Protective effects of *Ligustrum lucidum* fruit extract on acute butylated hydroxytoluene-induced oxidative stress in rats

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Abstract

Nuzhenzi, the fruit of *Ligustrum lucidum* Ait. (Oleaceae), is commonly used as tonic for kidney and liver in the traditional Chinese medicine prescription. The present study aimed to investigate the antioxidant activities of ethanol extract of *Ligustrum lucidum* fruits (ELL) and its effects on butylated hydroxytoluene (BHT)-induced oxidative stress in rats. Results showed that ELL possesses weak antioxidant activities. Compared to the BHT (1000 mg/kg)-treated group, results showed that ELL at 250, 500 and 1000 mg/kg significantly reduced the levels of blood urea nitrogen (BUN), serum glutamic pyruvic transaminase (sGPT), glutamic oxaloacetic transaminase (sGOT), alkaline phosphatase (sALP), lactate dehydrogenase (LDH), triglyceride (TG) and creatinine (Cr), as well as LDH in bronchoalveolar lavage fluid (BALF). It also significantly decreased the level of lipid peroxides in liver and lung. In addition, ELL significantly enhanced the levels of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in these organs. Histopathological evaluation of the tissues revealed that ELL reduced the incidence of lung lesions, while the liver and kidney tissues were not affected by BHT administration. Taken together, the protective effect of ELL against acute BHT-induced oxidative stress in rats could be through the upregulation of antioxidant enzymes.

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Keywords: *Ligustrum lucidum*; Butylated hydroxytoluene; Antioxidant enzymes; Oxidative stress

1. Introduction

Nuzhenzi, the fruit of *Ligustrum lucidum* Ait. (Oleaceae), is well known as tonic for kidney and liver in the traditional Chinese medicine prescription (Shi et al., 1998). It was reported to possess immunomodulatory, anti-inflammatory, hepatoprotective, anti-tumor and anti-aging activities (He et al., 2001a). Ethanol extract of *Ligustrum lucidum* fruits and its secoiridoid glucosides, namely oleoside dimethylester, oleuropein, neoneuzhenide, lucidumoside B and lucidumoside C, were shown to inhibit the free radical induced hemolysis in red blood cells (He et al., 2001b). Oleuropein also exhibited antiviral activities against respiratory syncytial virus (RSV) and parainfluenza

type 3 virus (Para 3), whereas oleoside dimethylester, lucidumoside C and ligustrosides were active against Para 3 virus (Ma et al., 2001). ELL contains a large amount of oleanolic acid and ursolic acid, which have been demonstrated to be effective in treating inflammation, hepatotoxicity, pain and hyperlipidemic (Yim et al., 2001; Liu et al., 2003).

Butylated hydroxytoluene (BHT), a phenolic antioxidant, is generally recognized as safe food additive and preservative (Lanigan and Yamarik, 2002). However, when at high doses it is known to cause lactate dehydrogenase (LDH), serum glutamic pyruvic transaminase (sGPT) and glutamic oxaloacetic transaminase (sGOT) leakage, which consequently lead to pneumotoxicity, hepatotoxicity and nephrotoxicity in animals (Marino and Mitchell, 1972; Powell and Connolly, 1991; Reed et al., 2001). BHT-quinone methide (BHT-QM; 2,6-di-*tert*-butyl-4-methylene-cyclohexa-2,5-dienone), an intermediate metabolite of BHT, is metabolized by cytochrome P450 in liver and lung in mice (Witschi et al., 1989).

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An overproduction of BHT-QM can covalently bind to protein and nucleic acid, which consequently lead to acute hepatotoxicity and pneumotoxicity. In normal condition, BHT-QM is conjugated and detoxicated by glutathione (GSH), but excess of BHT-QM can enhance the oxidative stress and deplete GSH (Mizutani et al., 1984; Sun et al., 2003). Oxidative stress is considered to play an important role in the cause of many diseases, including inflammation, aging and cancer (Cerutti, 1985; Lavrovsky et al., 2000).

