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The protective effect of quercetin, green tea or malt extracts against experimentally-induced lung fibrosis in rats

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The possible protective potentials of quercetin (50 mg/kg, p.o.), green tea extract (1 mg/kg, p.o.) malt extract (625 mg/kg, p.o.) and deprenyl (10 mg/kg, i.p.) against paraquat (PQ)-induced lung injury in rats were examined. PQ was administered twice a week (20 mg/kg, i.p.) with or without daily pretreatment with any of the chosen agents for 3 successive weeks. Changes in the enzymatic activities of myeloperoxidase (MPO), superoxide dismutase (SOD) and lactate dehydrogenase (LDH) as well as reduced glutathione (GSH), protein thiols (Pr-SHs) and nitric oxide (NO) contents of the lungs were determined. In addition, estimation of lung content of thiobarbituric acid reactive substances (TBARS) measured as malondialdehyde. Moreover, histopathological examination of the lung tissue was performed. On the biochemical level, PQ provoked remarkable lung damage noted by elevation of neutrophils MPO activity accompanied by decreased activities of cytosolic SOD and LDH, depletion of GSH and Pr-SHs contents as well as increased production of NO and TBARS. Furthermore, histopathological examination revealed marked edema, subpleural hemorrhage, acute inflammation and lymphocytic infiltration. Treatment significantly protected against most of PQ-induced lung biochemical and histopathological changes. It could be concluded that quercetin, green tea, malt extract and deprenyl offered remarkable protection against PQ-induced lung injury.

Key words: Paraquat, quercetin, green tea, malt, deprenyl, lung, rats.

INTRODUCTION

Paraquat (PQ) is a 1, 1'-dimethyl-4, 4'-bipyridylium herbi-cide that is very toxic to all living organisms. Both in acute and chronic intoxication primary injuries to mammals occur in the lungs, which selectively accumulate paraquat .It generates free radicals and leads to acute or chronic lung injury. Free radicals are often associated with fibro-genesis, which occurs in various disease states (Candan and Alagözlü, 2001).

Exposure of humans and animals to toxic doses of paraquat is known to damage the lung leading to pulmo-nary edema and hypertension, acute respiratory distress syndrome, and progressive lung fibrosis (Kimbrough and Gaines, 1970; Salmona et al., 1992). PQ is considered as a potent redox cycler readily converted to a free radical which generates superoxide anions and other redox pro-

ducts (Amanov et al., 1994). Intoxication of rats with paraquat was accompanied by accumulation in lungs, brain, heart, liver or kidney of malondialdehyde (MDA) (the compounds reacting with 2-thiobarbituric acid), indi-cating that the intoxication stimulated lipid peroxidation (LPO) in biomembranes (Yumino, et al., 2002). PQ-induced lung injury was then utilized as a model for induction of lung fibrosis (Salmona et al., 1992).

Pulmonary fibrosis is a disorder characterized by com-plex inflammatory processes that result in excessive fibroblast proliferation and progressive deposition of connective tissue in the pulmonary parenchyma (Crestani et al., 2007). This disorder leads to severe deterioration of lung function, with limiting symptoms and poor quality of life. Unfortunately, despite the severity of the disease, the treatments currently available for pulmonary fibrosis provide only minimal benefits and have significant side effects. An early feature of PQ toxicity is the influx of

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inflammatory cells, releasing proteolytic enzymes and oxygen free radicals, which can destroy the lung epithelium and result in pulmonary fibrosis. Therefore, the ability to suppress early lung injury seems to be an appropriate therapy of pulmonary damage before the development of irreversible fibrosis (Venkatesan, 2000).

Deprenyl, a monoamine oxidase (MAO) inhibitor possesses antioxidant effect by preventing free radical formation and enhancing antioxidant defenses, as well as anti-apoptotic and neurotrophic effects (Shimazu et al., 2003). Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) is a dietary flavonoid ubiquitous in nature. It is found in many plants, such as onions, broccoli, and tea. A significant number of chemical properties and pharmacological effects have been attributed to this flavonoid (Ross and Kasum, 2002). Quercetin exhibits potent antioxidant effects by combining with free radical species to form less reactive phenoxy radicals. The intake of dietary flavornoids has been related to a reduced risk for several diseases like cardiovascular and chronic inflammatory diseases (Johnson and Loo, 2000). These positive health effects associated with the intake of flavonoids have been ascribed to their well-known antioxidant properties and to inhibiting effects on a wide range of enzymes (Nijveldt et al., 2001). Furthermore, anti-inflammatory effects of flavonoids could contribute to these beneficial effects (Rice-Evans et al., 1996). Green tea, from the steamed dried leaves of camellia sinesis, is widely consumed in eastern Asia. The green tea polyphenols are natural plant flavonoids that comprise many types of catechins, among these, epigallocatechin-3-gallate (EGCG) is the major polyphenol component and it is primarily responsible for the beneficiary effects of green tea. Substantial evidence suggests that EGCG elicit anti-oxidant properties by attenuating the LPO caused by various forms of free radicals (Guo et al., 1996). Barley (Hordeum vulgare) is widely used as food; it is an important crop in Northern Europe, where it is used both for feed and in the malting industry (Finnie et al., 2002). Malt extract is prepared from malted grain of barley; it contains 50% maltose with dextrin, glucose and hordein protein. Their benefits stem from containing several active constituents as β-glucans and tocols as well as phenolic antioxidants as pr-oanthocyanidins, flavones (McMurrough and Madigan, 1996; Goupy et al., 1999), etc. Since the lung is the org-an exposed to the highest oxygen concentration, it is particularly at risk to the toxic effects of oxygen (Clark and Lambertson, 1971). Several lung diseases have be-en associated with oxidative stress and linked to oxidant insults; therefore the lungs and tissues of the respiratory tract require a specific defense system against oxidants and free radicals. Pulmonary fibrosis is a pathological state that is characterized by increased synthesis and deposition of extracellular matrix (ECM) in the distal air-space, and is probably initiated by acute or chronic lung injury (Thannickal et al, 2004). The current treatment of pulmonary fibrosis involves antifibrotic and immunosuppressive agents, with very less achievement (Lasky and Ortiz, 2001). Therefore, the successful treatment of pulmonary fibrosis remains an uphill task, and development of drug regimens for this condition remains an imperative challenge. The aim of the current study was to examine the possible protective potentials of the above mentioned agents against lung injury induced by low-dose PQ administration and to elucidate the mechanism(s) underlying these effects, if any. To achieve these objectives, PQ was administered twice a week with or without daily pretreatment with one of the selected agents. Protective effects of test agents against PQ-induced lung injury were examined by determination of the activities of neutrophils myeloperoxidase (MPO), cytosolic superoxide dismutase (SOD) and lactate dehydrogenase (LDH) as well as nitric oxide (NO), reduced glutathione (GSH) and protein thiols (Pr-SHs) contents in the lungs. Finally, the level of thiobarbituric acid reactive substances (TBARS) was estimated in the lungs as an index of lipid peroxidetion.

