence of reduced MPO activity in the lungs (9,11).

Oxygen toxicity has a key role in the development of BPD (1–7). High concentrations of inspired oxygen can damage lung cells by the overproduction of cytotoxic reactive oxygen species (ROS) such as superoxide free radical, hydrogen peroxide, and hydroxyl free radical. After hyperoxia, additional chemotactic effects of oxygen on inflammatory cells occur as well as the effects of cytokines and chemotactic factors. Inflammatory cells, neutrophils, and macrophages migrate to the lungs and are activated by hyperoxia, and ROS are produced by the cellular metabolism of molecular oxygen (35). ROS cause oxidative stress by oxidation of lipids, proteins, and DNA at the cellular level (36,37). Moreover, preterm infants are very prone to oxidative stress. They have immature and inadequate antioxidant enzyme system activity, such as superoxide dismutase, catalase, and glutathione peroxidase, as well as nutrient deficiencies such as vitamins A and E, iron, copper, zinc, and selenium (36–38). Thus, ROS overwhelm the neonate's immature antioxidant system and cause lung damage (36). Premature infants

with BPD also have increased lipid peroxidation. MDA, a reliable marker of lipid peroxidation and oxidative stress in the tissues, is the breakdown product of oxidation of polyunsaturated fatty acids by ROS (26,25). Therefore, ROS can be evaluated by the measurement of MDA and TAS/TOS levels in tissues (25,26,33). Ginger can be considered as a storehouse of antioxidants. The bioactive ingredients of ginger such as gingerols, shogaols, and zingerone have been shown to have antioxidant activity by inhibiting oxidase enzymes such as xanthine oxidase (8,9). Additionally, ginger enhances the activities of antioxidant enzymes and reduces the lipid peroxidation of cell membranes via ROS scavenging properties (35,39,40). In the present study, treatment with ginger resulted in decreased levels of MDA in addition to increased levels of TAS and reduced levels of TOS in the rat pups subjected to hyperoxia. These data showed that ginger reduces oxidative stress and lipid peroxidation, and enhances endogenous antioxidant systems in the lungs.

In premature infants, characteristic pathological changes seen in the lungs after BPD formation can be characterized by decreased alveolarization and alveolar hypoplasia leading to fewer and larger alveoli, as shown by decreased RAC and thickening of alveolar septa with significantly increased fibrosis. Moreover, surfactant deficiency plays an important role in the pathogenesis of BPD. In pathologic specimens, a reduced lamellar body expression pattern indicates severe lung injury that demonstrates decreased type II cells (33,41). In the current study, increased RAC and decreased thickness of the alveolar septa with remarkably reduced fibrosis and decreased SMA immunostaining, as well as better density of the lamellar body protein staining pattern, were present in the ginger-treated group (9–17,42). Additionally, during the BPD process, increased apoptosis in lung tissue cells has been demonstrated (43). In the present study, apoptosis was evaluated by measuring tissue caspase-3 levels, and ginger administration reduced cell apoptosis. Consequently, ginger protected lung injury with better alveolarization and decreased fibrosis and apoptosis.

In conclusion, our results determined for the first time that ginger significantly attenuated lung damage via inhibition of oxidative stress as well as augmentation of endogenous antioxidants, suppression of proinflammatory mediators and inflammation, institution of better alveolarization, and reduction of fibrosis and apoptosis. Therefore, ginger with its fewer side effects may be a promising candidate agent for maintaining greater functional lung activity of preterm infants afflicted with BPD. However, further studies are warranted to evaluate the efficacy of ginger in the prevention of lung injury prior to any clinical application.

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