Besides being regularly use as tonic in the traditional Chinese medicine, fruits of *Ligustrum lucidum* also possess the potential of developing into other health products. Hence, in this study, our aim was to examine the antioxidant activities of ELL and its pharmacological effects on BHT-induced acute oxidative stress in rats.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), Tris-HCl, thiobarbituric acid (TBA), ferrous chloride, ascorbate, xanthine, xanthine oxidase, ethylenediaminetetraacetic acid (EDTA), nitroblue tetrazolium (NBT) and 5,5'-dithio(bis)2-nitrobenzoic acid (DTNB) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of plant extract

Fruits of *Ligustrum lucidum* were obtained from the traditional Chinese pharmacy in Kaohsiung (Taiwan). It was then authenticated by Professor C.C. Lin, Kaohsiung Medical University, Taiwan. To prepare the *Ligustrum lucidum* extract, 100 g of *Ligustrum lucidum* fruits was thoroughly powdered and then extracted with 500 ml of 95% ethanol for 3 h in the hot water bath. After the supernatant of the first extraction was removed, an additional 500 ml of 95% ethanol was added, the sample was further subjected to another 3 h of extraction. The same procedure was repeated for three times. The decoction obtained from the three separate extractions was mixed, filtered, concentrated and lyophilized. The yield of dried *Ligustrum lucidum* fruit extract (ELL) was about 20%, which was collected and stored at -21°C until use.

2.3. Free radical scavenging activity assay

Free radical scavenging activity was assayed spectrophotometrically by the reduction of cytochrome *c* method as described by McCord and Fridovich (1969).

2.4. Xanthine oxidase inhibition test

Xanthine oxidase inhibition activity was estimated by the formation of uric acid from xanthine-xanthine oxidase system (Chang et al., 1994).

2.5. Anti-lipid peroxidation assay

The effect of ELL on rat liver homogenate induced with FeCl_2 -ascorbic acid for lipid peroxidation was determined by malondialdehyde-thiobarbituric acid (MDA-TBA) adduct according to Ohkawa et al. (1979).

2.6. Animals

Male Wistar rats, age at 6 weeks old, were purchased from the National Laboratory of Animal Breeding and Research Center (Taipei, Taiwan). They were housed in a controlled environment with temperature maintained at $22 \pm 3^{\circ}\text{C}$ and humidity at $55 \pm 5\%$ under a 12:12 h light/dark cycle. Animals were fed a standard laboratory diet and tap water *ad libitum*. After a week of adaptation, animals were subjected to various treatments for BHT-induced studies. All experimental protocols were performed according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, publication No. 86-23, revised 1985).

2.7. Animal treatments

The experiment was carried out according to procedures as described by Nakagawa et al. (1984) with minor modification. In brief, animals were randomly divided into five groups of nine animals each, namely group A: vehicle control; group B: BHT-treated; group C: BHT + ELL 250 mg/kg; group D: BHT + ELL 500 mg/kg and group E: BHT + ELL 1000 mg/kg. Groups A and B were orally given 10 ml/kg of distilled water for 7 days, while other groups were intragastrically administered with different doses of ELL. At the end of the 7th day, 1000 mg/kg of BHT dissolving in olive oil was given to groups B, C, D and E by gavage 6 h after the extracts were administered. At the same time, rats in group A were administrated with the same volume of olive oil. All animals were anesthetized with ether and sacrificed 18 h later. The thorax was opened by a median incision and the trachea was cannulated with a plastic catheter attached to a 10 ml syringe. The bronchoalveolar lavage fluid (BALF) was obtained by washing the lung across the trachea with 10 ml saline. It was then centrifuged at $300 \times g$ at 4°C for 10 min to obtain the supernatant for biochemical assays. Blood samples were collected for biochemical studies. The liver, lung and kidney were dissected and sectioned for histopathological observations. Part of these tissues were collected and stored at -80°C until use for biochemical assays.

2.8. Serum and bronchoalveolar lavage fluid (BALF) assessment

Blood collected was centrifuged at $1600 \times g$ at 4°C for 15 min to obtain the serum, which was then subjected to serum glutamic pyruvic transaminase (sGPT), glutamic oxaloacetic transaminase (sGOT), alkaline phosphatase (sALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine (Cr), cholesterol and triglyceride (TG) analyses. LDH in BALF were also measured. These analyses were carried out according

to the manufacturer's procedures using a Hitachi 7050 auto-analyser with commercial kits.

