Modulatory Effects of Curcumin and Green Tea Extract against Experimentally Induced Pulmonary Fibrosis: A Comparison with *N*-Acetyl Cysteine

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ABSTRACT: The study was aimed to investigate the protective effect of green tea extract (GTE), curcumin, and *N*-acetyl cysteine (NAC) on experimentally induced pulmonary fibrosis. Curcumin (200 mg/kg b.w), GTE (150 mg/kg b.w), and NAC (490 mg/kg b.w) were administered orally for 14 days with concomitant administration of cyclophosphamide (CP). Lung fibrosis was assessed by measuring hydroxyproline and elastin levels and confirmed by histopathological examination. Oxidative stress was also observed in the CP group. Lung myeloperoxidase activity was significantly decreased in animals of the CP group. *N*-acetyl-β-D-glucosaminidase, leukotriene C_4, and protein were increased in bronchoalveolar lavage fluid (BALF). Transforming growth factor-β, interleukin -1β, and histamine were increased in both serum and BALF. All modulators markedly attenuated the altered biochemical parameters as compared to CP-treated rats. These results suggest the possibility of using these treatments as protective agents with chemotherapy and as protective agents for lung fibrosis. © 2012 Wiley Periodicals, Inc. J Biochem Mol Toxicol 26:461–468, 2012; View this article online at wileyonlinelibrary.com. DOI 10.1002/jbt.21447

KEYWORDS: **Bronchoalveolar Lavage Fluid; Curcumin; Cyclophosphamide; Green Tea Extract Lung Fibrosis**

INTRODUCTION

Lung is a target organ for toxicity caused by a variety of xenobiotics due to environmental exposure to mineral dusts, airborne pollutants, cigarette smoke, or pharmacologic therapy with anticancer drugs that lead to acute and chronic inflammatory lung diseases. Under many conditions, this damage is repaired without any residual effects. However, the repair process can become deranged, resulting in the deposition of excess or abnormal collagen characteristic of fibrosis [1,2].

Five million individuals worldwide may suffer from idiopathic pulmonary fibrosis [3], which is a chronic, progressive lung disorder of unknown etiology in which excessive deposition of collagen-rich extracellular matrix (ECM) in the alveoli and interstitial tissues of the lung leads to impaired gas exchange [4]. The disease is associated with release of oxygen radicals and some mediators such as tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), platelets-derived growth factor, insulin-like growth factor-1 (IGF-I), and interleukins 1, 4, 8, and 13 as well as leukotrienes. The symptoms of the disease include dyspnea, nonproductive cough, fever, and damage to the lung cells [5].

Several models of experimental lung fibrosis are documented such as bleomycin, paraquat, and cyclophosphamide (CP) models [6–8].

CP, a chemotherapeutic agent commonly used in the treatment of lymphomas and leukemias, produces diffuse interstitial and alveolar edema and inflammatory changes that progress to lung fibrosis in animals and human [9]. The important factor for the therapeutic and the toxic effects of CP is the requirement of the metabolic activation by the hepatic microsomal cytochrome P450 mixed functional oxidase system [10]. Phosphoramide mustard and acrolein are the two active metabolites of CP [11]. CP antineoplastic effects are associated with the phosphoramide mustard, whereas the acrolein is linked with its toxic side effects [12]. Acrolein interferes with the tissue antioxidant defense system, produces highly reactive oxygen free radicals, and is mutagenic to mammalian cells [13].

Curcumin, a widely used yellow curry powder from turmeric (*Curcuma longa*) in Indian and other Asian cuisines has been used as a therapeutic agent because of its attractive combination of properties that

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include antiinflammatory [14], antioxidant [15], and anticancer qualities [16].

Green tea extract (GTE) has been reported to be useful in the prevention of cancer [17] and cardiovascular diseases [18] as well as antioxidant, scavenging reactive oxygen, and nitrogen species [19]. In addition, GTE has been found to modulate cytokine expression related to inflammation such as TNF-α by inhibiting the activation of nuclear factor KB (NF-κB) and activator protein 1 [20].

N-Acetyl cysteine (NAC), a well-known antioxidant, exerts direct antioxidant properties as a free radical scavenger and increases glutathione (GSH) levels as an L-cysteine prodrug in airway cells under oxidative stress [21]. Short-term studies using oral and intravenous NAC administration in patients with pulmonary fibrosis demonstrated that NAC improved antioxidant status of the lung by elevating GSH levels, as well as restoring pulmonary function tests with a low incidence of adverse effects [22, 23].

In the present study, we attempted to gain a better understanding of the biochemical changes associated with lung fibrosis and to correlate the reported histological changes and other biochemical markers, which led to development of CP-induced lung fibrosis. And also we aimed to study the protective effects of curumin and GTE as natural products against CP-induced lung fibrosis.

MATERIALS AND METHODS

Chemicals and Drugs

CP (Endoxan®) was purchased from Baxter Oncology (Frankfurt, Germany). GTE was received as a gift from Mepaco Company (Cairo, Egypt). Curcumin and NAC were purchased from Sigma(St. Louis, MO). All other chemicals and solvents used were of the highest purity and analytical grade.