## MATERIALS AND METHODS

Animals

Male albino rats of 150-200 g body weight were used in the current investigation. They were obtained from the National Scientific Research Center (Giza, Egypt). They were fed standard pellet chow (El-Nasr Chemical Company, Cairo, Egypt) and allowed free access to water. This study was conducted in accordance with ethical procedures and policies approved by Animal Care and Use Committee of Faculty of Pharmacy Cairo University, Cairo, Egypt.

Chemicals

PQ, deprenyl and quercetin were purchased from Sigma-Aldrich Co. (St. Louis, MD, USA). Green tea powder (containing 80% polyphenols) was obtained from Tiba Pharmaceutical Industry Company, Egypt. Malt extract was obtained from Marine Chemicals, India. All agents were dissolved in saline except quercetin that was prepared in 1% tween 80 prior to administration. Reagents kit for the determination of LDH was purchased from Stanbio, USA. All other used chemicals in the present study were of pure analytical grade.

Methods

Biochemical experiments: After one week of acclimatization, rats were randomly allocated to six groups each containing 12-14 rats. One group served as normal and received either saline or 1% tween 80 (p.o.), daily for 3 weeks. The second group received PQ (i.p.) at a dose of 20 mg/kg twice (Monday and Thursday) a week during 3 weeks (Ishida et al., 2006). The remaining four groups received deprenyl [(10 mg/kg/day; i.p.) (Fuller et al., 1988), quercetin (50 mg/kg/day; p.o.) (Piantelli et al., 2006)], green tea extract (1 mg/kg/day; p.o.) (Levites et al., 2001) or malt extract (625 mg/kg/day; p.o.) (Hong and Maeng, 2004), respectively daily for 3 successive weeks. In addition to daily treatments with one of the test drugs, the last four groups received, twice a week, injections of PQ (20 mg/kg; i.p.). After 3 weeks of treatments, animals were sacrificed. Lungs were rapidly isolated, rinsed with ice-cold saline, blotted and weighed. They were then homogenized in ice-cold potassium chloride using Potter-Elvejham glass homogenizer. Four
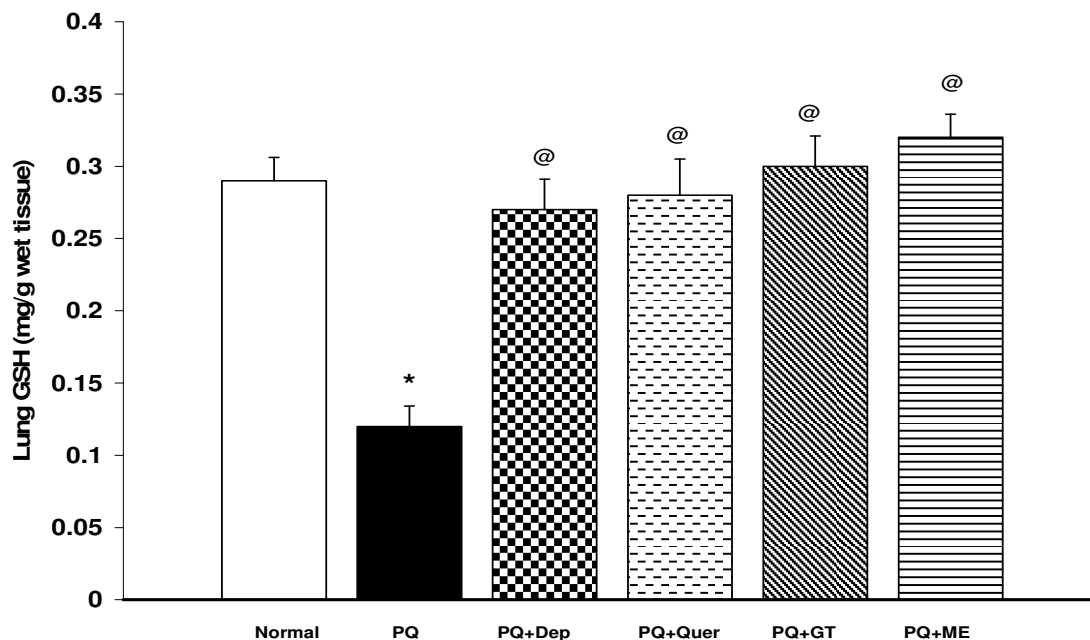
Figure 1. Effects of paraquat (PQ, 20 mg/kg, twice a week, i.p.), with or without 3 weeks daily treatments with deprenyl (Dep, 10 mg/kg, i.p.), quercetin (Quer, 50 mg/kg, p.o.), green tea extract (GT, 1 mg/kg, p.o.) or malt extract (ME, 625 mg/kg, p.o.) on lung glutathione (GSH) content in rats. PQ was administered as two i.p. injections/week to all groups except the normal one. The test drugs were administered daily for 3 weeks. The normal group received 1% tween 80 daily. Each bar with vertical line represents the mean of 7-14 animals ± SE. Statistical analysis was carried out by ANOVA followed by Tukey-Kramer multiple comparisons test.* Significantly different from normal group at $p < 0.05$.[@] Significantly different from PQ control group at $p < 0.05$.