2.9. Analysis of tissue homogenates

Liver, lung and kidney tissues were homogenized in four volumes of ice-cold 150 mM Tris–HCl (pH 7.4) using Potter–Elvehjem homogenizer. The homogenates were centrifuged at $1600 \times g$ for 15 min at 4 °C to obtain a supernatant for various biochemical analyses. Catalase (CAT) activity was determined according to Aebi (1984). Superoxide dismutase (SOD) activity was determined by the method of Beauchamp and Fridovich (1971). Total glutathione peroxidase (GPx) was done with method of Flohe and Gunzler (1984). Lipid peroxidation in the liver, kidney and lung homogenates was ascertained by the formation of malondialdehyde (MDA) and measured by the thiobarbituric acid reactive method according to Ohkawa et al. (1979). The protein concentration was measured by the method of Lowry et al. (1951).

2.10. Histopathological examinations

The liver, lung and kidney were fixed in 10% neutral buffered formalin, dehydrated with 50–100% ethanol solutions, and then embedded in paraffin. Tissues were sectioned at thickness 5 μ m, followed by staining with haematoxylin-eosin dye before subjecting to photomicroscopic observation.

2.11. Statistical analysis

All experimental data were expressed as means $\pm$ standard deviations (S.D.) of nine analyses. Statistical analysis was per-

formed by one-way analysis of variance (ANOVA), followed by Tukey's multiple range tests. A probability value of $P < 0.05$ was considered as significantly difference.

3. Results

3.1. Antioxidant activities of ELL

In the cytochrome c assay, the scavenging effect of ELL on the superoxide anions was found to increase with increasing sample concentrations. A concentrations 0.1–3.0 mg/ml, ELL showed a scavenging rate ranging from 26.39–77.78%. Its IC_{50} value was 1.58 mg/ml.

In the xanthine oxidase inhibition test, ELL demonstrated an inhibitory effect on xanthine oxidase activity in a concentration-dependent manner. The inhibitory activity of ELL at 1–10 mg/ml ranged from 24.22–59.45%. Its IC_{50} value was 7.54 mg/ml.

In anti-lipid peroxidation assay, the inhibition of MDA formation in rat liver homogenate increased with increasing concentration of ELL. At concentrations 1–10 mg/ml, the anti-lipid peroxidation activity ranged from 31.82–72.39%, with an IC_{50} value of 4.64 mg/ml.

3.2. Effects of ELL on the levels of sGPT, sGOT, sALP, BUN, Cr, LDH, cholesterol and TG, and BALF LDH in BHT-treated rats

Compared to the control group, results showed that, with the exception of cholesterol, the levels of sGPT, sGOT, sALP, BUN, Cr, LDH, TG, and BALF LDH were significantly increased in rats with oxidative stress induced by BHT (Tables 1 and 2). Pre-administration of ELL at 250, 500 and 1000 mg/kg significantly

Table 1

Effects of *Ligustrum lucidum* fruit extract (ELL) on the levels of serum glutamic pyruvic transaminase (sGPT), glutamic oxaloacetic transaminase (sGOT), alkaline phosphatase (sALP), lactate dehydrogenase (LDH) and creatinine (Cr), and blood urea nitrogen (BUN) in rats with oxidative stress induced by BHT

Treatment	sGPT (U/l)	sGOT (U/l)	sALP (U/l)	BUN (mg/dl)	Cr (mg/dl)
Control	45.0 $\pm$ 2.2	115.1 $\pm$ 14.0	336.7 $\pm$ 16.6	14.79 $\pm$ 2.64	0.462 $\pm$ 0.030
BHT	65.6 $\pm$ 17.1 ^a	190.2 $\pm$ 50.3 ^a	692.3 $\pm$ 74.1 ^a	22.64 $\pm$ 2.69 ^a	0.646 $\pm$ 0.052 ^a
BHT + ELL (250 mg/kg)	52.1 $\pm$ 8.1 ^b	126.9 $\pm$ 11.3 ^b	551.1 $\pm$ 37.5 ^{bc}	22.06 $\pm$ 2.88 ^c	0.564 $\pm$ 0.036 ^{bc}
BHT + ELL (500 mg/kg)	50.0 $\pm$ 6.5 ^b	121.2 $\pm$ 13.6 ^b	546.6 $\pm$ 75.4 ^{bc}	21.50 $\pm$ 1.28 ^c	0.564 $\pm$ 0.032 ^{bc}
BHT + ELL (1000 mg/kg)	48.1 $\pm$ 4.3 ^b	113.3 $\pm$ 8.1 ^b	529.2 $\pm$ 58.9 ^{bc}	19.36 $\pm$ 1.62 ^{bc}	0.565 $\pm$ 0.043 ^{bc}

Data were presented as means $\pm$ S.D. from nine independent analyses. Values within the same column with the different superscript letters were significantly different at $P < 0.05$ as analysed by Tukey's multiple range tests. ^{a,c}Significantly different from control group; ^bsignificantly different from BHT treatment.