Green tea powdered extract contained tea polyphenols (by UV) 82.6% and total catechins (by HPLC) 56.89%. The percentage of catechins in GTE was epigallocatechin-gallate 34.07%, epigallocatechin 29.8%, epicatechin 19.32%, gallocatechin-gallate 6.29%, and epicatechin-gallate 3.5% of total catechine content.

Experimental Design

One hundred two male albino Wistar rats (180–220 g) were used in the study. Animals had free access to water and standard rat chow throughout the study. All the procedures in this study are in accordance with the guidelines for the care and use of laboratory animals as adopted by the Ethics Committee of the Faculty of Pharmacy Cairo University.

Rats were divided into six groups. Group I (n = 8): Rats received normal saline, ip (vehicle for CP) and served as a normal control group. Group II (n = 20): Rats received CP dissolved in normal saline (150 mg/kg b.w., ip) for 2 consecutive days, then were kept for 7 days [24] and served as a lung fibrosis–induced group. Group III (n = 18): Rats were given 150 mg/kg b.w GTE [25]. Group IV (n = 18): Rats received 490 mg/kg b.w NAC [26]. Group V (n = 18): Rats received 200 mg/kg b.w curcumin dissolved in 1% carboxy methyl cellulose (CMC) [27]. Group VI (n = 20): Rats received 1% (CMC) (vehicle for curcumin). Groups from III to VI received an oral daily dose of their corresponding treatments for 14 days with concomitant administration of CP on days 7 and 8 with the same dosage regimen as in group II.

Collection of Blood, Bronchoalveolar Lavage Fluid, and Lung Tissues

At the end of the experimental period, all the animals were sacrificed by cervical decapitation. Blood of each rat was collected into a dry test tube and centrifuged at 3000 rpm for 15 min for separation of serum. After sacrificing the animals, thoracic viscera was exposed; a needle was inserted in the right ventricle, and the lung was washed with ice-cold physiological saline. The trachea was cannulated, and the lung was lavaged with ice-cold sterile physiological saline three times at a volume of 3 mL/wash. The bronchoalveolar lavage fluid (BALF) from each rat was centrifuged at 3000 rpm for 10 min to sediment the cells prior to biochemical assays.

Lungs were excised and washed with normal saline and dried, then two portions of lung were cut: The first one was fixed in 10% formol saline and used for histopathological analysis, and the other one was used for hydroxyproline determination. The remaining lung was homogenized in ice-cold double distilled water to make 10% homogenate. A suitable aliquot of homogenate was mixed equally with phosphate hexadecyltrimethyl ammonium bromide buffer pH 6, centrifuged at 10,000 rpm at 4°C for 15 min. The resulting supernatant was used for the determination of myloperoxidase (MPO) activity.

A second aliquot was mixed equally with 30% cold $ZnSO_4$, centrifuged at 17,000 rpm at 4°C for 15 min, and the resulting supernatant was used for the determination of nitric oxide (NO) level as NO_2/NO_3.

To determine the lung oxidant status, a suitable aliquot of homogenate was mixed equally either with 2.3% KCl or 7.5% sulfosalicylic acid, centrifuged at 3000 rpm, and the resulting supernatants were used for the determination of malodialdehyde (MDA) and reduced GSH levels, respectively.

Biochemical Measurements

Determination of Lung Fibrotic Markers

Collagen and elastin are the only proteins that have been found to contain hydroxyproline [28]. Since collagen is by far the most abundant protein in lung tissue, comprising 60–70% of the tissue mass [29]. Analysis of the hydroxyproline content of lung tissue has provided a reliable index of the quantity of collagen [30]. Hydroxyproline was determined according to the method described by Woessner [31]. Lung specimens were hydrolyzed by 6 N HCl at 100°C for 24 h. After neutralization, hydroxyproline was oxidized by buffered chloramine T reagent at room temperature. The chromophore was developed by heating with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde) at 60°C and then was detected at 555 nm.

The percentage of elastin is calculated from the following expression: [(micrograms of hydroxyproline)/(micrograms of sample) × factor] × 100. This factor is 43.4 for rat elastin [32].

Analysis of the hydroxyproline content of lung tissue has generally provided a reliable index of the quantity of collagen [30]

Determination of Lung Oxidative, Nitrosative Status, and MPO Activity

Lung GSH, MDA, NO levels, and MPO activity were determined according to the methods of Beutler et al. [33], Mihara and Uchiyama [34], Miranda et al. [35], and Bradley et al [36], respectively.

Determination of Inflammatory Markers in Serum and Lavage

Transforming growth factor-β1 (TGF-β1) may play a key role during the process of pulmonary fibrosis by increasing the synthesis and secretion of ECM protein through stimulation of its gene transcription and by decreasing degradation of ECM through inhibition of secretion of protein kinases [37].

In lung fibroblasts, leukotrienes stimulate collagen synthesis, chemotaxis, and transform into myofibroblasts [38]. IL-1β is believed to have an important role in the generation of acute inflammatory responses and a central role in experimental lung fibrosis [39]. Histamine is a vasoactive chemotactic mediator released from mast cells and has been reported as a putative mediator in the chain of events leading to fibrosis [40,41].