homogenate was freeze-sawed twice then sonicated in presence of aliquots of each homogenate were used for estimation of GSH, Pr-SHs, TBARS and NO, respectively. Another aliquot of each 0.5% hexadecyltrimethylammoium bromide (to release MPO from neutrophils) and used for estimation of MPO activity. The rest of the homogenate was centrifuged at 105000 xg for 20 min at 4ºC using a Dupont-Sorvall ultra-centrifuge (USA) to obtain the cytosolic fraction. The cytosols were used for the estimation of the activities of SOD and LDH. Estimation of GSH and Pr-SHs contents was performed spectrophotometrically at 412 nm, using Ellman's reagent (Koster et al., 1986; Ahmed et al., 1991) and expressed as mg/g wet tissue and µmol/g wet tissue, respectively. NO: estimated as nitrate/nitrite, was determined spectrophotometrically at 540 nm, using Greiss reagent (Miranda et al., 2001) and expressed as µmol/g wet tissue. Lipid peroxidation products were estimated by the determination of the content of TBARS that was measured as malondialdehyde (Uchiyama and Mihara, 1978) and expressed as nmol/g wet tissue. MPO activity was determined kinetically by measuring rate of hydrogen peroxide-dependent oxidation of O-dianisidine catalyzed by MPO (Krawisz et al., 1984) and expressed as units/g wet tissue. Cytosolic SOD activity was estimated kinetically using pyrogallol according to the method of Marklund and Marklund, 1974 and expressed as units/g wet tissue.   The activity of LDH was determined kinetically by measurement of the rate of NAD reduction to NADH at 340 nm (Buhl and Jackson, 1978) and expressed as units/g wet tissue.

Histopathological Experiments: The representative sections of lungs (n = 6) were prepared by formalin fixation, routinely process-sed and embedded in paraffin. Three-micrometer-thick sections were placed on slides and stained with hematoxylin and eosin

(H&E). The slides were then investigated under light microscope (Nikon XDS-1B). Sections were examined to investigate the toxic effect of PQ; the pulmonary haemorrhage and edema in PQ-poisoned rat lungs.

Statistical analysis

Comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by "Tukey-Kramer multiple comparisons test". The level of significance was set at p < 0.05. "GraphPad Software InStat (version 2)" was used to carry out these statistical tests.

RESULTS

In the current study, there was no significant difference between the two groups of normal rats receiving either saline or tween 80, so the two groups were pooled in one group and served as normal control. PQ produced marked depletion of GSH and Pr-SHs contents by 41 and 62 %, respectively as compared to the respective normal values (Figures 1 and 2). Treatments with all of the chosen test drugs significantly prevented PQ-induced GSH depletion (Figure 1), but no significant effect was noted on Pr-SHs levels except with malt extract which produced a significant increase by 1.5 fold as compared to the normal group (Figure 2). PQ administration signifi-
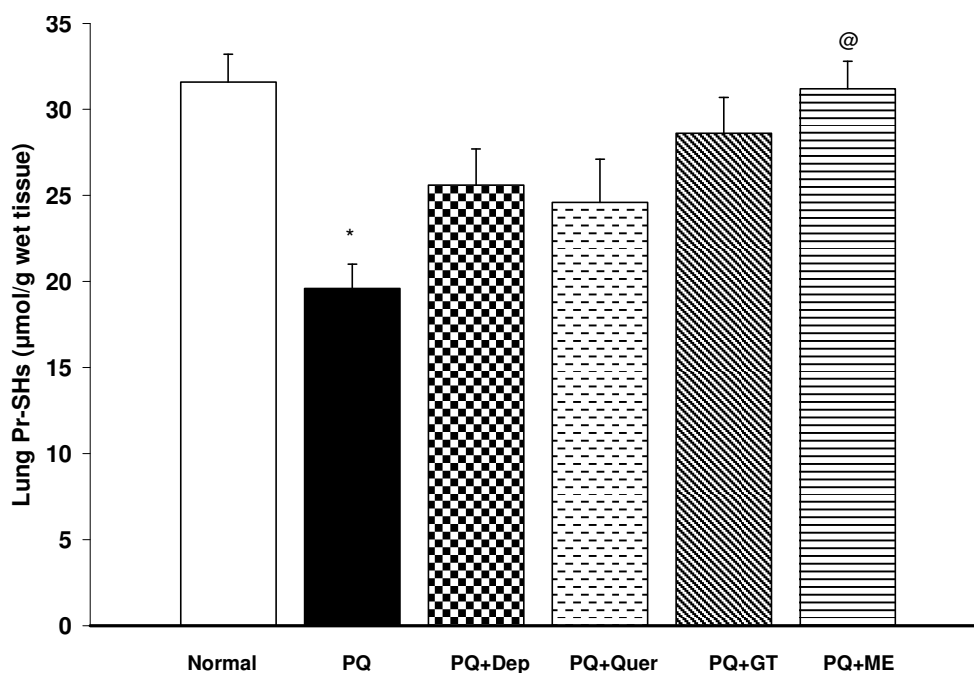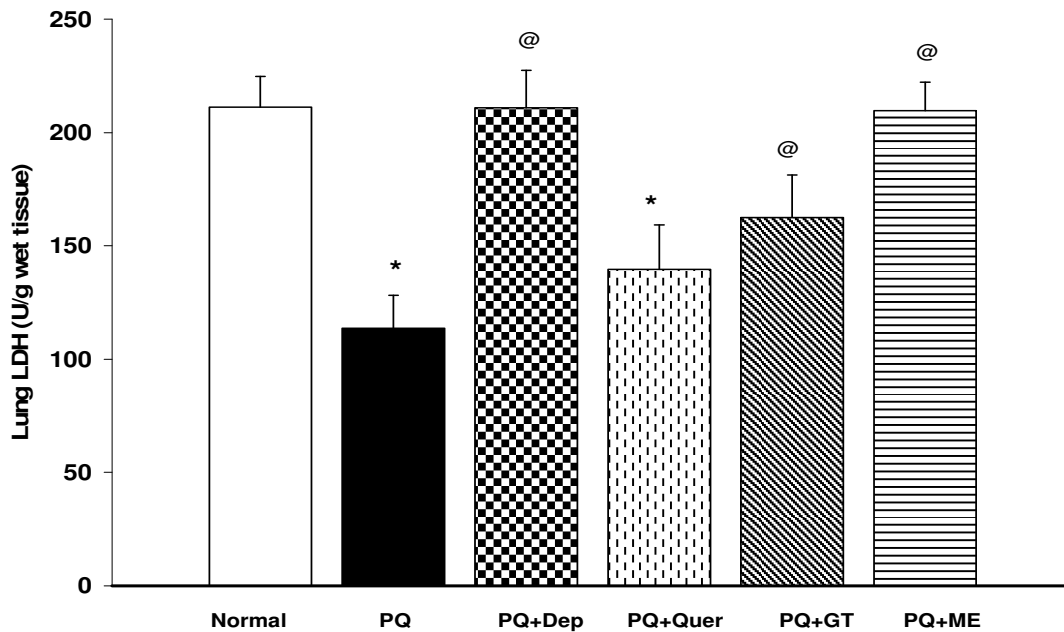
Figure 2. Effects of paraquat (PQ, 20 mg/kg, twice a week, i.p.), with or without 3 weeks daily treatments with deprenyl (Dep, 10 mg/kg, i.p.), quercetin (Quer, 50 mg/kg, p.o.), green tea extract (GT, 1 mg/kg, p.o.) or malt extract (ME, 625 mg/kg, p.o.) on lung protein thiols (Pr-SHs) content in rats.PQ was administered as two i.p. injections/week to all groups except the normal one. The test drugs were administered daily for 3 weeks. The normal group received 1% tween 80 daily. Each bar with vertical line represents the mean of 7-14 animals ± SE.Statistical analysis was carried out by ANOVA followed by Tukey-Kramer multiple comparisons test.*Significantly different from normal group at $p < 0.05$.[@] Significantly different from PQ control group at $p < 0.05$.