Table 2

Effects of *Ligustrum lucidum* fruit extract (ELL) on the levels of serum lactate dehydrogenase (LDH), cholesterol and triglyceride (TG), and bronchoalveolar lavage fluid (BALF) LDH in rats with oxidative stress induced by BHT

Treatment	Serum			BALF
	log LDH (U/l)	Cholesterol (mg/dl)	log TG (mg/dl)	log LDH (U/l)
Control	2.77 $\pm$ 0.25	64.86 $\pm$ 9.99	1.80 $\pm$ 0.06	1.55 $\pm$ 0.14
BHT	3.27 $\pm$ 0.02 ^a	75.89 $\pm$ 12.97	2.30 $\pm$ 0.14 ^a	1.72 $\pm$ 0.30 ^a
BHT + ELL (250 mg/kg)	3.06 $\pm$ 0.08 ^{bc}	68.00 $\pm$ 10.63	1.86 $\pm$ 0.30 ^b	1.62 $\pm$ 0.11 ^b
BHT + ELL (500 mg/kg)	2.98 $\pm$ 0.09 ^b	67.00 $\pm$ 8.51	1.82 $\pm$ 0.15 ^b	1.59 $\pm$ 0.10 ^b
BHT + ELL (1000 mg/kg)	2.90 $\pm$ 0.06 ^b	61.44 $\pm$ 8.25 ^b	1.78 $\pm$ 0.30 ^b	1.55 $\pm$ 0.07 ^b

Data were presented as means $\pm$ S.D. from nine independent analyses. Values within the same column with the different superscript letters were significantly different at $P < 0.05$ as analysed by Tukey's multiple range tests. ^{a,c}Significantly different from control group; ^bsignificantly different from BHT treatment.

Table 3
Effects of *Ligustrum lucidum* fruit extract (ELL) on the levels of antioxidant enzymes and lipid peroxidation in the liver, kidney and lung of rats with oxidative stress induced by BHT

Treatment	Control	BHT	BHT + ELL (250 mg/kg)	BHT + ELL (500 mg/kg)	BHT + ELL (1000 mg/kg)
log MDA (nmol/g protein)					
Liver	2.74 ± 0.20	2.59 ± 0.25	2.12 ± 0.22 ^{bc}	2.03 ± 0.37 ^{bc}	1.91 ± 0.43 ^{bc}
Kidney	2.85 ± 0.14	2.70 ± 0.18	2.26 ± 0.69 ^c	2.42 ± 0.23	2.27 ± 0.35 ^c
Lung	2.86 ± 0.09	3.06 ± 0.15 ^a	2.93 ± 0.09	2.88 ± 0.12	2.88 ± 0.19 ^b
SOD (U/mg protein)					
Liver	7.34 ± 1.23	5.32 ± 0.77 ^a	6.77 ± 1.23	7.98 ± 0.85 ^b	8.02 ± 1.61 ^b
Kidney	8.71 ± 1.19	6.13 ± 1.14 ^a	6.49 ± 0.90 ^{cd}	6.31 ± 1.44 ^{ce}	9.29 ± 0.76 ^{bde}
Lung	2.05 ± 0.53 ^a	0.94 ± 0.31 ^a	1.45 ± 0.30 ^c	1.55 ± 0.38 ^b	1.88 ± 0.41 ^b
CAT (U/mg protein)					
Liver	1002.92 ± 186.44	815.94 ± 88.17 ^a	1124.80 ± 193.34 ^b	1364.92 ± 272.78 ^{bc}	1578.48 ± 276.39 ^{bc}
Kidney	144.06 ± 27.92	78.71 ± 12.21 ^a	123.17 ± 27.88 ^d	137.70 ± 32.11 ^{be}	218.24 ± 75.46 ^{bde}
Lung	485.87 ± 44.28	348.99 ± 56.88 ^a	434.65 ± 82.93	517.58 ± 146.39 ^b	539.27 ± 90.88 ^b
GPx (U/mg protein)					
Liver	3.64 ± 0.21	2.70 ± 0.43 ^a	2.66 ± 0.50 ^c	3.26 ± 0.44 ^d	3.52 ± 0.45 ^{bd}
Kidney	2.99 ± 0.25	1.83 ± 0.34 ^a	2.48 ± 0.44	2.87 ± 0.91 ^b	3.10 ± 0.43 ^b
Lung	1.32 ± 0.16	0.99 ± 0.29 ^a	1.52 ± 0.18 ^{bd}	1.53 ± 0.09 ^{be}	1.86 ± 0.13 ^{bde}