TGF-β1, interleukin-1β (IL-1β), and histamine were determined in both serum and BALF using ELISA kits supplied by WKEA Med Supplies Crop. (New York) and BioVendor Research and Diagnostic (Candler, NC) and DIAsource ImmunoAssays(Louvain-La-Neuve, Belgium), respectively. BALF leukotriene C4 ($LT\text{-}C_4$) was determined using an ELISA kit supplied by Neogen (Lansing, MI).

N-Acetyl-β-D-glucosaminidase (NAG) activity in lavage fluid was measured by a colorimetric method based on the enzymatic hydrolysis of the substrate *p*-nitrophoneyl-*N*-acetyl-β-D-glucusaminide by NAG in 0.1 M citrate buffer pH 4.4 at 37°C for 15 min and the produced yellow color in alkaline medium by the liberated *p*-nitrophenol was measured at 405 nm [42].

Total protein content in BALF fluid was determined according to the method of Lowry et al. [43].

Histopathological Examination

The lung sections were stained by hematoxylin and eosin stains, examined through the light microscope.

Statistical Analysis

Comparisons between the mean of the control and treated groups were carried out using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer post hoc test. In all cases, a probability level of $P < 0.05$ was considered to indicate a significant difference.

RESULTS

Analysis of Lung Fibrotic Markers

Lung hydroxyproline and elastin (Figure 1A) was significantly increased in group II (CP-treated group) and group VI (CMC + CP-treated group) when compared with the normal control group ($P < 0.05$). Curcumin, NAC, and GTE significantly decrease their content in lung as compared to CP-treated rats.

Analysis of Lung Inflammatory Markers

Curcumin, GTE, and NAC pretreatment markedly normalized the elevated NO level and the reduced MPO activity that observed in lungs of CP-treated rats (Figure 1B).

Analysis of Lung Oxidant Stress Markers

It was observed that CP administration significantly caused lung oxidative stress as evidenced by the increase in lung MDA and decrease in GSH levels in groups II and VI. Curcumin, GTE, and NAC normalized these parameters (Figure 1C).

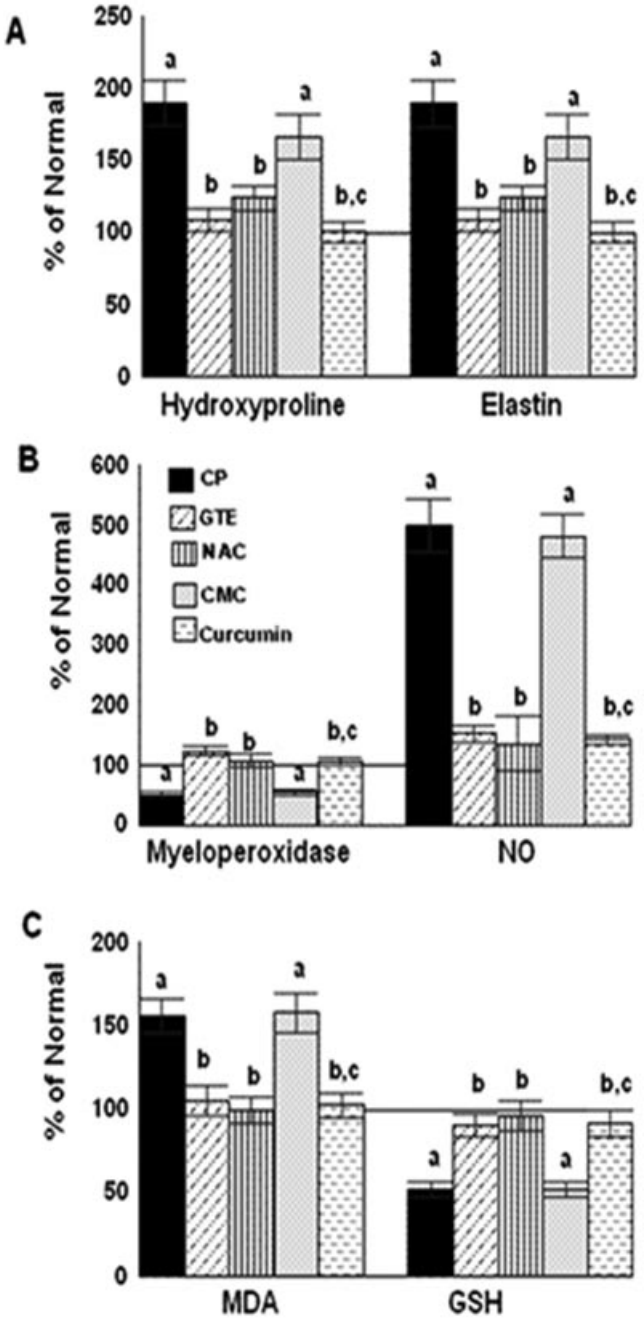

FIGURE 1. Effect of curcumin, GTE, and NAC on lung: hydroxyproline and elastin (A), MPO activity and NO level (B) and MDA and GSH and NO levels (C) in CP-induced lung fibrosis. Values are mean ± SEM of 8–12 observations per group and calculated as percentage of normal control. (a) Significant difference from the normal control group; (b) significant difference from the lung fibrotic group (CP); (c) significant difference from the CMC group, $P < 0.05$ (one-way ANOVA followed by the Tukey–Kramer post hoc test). Normal values for hydroxyl proline 628.27 μg/g, elastin 2.72 μg/g, MPO 1.51 IU/mL, NO 297 nmol/g, MDA 195.3 nmol/g , and GSH 352.93 μg/g.