cantly increased the formation of TBARS by 2 fold as compared to the normal group. Treatments with deprenyl, quercetin, green tea and malt extracts reduced PQ-induced increase in lipid peroxidation by 57, 64, 67 and 54% respectively as compared to control PQ group (Figure 3).In the current investigation, PQ administration strongly suppressed the activities of cytosolic LDH and SOD by 54 and 39%, respectively, as compared to the respective normal values (Figures 4 and 5). Treatments with deprenyl and malt extract significantly prevented PQ-induced inhibition of cytosolic LDH activity. Whereas, green tea protected against PQ-induced inhibition of LDH activity by 70% as compared to PQ control group (Figure 4). On the other hand, PQ-induced inhibition of SOD activity was prevented by treatments with deprenyl, quercetin, green tea and malt extract by 53, 50, 45 and 42% respectively as compared to the corresponding control group (Figure 5). In the present investigation, PQ administration for 3 successive weeks resulted in almost 2.5 fold increase in MPO activity as compared to the normal group (Figure 6). Treatment with deprenyl, quercetin, green tea and malt extracts significantly protected against PQ-induced increase in MPO activity by 50, 48, 40 and 42%, respectively as compared to control PQ respective

value (Figure 6). Finally, administration of PQ resulted in almost 2 fold increase in NO level as compared to the normal group (Figure 7). Treatment with deprenyl, quercetin and malt extract significantly protected against PQ-induced increase in NO level by 55, 54 and 53% respectively as compared to control PQ respective value; on the other hand green tea did not significantly lower elevated NO levels as compared to control PQ group (Figure 7). Concerning the histopathological findings, PQ produced a marked pulmonary edema and hemorrhage (+++) as compared with the normal control group. Animals that received PQ showed the expected typical patterns of lung histopathology, namely: marked edema, subpleural hemorrhage, acute inflammation, perivascular mononuclear cell infiltrates, sloughing of alveolar and bronchiolar lining cells, and diffuse interstitial fibrosis. The alveolar spaces contain edematous fluid, erythrocytes, macrophages and fibrin. The most prominent finding was infiltration of the alveolar walls by mononuclear cells. Treatment with deprenyl and quercetin showed a mode-moderate heamorrhage and edema (++), as compared to the PQ control group. On the other hand, green tea and malt extracts showed very mild edema and hemorrhage (+), as compared to the PQ- treated group (Figure 8).
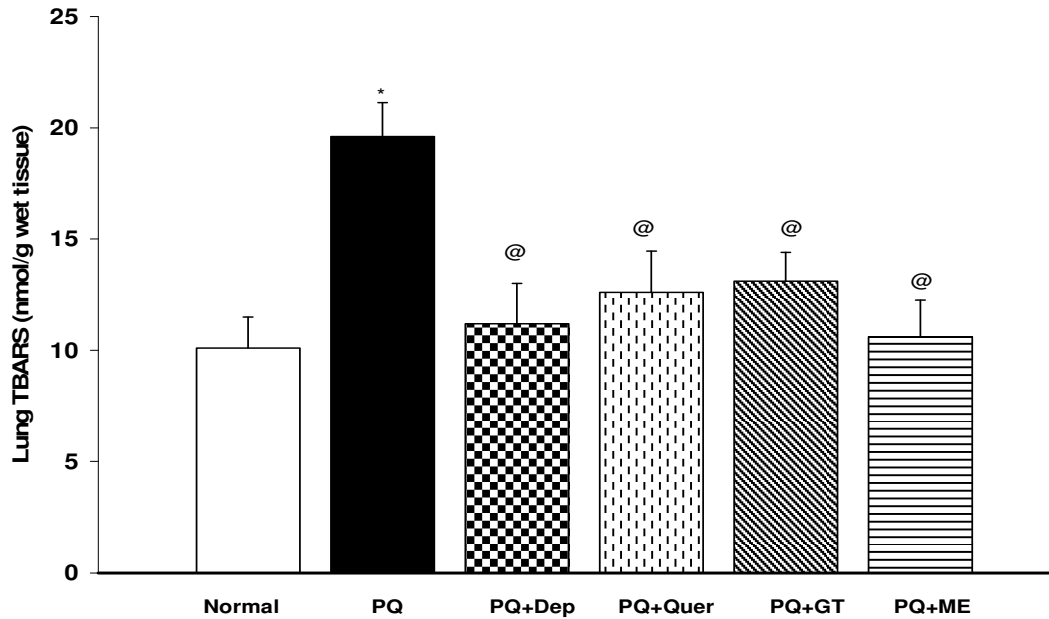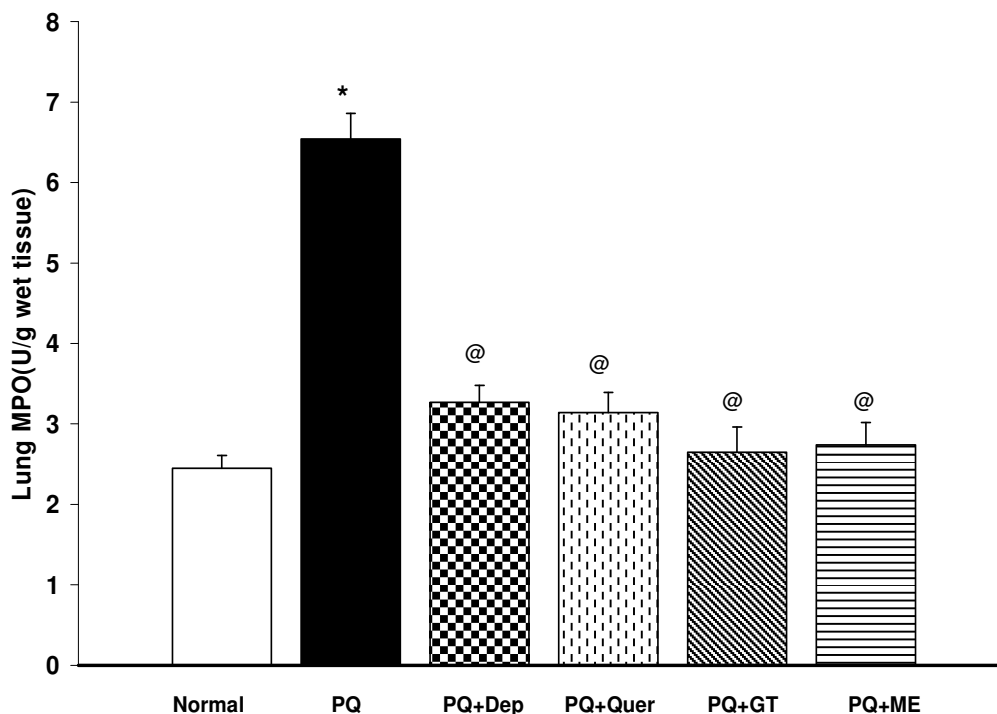
Figure 3. Effects of paraquat (PQ, 20 mg/kg, twice a week, i.p.), with or without 3 weeks daily treatments with deprenyl (Dep, 10 mg/kg, i.p.), quercetin (Quer, 50 mg/kg, p.o.), green tea extract (GT, 1 mg/kg, p.o.) or malt extract (ME, 625 mg/kg, p.o.) on lung thiobarbituric acid reactive substances (TBARS) content in rats. PQ was administered as two i.p. injections/week to all groups except the normal one. The test drugs were administered daily for 3 weeks. The normal group received 1% tween 80 daily. Each bar with vertical line represents the mean of 7-14 animals ± SE.Statistical analysis was carried out by ANOVA followed by Tukey-Kramer multiple comparisons test.* Significantly different from normal group at $p < 0.05$.@ Significantly different from PQ control group at $p < 0.05$.