Data were presented as means ± S.D from nine independent analyses. Values of measurement within the same tissue with the different superscript letters were significantly different at $P < 0.05$ as analyzed by Tukey's multiple range tests. ^{a,c}Significantly different from control group. ^bSignificantly different from BHT treatment. ^{d,e}Significantly different from BHT treated with ELL group.

inhibited the BHT-induced increase in the levels of these biomolecules. No difference was noted in BUN and cholesterol levels between BHT alone and BHT plus ELL at 250 and 500 mg/kg. However, a significant decrease in these two components was noted at 1000 mg/kg ELL.

3.3. Effects of ELL on the levels of antioxidant enzymes in tissues of BHT-treated rats

BHT administration significantly decreased the levels of SOD, CAT and GPx in liver, kidney and lung (Table 3). Pre-treatment with ELL significantly enhanced the activity of these antioxidant enzymes. Interestingly, administration of 1000 mg/kg ELL increased the SOD level in all tested tissues to that of the control group, whereas the levels of CAT and GPx were found to return to the level of control group at 500 and 1000 mg/kg ELL.

3.4. Effect of ELL on the level of MDA in tissues of BHT-treated rats

Compared to the control group, BHT at 1000 mg/kg significantly increased the level of MDA in lung tissue, while the level in the liver and kidney was not affected (Table 3). Pre-treatment with ELL at 500 and 1000 mg/kg reversed the MDA concentration to the normal level in lung. Compared to the BHT-treated group, administration of 250, 500 and 1000 mg/kg ELL significantly decreased the MDA level in liver, while the level in the kidney was moderately affected.

3.5. Histopathological observation

Histopathological results showed that BHT at 1000 mg/kg caused a significant damage in lung as indicated by a serious

change in the tissue appearance, with inflammation and structure destruction in the tissue as compared with the control group (Fig. 1). However, liver and kidney tissues were not affected (Figs. 2 and 3). Interestingly, pre-treatment with ELL significantly reversed the lung condition in the BHT-treated rats.

4. Discussion

The present study demonstrated that ELL possesses weak antioxidant activities, but shows effective protection against BHT-induced oxidative damage in rats. This observation can be supported by the significant decrease in the levels of sGPT, sGOT, sALP, BUN, Cr, LDH and TG, and BALF LDH. Besides decreasing the level of lipid peroxides in liver and lung, pre-treatment of ELL led to an increase in CAT, SOD and GPx levels in these organs in BHT-treated rats. The histopathological observation also revealed that ELL could reduce the incidence of lung lesions. These results support that ELL could have protective effect against acute BHT-induced oxidative stress in rats, its mechanism of action could be through the enhancement of antioxidant enzymes production.

Oxidant-induced injury to the plasma membrane of cells can evoke a wide variety of responses. Biological membranes contain a large amount of polyunsaturated fatty acids, which are particularly susceptible to peroxidative attacks to produce lipid peroxides. Lipid peroxides have been used as a sensitive indicator of oxidant-induced cell injury (Hickey et al., 2001). In this study, we showed that BHT at 1000 mg/kg significantly increased the level of lipid peroxides and caused serious histological lesion to the lung tissue. Surprisingly, no obvious changes were noted in the liver and kidney tissues. This could be due to the variation in the metabolic stage and duration of being exposed to BHT or BHT-QM.

Fig. 1. Effect of *Ligustrum lucidum* fruit extract (ELL) on acute BHT-induced oxidative stress in the lung of rats. Lung sections were stained with haematoxylin and eosin (H&E-stained $\times 200$). (A) Control; (B) BHT-treated; (C) BHT + ELL (250 mg/kg); (D) BHT + ELL (500 mg/kg); (E) BHT + ELL (1000 mg/kg).