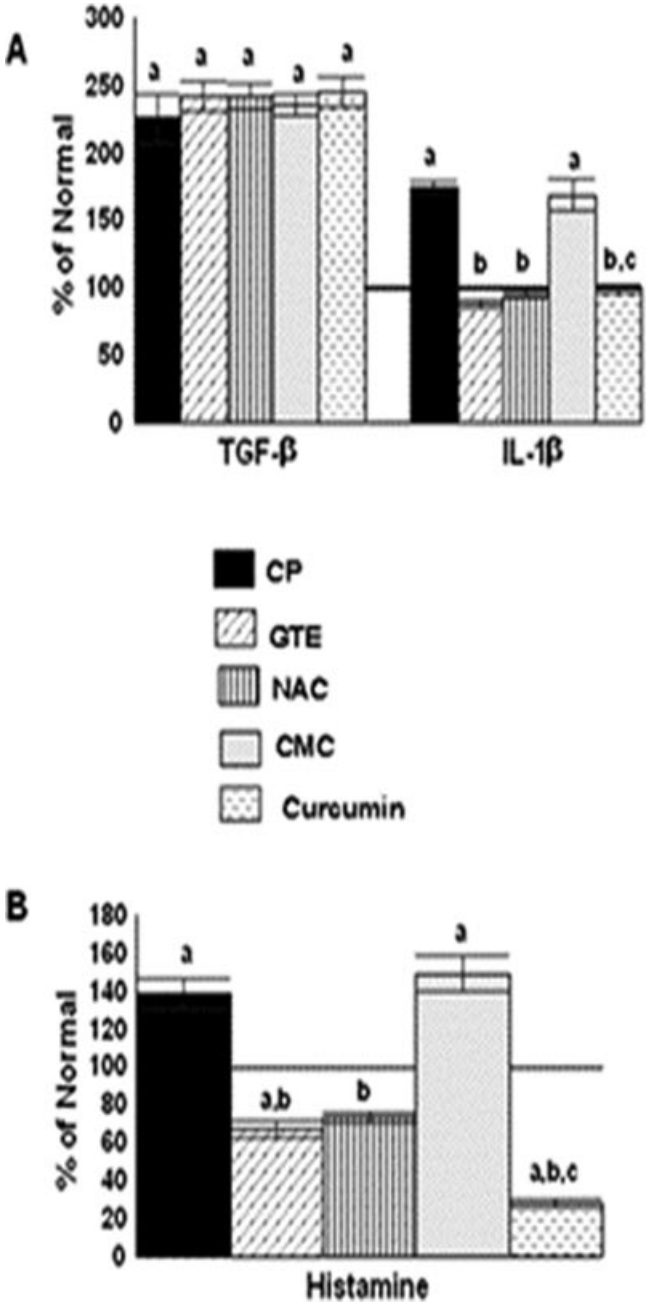

FIGURE 2. Serum TGF-β, IL-1β, and histamine in CP-induced lung fibrosis. Values are mean ± SEM of 8–12 observations per group and calculated as percentage of normal control. (a) Significant difference from the normal control group; (b) significant difference from the lung fibrotic group (CP); (c) significant difference from the CMC group, $P < 0.05$ (one-way ANOVA followed by the Tukey–Kramer post hoc test). Normal values for TGF-β 84.16 ng/L, IL-1β 304.93 pg/mL, and histamine 37.45 ng/mL.

Analysis of Inflammatory Markers in Serum

Serum TGF-β, IL-1β (Figure 2A), and histamine (Figure 2B) were increased significantly ($P < 0.05$) after CP administration. Pretreatment with curcumin, NAC, and GTE consistently resulted in a reduction of serum

TABLE 1. Effects of Curcumin, GTE, and NAC on TGF-β, Histamine, IL-1β, LT-C_4, Total Protein Levels and NAG Activity BALF in CP-Induced Lung Fibrosis

	Groups					
Parameter	*Normal*	*CP*	*GTE*	*NAC*	*CMC*	*Curcumin*
TGF-β (ng/L)	96.16 ± 9.44	137.94 ± 4.57^{a}	132.31 ± 4.35^{a}	142.25 ± 3.82^{a}	137.42 ± 8.31^{a}	136.81 ± 8.68^{a}
Histamine (ng/mL)	29.91 ± 1.98	56.81 ± 4.00^{a}	$2.04 \pm 0.23^{a,b}$	$9.36 \pm 0.77^{a,b}$	51.90 ± 4.40^{a}	$11.00 \pm 0.87^{b,c}$
IL-1β (pg/mL)	365.56 ± 32.4	596.66 ± 26.26^{a}	405.26 ± 22.84^{b}	362.12 ± 22.84^{b}	621.04 ± 34.46^{a}	$424.2 \pm 28.21^{b,c}$
LT-C4 (ng/mL)	0.866 ± 0.067	3.06 ± 0.28^{a}	0.85 ± 0.048^{b}	1.29 ± 0.1283^{b}	2.66 ± 0.217^{a}	$0.78 \pm 0.078^{b,c}$
Total protein (μg%)	32.35 ± 3.11	81.72 ± 7.62^{a}	51.32 ± 4.25^{b}	41.32 ± 3.41^{b}	96.26 ± 19.59^{a}	$37.00 \pm 3.13^{b,c}$
NAG (nmol/min/mL)	6.128 ± 0.545	13.15 ± 1.13^{a}	6.36 ± 0.55^{b}	6.99 ± 0.708^{b}	12.89 ± 0.969^{a}	$81.53 \pm 8.40^{b,c}$