Figure 4. Effects of paraquat (PQ, 20 mg/kg, twice a week, i.p.), with or without 3 weeks daily treatments with deprenyl (Dep, 10 mg/kg, i.p.), quercetin (Quer, 50 mg/kg, p.o.), green tea extract (GT, 1 mg/kg, p.o.) or malt extract (ME, 625 mg/kg, p.o.) on lung lactate dehydrogenase (LDH) activity in rats. PQ was administered as two i.p. injections/week to all groups except the normal one. The test drugs were administered daily for 3 weeks. The normal group received 1% tween 80 daily. Each bar with vertical line represents the mean of 7-14 animals ± SE.Statistical analysis was carried out by ANOVA followed by Tukey-Kramer multiple comparisons test.* Significantly different from normal group at $p < 0.05$.@ Significantly different from PQ control group at $p < 0.05$.
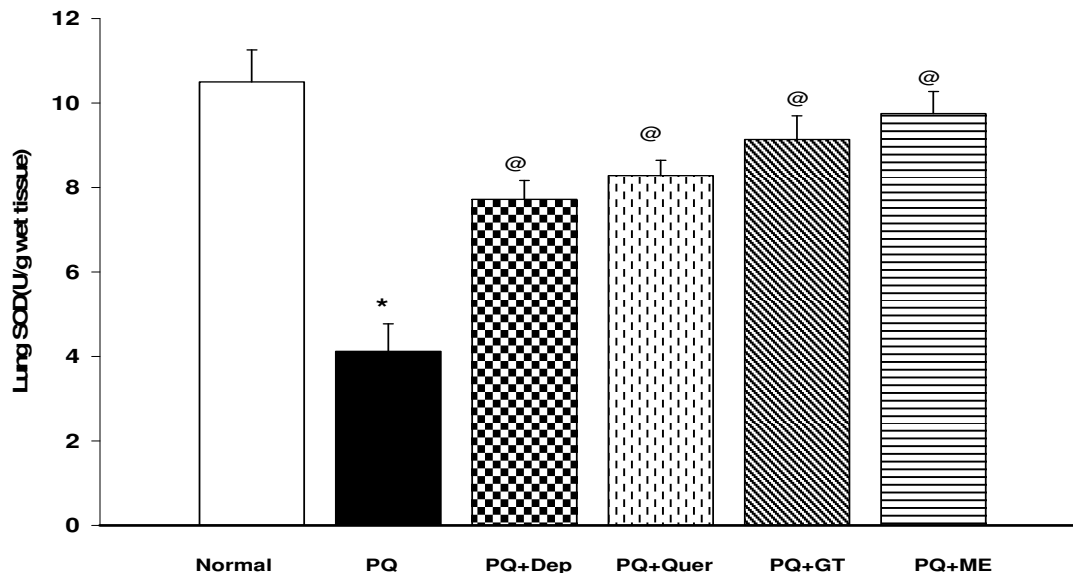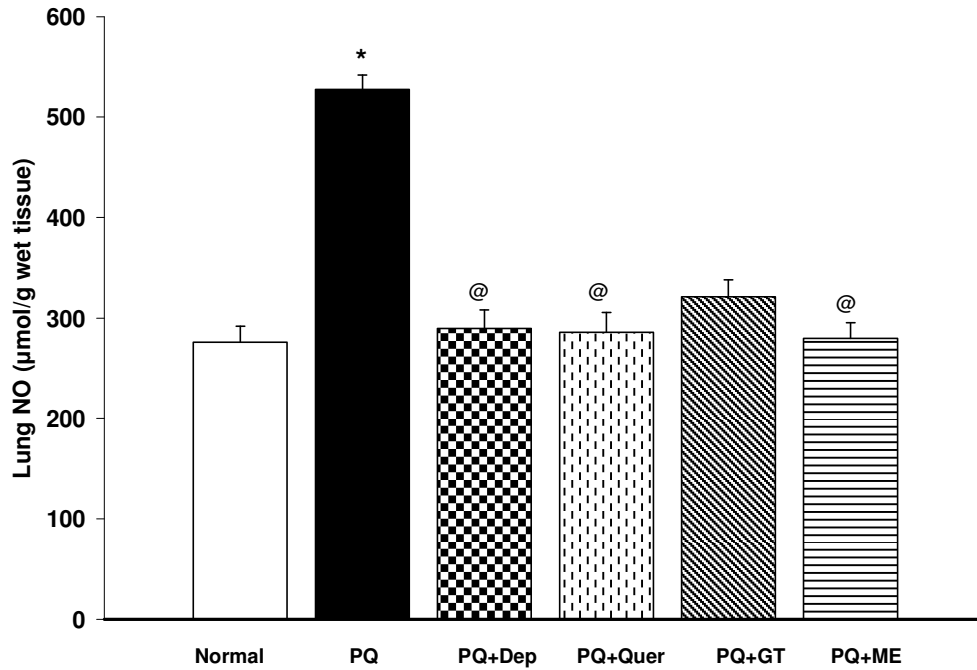
Figure 5. Effects of paraquat (PQ, 20 mg/kg, twice a week, i.p.), with or without 3 weeks daily treatments with deprenyl (Dep, 10 mg/kg, i.p.), quercetin (Quer, 50 mg/kg, p.o.), green tea extract (GT, 1 mg/kg, p.o.) or malt extract (ME, 625 mg/kg, p.o.) on lung superoxide dismutase (SOD) activity in rats. PQ was administered as two i.p. injections/week to all groups except the normal one. The test drugs were administered daily for 3 weeks. The normal group received 1% tween 80 daily. Each bar with vertical line represents the mean of 7-14 animals ± SE.Statistical analysis was carried out by ANOVA followed by Tukey-Kramer multiple comparisons test.* Significantly different from normal group at $p < 0.05$.@ Significantly different from PQ control group at $p < 0.05$.
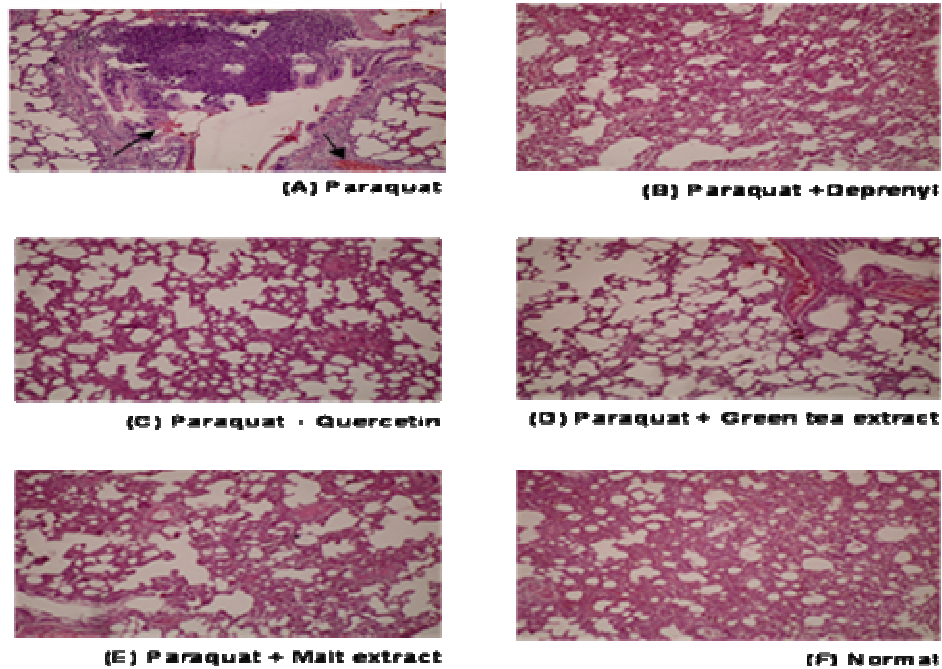