Hepatic cells participate in a variety of metabolic activities and contain a number of enzymes. In the stage of liver injury, the transport function of hepatocytes is disturbed, which can result in the leakage of plasma membrane, thereby causing an increase in sGPT and sGOT levels. In addition, a defective excretion of bile by the liver was reflected by an increase in the level of sALP. The distribution of LDH throughout the body is also recognized as one of the markers for liver and muscle lesions (Rajesh and Latha, 2004; Yanardag and Tunali, 2006). In this study, the increase in sGPT, sGOT, sALP and LDH levels indicated that BHT could induce hepatic dysfunction. ELL showed protective effect against BHT-induced hepatic dysfunction through decreasing the levels of sGPT, sGOT, sALP and LDH.

The present study also showed that BHT significantly increased the level of LDH in BALF, which was associated with the incidence of lung lesions in BHT-treated rats. It is interesting to note that pre-treatment with ELL was able to

protect against BHT-induced lung injury. This can be shown by a decrease in the level of LDH and the alleviation lung tissue damage.

In general, changes in Cr concentration were more prominent than the level of BUN during the earlier phases of kidney disease (Erdem et al., 2000). BUN only begins to rise after a marked renal parenchyma injury occurs. In this study, the increase in the level of Cr and BUN indicated that BHT could induce renal dysfunction, which was alleviated by pre-treatment with ELL as shown by a decrease in Cr and BUN levels. This suggests that ELL may possess protective effect against BHT-induced renal dysfunction.

Hyperlipidemia is considered as an important risk factor of atherosclerosis due to impairment of endothelial function. This is resulted from the reduction of nitric oxide availability and an increase in the production of oxygen free radicals (Bartus et al., 2005). In addition, changes in the enzymatic antioxidative defense systems such as SOD, CAT, GPx and GSH

Fig. 2. Effect of *Ligustrum lucidum* fruit extract (ELL) on acute BHT-induced oxidative stress in the liver of rats. Liver sections were stained with haematoxylin and eosin (H&E-stained $\times 200$). (A) Control; (B) BHT-treated; (C) BHT + ELL (250 mg/kg); (D) BHT + ELL (500 mg/kg); (E) BHT + ELL (1000 mg/kg).

during hyperlipidemia, also suggest a reduced resistance to reactive oxidant-mediated damage (Oh et al., 2006). An increase in the level of serum TG indicated that BHT might induce the risk of atherosclerosis development. Pre-administration of ELL significantly decrease the levels of serum TG and cholesterol, suggesting ELL might play a role in protecting against atherosclerosis development.

The endogenous antioxidant enzymes are responsible for the detoxification of deleterious oxygen radicals. CAT is a hemo-protein which catalyses the reduction of hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Rajasekaran et al., 2005). SOD constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 (Hassan, 1988). GPx plays an important role in the reduction of H_2O_2 in the presence of reduced GSH. The detoxifying action of GPx against H_2O_2 protects cell membrane against oxidative damage (Jung et al., 2005). The antioxidant enzymes CAT and

GPx protect SOD against inactivation by H_2O_2 . Reciprocally, SOD protects CAT and GPx against superoxide anion. Thus, the balance of these enzymatic systems is essential to dispose the superoxide anion and peroxides generated in the tissues (Selvakumar et al., 2004). The reduction in the activities of these enzymes and increase in lipid peroxides could reflect the adverse effects of BHT in the antioxidant system in liver, kidney and lung. After ELL treatment, a significant increase in the levels of these antioxidant enzymes with a corresponding decrease in the level of lipid peroxides in the liver, kidney and lung. This finding supports the protective effect of ELL against BHT-induced oxidative stress.

In conclusion, ELL treatment could prevent the acute BHT-induced oxidative stress in rats as demonstrated by the decrease in sGPT, sGOT, sALP and LDH concentrations, and BALF LDH, as well as an increase in levels of antioxidant enzymes in liver, kidney and lung. ELL also effectively protected against lung injury in the BHT-treated rats, which was further supported

Fig. 3. Effect of *Ligustrum lucidum* fruit extract (ELL) on acute BHT-induced oxidative stress in the kidney of rats. Kidney sections were stained with haematoxylin and eosin (H&E-stained $\times 200$). (A) Control; (B) BHT-treated; (C) BHT + ELL (250 mg/kg); (D) BHT + ELL (500 mg/kg); (E) BHT + ELL (1000 mg/kg).

by the histopathological observation. These results conclude that the protective effect of ELL against acute BHT-induced oxidative stress in rats could be through the upregulation of antioxidant enzymes.

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