Values are mean $\pm$ SEM of 8–12 observations per group.
[a]Significant difference from the normal control group, $P < 0.05$ (one-way ANOVA followed by Tukey-Kramer post hoc test).
[b]Significant difference from the lung fibrotic group (CP), $P < 0.05$ (one-way ANOVA followed by Tukey-Kramer post hoc test).
[c]Significant difference from CMC group, $P < 0.05$ (one-way ANOVA followed by the Tukey–Kramer post hoc test).

TABLE 2. Severity of the Reaction in the Lung According to Histopathological Alterations

	Groups					
Histopathological Alteration	*Normal Control*	*CP*	*NAC*	*GTE*	*CMC*	*CURCUMIN*
Fibrosis	–	+++	+	+	++	+
Collagen proliferation	–	+++	+	+	++	+

+++ indicates sever, ++ indicates moderate, + indicates mild, and – indicates nil.

IL-1β, but they did not alter the elevated level of TGF-β. The histamine level decreased significantly as compared either to the normal control or CP group.

Analysis of Inflammatory Markers in BALF

TGF-β, IL-1β, histamine, LT-C_4, total protein, and *N*-acetyl-β-D-glucosaminidase activity in BALF were increased significantly ($P < 0.05$) after CP administration. Curcumin, NAC, and GTE pretreatment resulted in excellent reduction in the elevated IL-1β, LT-C_4, histamine, total protein levels, and NAG activity. However, they did not affect the elevated TGF-β (Table 1).

Lung Histopathological Examination

In the control group (Figure 3A), there was no histopathological alteration with a normal histological structure of the bronchiole, air alveoli, and interstitial blood vessels. Focal fibrosis with collagen fibers were detected between the bronchiole and blood vessels (Figure 3B), as well as between adjacent blood vessels (Figure 3C) in group II. In group VI, focal fibrosis was observed in between the air alveoli, blood vessel, and bronchiole (Figure 3D). In curcmin, NAC, and GTE groups (Figure 3E–3G), respectively, few collagens as well as few fibroblastic cells proliferation were detected in a focal manner between the bronchiole and blood vessels.

The severity of the reaction in the lung according to histopathological alterations is demonstrated in Table 2.

DISCUSSION

Lung fibrosis was evidenced in the present study by the increased levels of lung hydroxyproline and elastin, correlating well with the histopathological evidence of fibrosis in most animals in the CP group. Our results are in good agreement with those reported in experimental animals exposed to a variety of lung toxicants [44, 45].

The observed decrease in lung GSH and an increase in MDA levels in the CP group might be due to the increased production of free radicals and a subsequent increase in lipid peroxidation.

Although it was expected that lung MPO activity would increase after CP administration due to accumulation of neutrophils and as previous studies showing [7, 46], our study showed that the MPO activity was decreased significantly after CP administration. This result may be due to the observed increase in the lung NO level, as NO is an inhibitor to MPO enzyme [47].

The observed increase in the TGF-β level in CP-treated rats may be due to the increased NO also, which stimulates the transcription factor (NF-κB) to promote TGF-β1 gene expression [48].

Acrolein generation during CP metabolism and the severe inflammatory reaction in consequence to the