Figure 6. Effects of paraquat (PQ, 20 mg/kg, twice a week, i.p.), with or without 3 weeks daily treatments with deprenyl (Dep, 10 mg/kg, i.p.), quercetin (Quer, 50 mg/kg, p.o.), green tea extract (GT, 1 mg/kg, p.o.) or malt extract (ME, 625 mg/kg, p.o.) on lung myeloperoxidase (MPO) activity in rats. PQ was administered as two i.p. injections/week to all groups except the normal one. The test drugs were administered daily for 3 weeks. The normal group received 1% tween 80 daily. Each bar with vertical line represents the mean of 7-14 animals ± SE.Statistical analysis was carried out by ANOVA followed by Tukey-Kramer multiple comparisons test.* Significantly different from normal group at $p < 0.05$.@ Significantly different from PQ control group at $p < 0.05$.

Figure 7. Effects of paraquat (PQ, 20 mg/kg, twice a week, i.p.), with or without 3 weeks daily treatments with deprenyl (Dep, 10 mg/kg, i.p.), quercetin (Quer, 50 mg/kg, p.o.), green tea extract (GT, 1 mg/kg, p.o.) or malt extract (ME, 625 mg/kg, p.o.) on lung nitric oxide (NO) level in rats. PQ was administered as two i.p. injections/week to all groups except the normal one. The test drugs were administered daily for 3 weeks. The normal group received 1% tween 80 daily. Each bar with vertical line represents the mean of 7-14 animals ± SE.Statistical analysis was carried out by ANOVA followed by Tukey-Kramer multiple comparisons test.* Significantly different from normal group at $p < 0.05$.[@] Significantly different from PQ control group at $p < 0.05$.