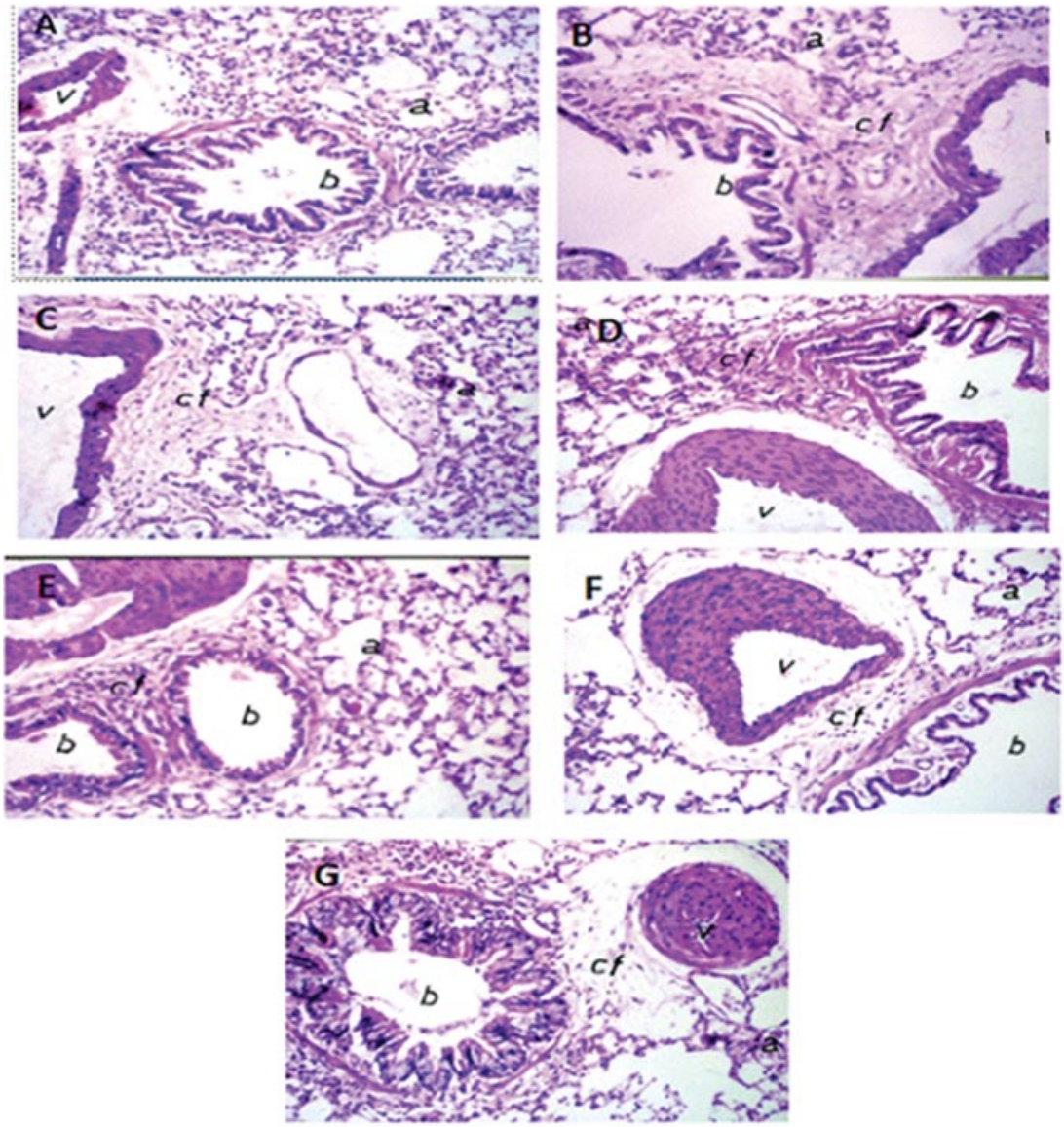

FIGURE 3. Light micrographs of lung sections stained with hematoxylin and eosin from the control group (A) showing the normal histological structure of the bronchiole (b), air alveoli (a) , and interstitial blood vessels (v), the CP group (B,C) showing collagen fibers and fibrosis between the bronchiole (b) and blood vessels (v) in focal manner in photograph B and focal fibrosis with collagen proliferation (cf) in between the blood vessels of stroma (v), the CMC group (D) showing focal fibrosis (cf) between air alveoli (a), blood vessels (v), and bronchioles (b) and curcumin, NAC, and GTE groups (E, F, G, respectively) showing mild collagen proliferation (cf) in between bronchioles (b).

enhanced lipid peroxidation leads to the increase in NAG activity and elevated total protein and inflammatory markers levels.

In our study, treatment with curcumin resulted in decreased oxidative and nitrosative stress and decreased inflammatory markers, thus indicating its potent antioxidant and antiinflammatory properties. The membrane-stabilizing action of curcumin, as evidenced by the observed decrease in NAG activity, prevented the damaging action of CP. Thus, curcumin with its high lipophilicity could localize within the membranous subcellular fraction and inhibit the propagation phase of membrane lipid peroxidation. Curcumin decreased the elevation in the lung hydroxyproline content as compared to the CP group. Similarly, it also decreased the excessive deposition of lung collagen in bleomycin-induced pulmonary fibrosis in rats [49]; the suggested mechanism may be inhibition of collagen gene transcription [50].

GTE alleviated the biochemical changes in the CP-treated rat lung. Previous studies indicated that the presence of a gallate (G) ring and either a catechol (B) ring or a pyrogallo was important for the antioxidant activities of catechins [51]. Our results showed that GTE

attenuated the CP-induced increase in IL-1β, which is in agreement with previous studies, suggesting that green tea regulates the IL-1β and TNF-α expression in transcriptional machinery [20,52].

Histological staining showed that GTE clearly reduced the patchy fibrosis; also quantitative determination of hydroxyproline confirmed the reduction of collagen deposition. The antifibrotic effect might be through the inhibition exerted by epigallocatechin-gallate on gelatinases involved in the damage to lung extracellular matrix, matrix metalloproteinases (MMP-2), and (MMP-9) [53].

In the present study, administration of NAC attenuated CP-induced lung fibrosis. Possible explanations for this antifibrotic effect may be the antioxidant efficacy and inhibition of fibroblast proliferation. Our data are consistent with a previous study that uses NAC in attenuation of experimentally induced lung fibrosis [54].

We found that our treatments decreased the histamine level as compared to CP and control groups. Previous studies explain that by suppressing compound 48/80 induced rat peritoneal mast cell degranulation and histamine release [55–57].

In conclusion, curcumin, GTE, and NAC have the potential to moderate CP-induced lung injury in rats Thus these compounds may have a wide application in suppressing drug- or chemical-induced lung injury.

REFERENCES

1. Kehrer JP, Kacew S. Systematically applied chemicals that damage lung tissue. Toxicology 1985;35:251–293.
2. Venkatesan N, Punithavathi D, Babu M. Protection from acute and chronic lung diseases by curcumin. Adv Exp Med Biol 2007;595:379–405.
3. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 2000;161:646–664.
4. Gal AA, Staton GW Jr. Current concepts in the classification of interstitial lung disease. Am J Clin Pathol 2005; 123(suppl):S67–S81.
5. Daba MH, El-Tahir KE, Al-Arifi MN, Gubara OA. Drug-induced pulmonary fibrosis. Saudi Med J 2004;25:700-706.
6. Sener G, Topaloglu N, Sehirli AO, Ercan F, Gedik N. Resveratrol alleviates bleomycin-induced lung injury in rats. Pulm Pharmacol Ther 2007;20:642–649.
7. Venkatesan N, Punithavathi D, Chandrakasan G. Biochemical and connective tissue changes in cyclophosphamide-induced lung fibrosis in rats. Biochem Pharmacol 1998;56:895–904.
8. Copland GM, Kolin A, Shulman HS. Fatal pulmonary intra-alveolar fibrosis after paraquat ingestion. N Engl J Med 1974;291:290–292.
9. Kehrer JP, Margolin SB. Pirfenidone diminishes cyclophosphamide-induced lung fibrosis in mice. Toxicol Lett 1997;90:125–132.
10. Sladek NE, Metabolism of oxazaphosphorines. Pharmacol Ther 1988;37:301–355.
11. Ludeman SM. The chemistry of the metabolites of cyclophosphamide. Curr Pharm Des 1999;5:627–643.
12. Kern JC, Kehrer JP. Acrolein-induced cell death: a caspaseinfluenced decision between apoptosis and oncosis/necrosis. Chem Biol Interact 2002;139:79–95.
13. Kawanishi M, Matsuda T, Nakayama A, Takebe H, Matsui S, Yagi T. Molecular analysis of mutations induced by acrolein in human fibroblast cells using SUPF shuttle vector plasmids. Mut Res Gen Toxicol Environ Mutagen 1998;417: 65–73.
14. Rao TS, Basu N, Siddiqui HH. Anti-inflammatory activity of curcumin analogues. Indian J Med Res 1982;75:574–578.
15. Sharma OP. Antioxidant activity of cureumin and related compounds. Biochem Pharmacol 1976;125:1811–1812.
16. Gescher A, Pastorino U, Plummer SM, Manson MM. Suppression of tumour development by substances derived from the diet—mechanisms and clinical implications. Br J Clin Pharmacol 1998;45:1–12.
17. Isemura M, Saeki K, Kimura T, Hayakawa S, Minami T, Sazuka M. Tea catechins and related polyphenols as anti-cancer agents. Biofactors 2000;13:81–85.
18. Sueoka N, Suganuma M, Sueoka E, Okabe S, Matsuyama S, Imai K, Nakachi K, Fujiki H. A new function of green tea: prevention of lifestyle-related disease. Ann N Y Acad Sci 2001; 928:274–280.
19. Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr 2003;133:3275S–3284S.
20. Okabe S, Ochiai Y, Aida M, Park K, Kim SJ, Nomura T, Suganuma M, Fujiki H. Mechanistic aspects of green tea as a cancer preventive: effect of components on human stomach cancer cell lines. Jpn J Cancer Res 1999;90:733–739.
21. Cortijo J, Marti-Cabrera M, de la Asuncion JG, Pallardo FV, Esteras A, Bruseghini L, Vina J, Morcillo EJ. Contraction of human airways by oxidative stress protection by *N*-acetylcysteine. Free Radic Biol Med 1999;27:392–400.
22. Meyer A, Buhl R, Magnussen H. The effect of oral *N*-acetylcysteine on lung glutathione levels in idiopathic pulmonary fibrosis. Eur Respir J 1994;7:431–436.
23. Behr J, KM, Degenkolb B, Krombach F, Vogelmeier C. Antioxidative and clinical effects of high-dose *N*-acetylcysteine in fibrosing alveolitis. Adjunctive therapy to maintenance immunosuppression. Am J Respir Crit Care +Med 1997;165:1897–1901.
24. Senthilkumar S, Yogeeta SK, Subashini R, Devaki T. Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats. Chem Biol Interact 2006;160:252–260.
25. Kim HR, Park BK, Oh YM, Lee YS, Lee DS, Kim HK, Kim JY, Shim TS, Lee SD. Green tea extract inhibits paraquat-induced pulmonary fibrosis by suppression of oxidative stress and endothelin-l expression. Lung 2006;184:287–295.
26. Blesa S, Cortijo J, Mata M, Serrano A, Closa D, Santangelo F, Estrela JM, Suchankova J, Morcillo EJ. Oral *N*-acetylcysteine attenuates the rat pulmonary inflammatory response to antigen. Eur Respir J 2003;21:394–400.
27. Venkatesan N, Chandrakasan G. Modulation of cyclophosphamide-induced early lung injury by