Figure 8. Effects of paraquat (PQ, 20 mg/kg, twice a week. i.p), with or without 3 weeks daily treatments with: deprenyl (10 mg/kg. i.p) (B), quercetin (40 mg/kg, p.o.) (C), green tea extract (1 mg/kg, p.o) (D), or malt extract (625 mg/kg. p.o) (E), on lung histopathology. PQ-treated group showed heamorrhage (arrows) and severe edema (appeared vacuoles). Effect or different drugs shown. The normal brain histology is shown in (F). (H&EX400)

DISCUSSION

An early feature of PQ toxicity is the influx of inflamematory cells, releasing proteolytic enzymes and oxygen free radicals, which can destroy the lung epithelium and result in pulmonary fibrosis. Therefore, the ability to suppress early lung injury seems to be an appropriate therapy of pulmonary damage before the development of irreversible fibrosis (Venkatesan, 2000; Ishimoto et al., 2006). PQ is a strong pneumotoxicant, especially due to its accumulation in the lung through a polyamine uptake system and to its capacity to induce redox cycling, leading to oxidative stress-related damage (Dinis-Oliveira et al., 2007). It is concentrated in type II pneumocytes by an active process and passively diffuses into type I pneumocytes and other cells where it is converted to a paraquat radical (PQ^+.) by oxidizing NADPH (Forman et al., 1982). The paraquat radical reacts with oxygen which regenerates paraquat (PQ^{2+}) and forms superoxide radical generating other reactive species such as hydrogen peroxide and hydroxyl radicals. These reactive species cause lipid peroxidation, protein and DNA degradation which lead to cell destruction (Dusinska et al., 1998).In addition to in-creasing oxygen consumption, PQ dramatically reduces cellular NADPH concentrations (Witschi et al., 1977). Si-nce NADPH is required in multiple biological pathways, including the reduction of oxidized glutathione, the function of cytochrome P450 enzymes, and many biosynthetic reactions, depletion of this pyridine nucleotide can inhibit many essential cellular functions and contribute to toxicity (Smith, 1987). Several mammalian NADPH oxidases have been identified as potential inducers of paraquat redox cycling including cytochrome P450 reductase and nitric-oxide synthase (Day et al., 1999). These enzymes generate reduced paraquat radical that can act as an electron donor. Reacting rapidly with molecular oxygen, the paraquat radical recycles back to paraquat and in the process forms highly toxic oxidants including superoxide anion, hydrogen peroxide, hydroxyl radicals, and in the presence of nitric oxide, peroxynitrite (Dicker and Cederbaum, 1991). Cellular damage generated by these oxidants, including lipid peroxidation, may be important in paraquat-induced lung damage (Schweich et al., 1994).

Oxidative stress results from an oxidant/antioxidant imbalance in favour of oxidants. A large number of studies have demonstrated that increased oxidative burden occurs in airways diseases, shown by increased marks of oxidative stress in the airspaces and systemically in these patients (Blesa et al., 2003). There is now substantial evidence that oxidative stress plays an important role in the injurious and inflammatory responses in airways diseases such as asthma and chronic obstructive pulmonary disease (COPD) (MacNee, 2001).

In the present study, PQ increased the content of LPO and decreased GSH and Pr-SHs contents. These results were matched with the results of Candan and Alagözlü, 2001. Any pathological state that leads to increased pro-

duction and/or ineffective scavenging of reactive oxygen species may play a crucial role in determining tissue injury (Halliwell and Gutteridge, 1984).

Glutathione plays a crucial role in the cellular antioxidant defense system by scavenging free radicals and other reactive oxygen species, removing hydrogen and lipid peroxides and preventing oxidation of biomolecules (Wu et al., 2004). The oxidative stress caused by paraquat can lead to significant depletion of glutathione in tissues and reversely, inhibition of glutatione production (Nakagawa et al., 1995). Based on the above observations, it can be inferred that maintenance of glutathione content can ameliorate paraquat toxicity. GSH reacts directly with ROS and electrophilic metabolites. It protects essential thiol group from oxidation, and serves as a substrate for several enzymes including GPx. It is known to protect the cellular system against the toxic effects of lipid peroxidation (Lu, 1999).

Decreased GSH contents indicate increased oxidative stress. Furthermore, sustained oxidative challenge to the lung results in depletion of GSH and Pr-SHs. The alterations of anti-oxidant system particularly glutathione can be utilized as biomarkers during management of paraquat poisoning (Ray et al., 2007). Our results were matched with those of other researchers (Atzori et al., 1998), which indicate that the reduced ability of the glutathione redox cycle, leading to high oxidative stress, is closely associated with paraquat-induced cytotoxicity. Other researchers suggest that the reduced regenerative ability of oxidatively damaged proteins due to the inactivation of thioredoxin reductase and glutathione peroxidase by paraquat may contribute to increasing oxidative stress, leading to cell death (Takizawa et al., 2007). Consistent with this concept, the downfall in the content of GSH and Pr-SHs in this study might be due to enhanced utilization of this antioxidant for scavenging free radicals. Increased LPO products in cells can result in cellular dehydration, whole cell deformity and cell death. Thus, LPO is considered as the prime rationale that instigates lung injury. The most commonly used LPO markers are TBARS and hydroperoxides. Induction of lung fibrosis with PQ, in this study, resulted in elevated contents of TBARS signifying the increased production of oxygen radicals. Similar results of PQ-induced augmented LPO content was reported by other investigators (Candan and Alagözlü, 2001; Dinis-Oliveira et al., 2006). LPO damages membranes integrity leading to leakage of intracellular enzymes (Ghazi-Khansari et al., 2005). This could explain the decrease in cytosolic LDH activity noted by PQ in the current work. NO synthase (NOS) has been shown to participate in PQ-induced lung injury. NO reacts with $O^{-(2)}$ generated by PQ to produce the toxin peroxynitrite. Similar results were reported also by other investigators that confirm that toxicity of such redoxactive compounds involve a loss of NO-related activity (Day et al., 1999; Yeh et al., 2006). Moreover, production of pro-inflammatory cytokines has possibly been linked to PQ-induced inflammatory processes through ROS and NO; cytokines

increased in inducible NO synthase (iNOS) expression and nitrite accumulation which is potentiated by PQ. This increase could be completely prevented by NOS inhibitors (Tomita et al., 2001).It has been suggested that NO, originally known as an endothelial cell-derived vaso-relaxation factor, is important in the modulation of tissue inflammation (Rees et al., 1990). In an inflammatory setting or in the presence of endotoxin and cytokines, the calcium independent iNOS is expressed in many cell types including human lung epithelium (Moncada and Higgs, 1993). Excessive generation of NO by iNOS accounts for its proinflammatory and cytotoxic effects. In addition, NO is implicated as a major mediator responsible for PQ-induced lung injury. The mechanism by which NO triggers these events is not fully clear, but is possibly due to its rapid reaction with superoxide anion forming the strong oxidant peroxynitrite (Berisha et al.,1994).