curcumin, an anti-inflammatory antioxidant. Mol Cell Biochem 1995;142:79–87.
28. Prockop DJ, Udenfriend S. A specific method for the analysis of hydroxyproline in tissues and urine. Anal Biochem 1960;1:228–239.
29. Seyer JM, Hutcheson ET, Kang AH. Collagen polymorphism in idiopathic chronic pulmonary fibrosis. J Clin Invest 1976;57:1498–1507.
30. Kehrer JP, Lee YC, Solem SM. Comparison of in vitro and in vivo rates of collagen synthesis in normal and damaged lung tissue. Exp Lung Res 1986;10:187–201.
31. Woessner JF Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys 1961;93:440–447.
32. Neuman RE, Logan MA. The determination of collagen and elastin in tissues. J Biol Chem 1950;186:549–556.
33. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882–888.
34. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271–278.
35. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001;5:62–71.
36. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982;78:206–209.
37. Roberts AB, Flanders KC, Heine UI, Jakowlew S, Kondaiah P, Kim SJ, Sporn MB. Transforming growth factor-beta: multifunctional regulator of differentiation and development. Philos Trans R Soc Lond, B: Biol Sci 1990;327:145–154.
38. Wilborn J, Bailie M, Coffey M, Burdick M, Strieter R, Peters-Golden M. Constitutive activation of 5-lipoxygenase in the lungs of patients with idiopathic pulmonary fibrosis. J Clin Invest 1996;97:1827–1836.
39. Kalayarasan S, Sriram N, Sudhandiran G. Diallyl sulfide attenuates bleomycin-induced pulmonary fibrosis: critical role of iNOS, NF-kB, TNF-α and IL-1β. Life Sci 2008;82:1142–1153.
40. Stevens RL, Austen KF. Recent advances in the cellular and molecular biology of mast cells. Immunol Today 1989;10:381–386.
41. Aldenborg F, Nilsson K, Jarlshammar B, Bjermer L, Enerback L. Mast cells and biogenic amines in radiation-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 1993;8:112–117.
42. Horak E, Hopfer SM, Sunderman FW Jr. Spectrophotometric assay for urinary *N*-acetyl-β-D-glucosaminidase activity. Clin Chem 1981;27:1180–1185.
43. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–275.
44. Chen CM, Chou HC, Hsu HH, Wang LF. Transforming growth factor-β1 upregulation is independent of angiotensin in paraquat-induced lung fibrosis. Toxicology 2005;216:181–187.
45. Xu M, Deng B, Chow YL, Zhao ZZ, Hu B. Effects of curcumin in treatment of experimental pulmonary fibrosis: a comparison with hydrocortisone. J Ethnopharmacol 2007;112:292–299.
46. Venkatesan N. Pulmonary protective effects of curcumin against paraquat toxicity. Life Sci 2000;66:PL21–PL28.
47. Soule BP, Cambon C, Orfila C, Seguelas MH, Lepert JC, Rami J, Carre P, Pipy B. Therapeutic and clinical applications of nitroxide compounds. Antioxid Redox Signal 2007;9:1731–1743.
48. Gossart S, Cambon C, Orfila C, Seguelas MH, Lepert JC, Rami J, Carre P, Pipy B. Reactive oxygen intermediates as regulators of TNF-α production in rat lung inflammation induced by silica. J Immunol 1996;156:1540–1548.
49. Punithavathi D, Venkatesan N, Babu M. Curcumin inhibition of bleomycin-induced pulmonary fibrosis in rats. Br J Pharmacol 2000;131:169–172.
50. Kang HC, Nan JX, Park PH, Kim JY, Lee SH, Woo SW, Zhao YZ, Park EJ, Sohn DH. Curcumin inhibits collagen synthesis and hepatic stellate cell activation in vivo and in vitro. J Pharm Pharmacol 2002;54:119–126.
51. Mukai K, Mitani S, Ohara K, Nagaoka S. Structure–activity relationship of the tocopherol-regeneration reaction by catechins. Free Radic Biol Med 2005;38:1243–1256.
52. Sueoka N, Suganuma M, Sueoka E, Okabe S, Matsuyama S, Imai K, Nakachi K, Fujiki H. A new function of green tea: prevention of lifestyle-related diseases. Ann N Y Acad Sci 2001;928:274–8-20.
53. Selman M, King TE, Pardo A, Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med 2001;134:136–151.
54. Yildirim Z, Kotuk M, Iraz M, Kuku I, Ulu R, Armutcu F, Ozen S. Attenuation of bleomycin-induced lung fibrosis by oral sulfhydryl containing antioxidants in rats: erdosteine and *N*-acetylcysteine. Pulm Pharmacol Ther 2005;18:367–373.
55. Suzuki M, Nakamura T, Iyoki S, Fujiwara A, Watanabe Y, Mohri K, Isobe K, Ono K, Yano S. Elucidation of anti-allergic activities of curcumin-related compounds with a special reference to their anti-oxidative activities. Biol Pharm Bull 2005;28:1438–1443.
56. Matsuo N, Yamada K, Yamashita K, Shoji K, Mori M, Sugano M. Inhibitory effect of tea polyphenols on histamine and leukotriene B4 release from rat peritoneal exudate cells. In Vitro Cell Dev Biol Anim 1996;32:340–344.
57. Klugmann FB, Decorti G, Candussio L, Grill V, Mallardi F, Baldini L. Inhibitors of adriamycin-induced histamine release in vitro limit adriamycin cardiotoxicity in vivo. Br J Cancer 1986;54:743–748.