As SOD is considered as the 1st line of defense against superoxide anions produced heavily during PQ redox cycling (Yumino et al., 2002), its activity is expected to decrease with PQ administration due to its consumption in neutralizing the produced superoxide anions. In the current investigation, daily treatment of rats with deprenyl (10 mg/kg) prevented almost all of the biochemical changes induced by PQ in lung homogenates. Deprenyl is known to up-regulate activities of antioxidant enzymes such as SOD and catalase (CAT) in brain dopaminergic regions (Li et al., 1998). Furthermore, the drug was found to enhance anti-oxidant enzyme activities not only in brain but also in extra-brain tissues as heart, kidneys, adrenal glands and the spleen. Moreover, it was found in an increase of many humoral factors interferon-gamma (INF-γ), tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β) and enhancement of natural killer (NK) cell functions (Kitani
et al., 2002).

Deprenyl, by inducing antioxidant enzymes and decreasing the formation of reactive oxygen species, is able to combat an oxidative challenge implicated as a common causative factor in neurodegenerative diseases. In a dose substantially lower than required for MAO-B inhibition, deprenyl interferes with early apoptotic signalling events induced by various kinds of insults in cell cultures of neuroectodermal origin, thus protecting cells from apoptotic death (Magyar and Szende, 2004). Deprenyl requires metabolic conversion to a hitherto unidentified metabolite to exert its antiapoptotic effect, which serves to protect the integrity of the mitochondrion by inducing transcriptional and translational changes (Magyar et al., 2004). The antioxidant effects of deprenyl were collectively extrapolated in this study to investigate its antioxidant and anti-inflammatory effects on experimentally-induced lung fibrosis. Green tea extract daily administered for 3 weeks to experimental animals alleviated greatly the biochemical changes associated with PQ-induced lung fibrosis in the present study. Previous structure–activity studies indicated that the presence of a gallate (G)-ring and either a catechol (B) - ring or a pyrogallol

important for the antioxidant activities of catechins (Mukai et al., 2001). Several reports confirmed the inhibitory activity of EGCG on LPO in experimental animals (Chen et al., 2004).

Since transition metals such as iron may play a central role in the process of lipid peroxidation during experimentally-induced lung fibrosis (Gong et al., 2004), reducing the availability of iron may lessen the extent of lipid peroxidation and culminate pulmonary fibrosis.

The decreased LPO production observed in green tea treated group in the present study might also be due to its iron chelating activity, since green tea polyphenols have been shown to possess relatively potent metal chelating properties (Guo et al., 1996; Frei et al., 2003). The potential metal chelating activity could be attributed to the gallate moiety present in the C-ring (Kumamoto et al., 2001). This may be of major significance for treatment of PQ-induced pulmonary fibrosis.

The induction of antioxidant enzymes could be a critical approach for protecting cells against a variety of endogenous and exogenous toxic compounds such as ROS (Sen, 1995). The equilibrium between these enzymes is important for the effective removal of free radicals and oxidative stress in intracellular organelles. A notable descend in these enzymes activities was observed in PQ-induced damage, which might be due to increased LPO and over-production of ROS. This is maintained to near normal values upon treatment with green tea. This may be due to the direct action of EGCG on superoxide, hydroxyl and alkoxyl radical coupled with its ability to attenuate LPO, which in turn reduces free radical generation and oxidative stress during pulmonary fibrosis. This is in agreement with a previous report in another model of bleomycin-induced lung fibrosis (Sriram et al., 2008).

Treatment of rats with quercetin in the present experiment markedly protected against PQ-induced lung fibrosis. These effects were observed on most of the biochemical parameters. The antioxidant properties of quercetin, its effects on several enzyme systems, and effects on biological pathways involved in carcinogenesis, inflammation, and cardiovascular diseases have been largely studied (Middleton et al., 2000).

Quercetin supplementation significantly increased all the tested antioxidants. This may be due to the ability of quercetin to interact with hydroxyl, superoxide, alkoxyl and peroxyl radicals thereby subsequently scavenging them, inhibiting iNOS protein expression and chelation of transition metals ions (Terao, 1999). Hence, it is suggested that the quercetin treatment could have protected against the fibrogenic effects of PQ.

Certain studies confirm that quercetin and its metabolites were widely distributed in rat tissues, with the highest concentrations in lungs (3.98 and 15.3 nmol/g tissue for the 0.1 and 1% quercetin diet, respectively) (de Boer et al., 2005). Interestingly, several studies have demonstrated that dietary quercetin enhanced the antioxidant defense system by up regulating antioxidant enzymes (Nagata et al., 1999).

Using malt extract in the current investigation protected markedly against PQ induced lung fibrosis. Malt extract is prepared from barley grains (Sweetman, 2005). The beneficial and antioxidant effects of both malt extract and barley grains are well recognized (Goupy et al., 1999; Liu and Yao, 2007). The antioxidant potency of barley was manifested as increasing the glutathione peroxidase activity and decreasing TBARS content in serum of rats fed with a barley diet (Goupy et al., 1999; Zduńczyk et al., 2006). This could account for the present pulmonary protective effects noted by malt extract. Regarding the histopathological investigation, previous researchers reported similar pulmonary toxic effects of PQ. The pre-sent results are characterized by initial development of pulmonary edema, infiltration of inflammatory cells, alveolar hemorrhage, fibroblast proliferation and increased collagen deposition (Nerlich et al., 1984). Intraperitoneal administration of PQ to rodents is considered to thoroughly reproduce the histologic alterations that resemble the human pulmonary fibrosis. In our experiments, we have observed marked distortion of the alveolar space with hemorrhage coupled with large number of leukocyte invasion in PQ-induced rats. These histological changes could be attributed to increased oxidative stress (Grande et al., 1998). The rats treated with green tea and malt extract showed normal histology probably due to their potent antioxidant activity. Thus to conclude, it can be stated that the natural products used in this study, due to their antioxidant activity could form an effective drug regimen for pulmonary fibrosis. Further studies are required to elucidate multiple mechanisms that devise their protective effect against pulmonary fibrosis.

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