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Effect of *Sesbania grandiflora* on Lung Antioxidant Defense System in Cigarette Smoke Exposed Rats

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Abstract: This study is performed to investigate the harmful effects of cigarette smoke on lung antioxidant defense system and restorative property of *Sesbania grandiflora* in rat lung. Adult male WKY rats were exposed to cigarette smoke for a period of 90 days and consecutively treated with aqueous suspension of *S. grandiflora* (1000 mg kg⁻¹ b.w./day, p.o) for a period of 3 weeks. The level of lipid peroxide was evaluated as marker of lung damage. Antioxidant status of the lung was assessed from the levels of reduced glutathione, vitamin C and vitamin E and the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and glucose-6-phosphate dehydrogenase. The levels of copper, zinc, manganese and selenium in lung were also measured. Increased lipid peroxidation was evident from the diminished levels of both enzymatic and non-enzymatic antioxidants. Alterations in the levels of trace elements with elevation of copper and depletion of zinc, manganese and selenium were also observed. *S. grandiflora* administration ameliorated the antioxidant status and maintained the levels of trace elements. These results suggest that chronic cigarette smoke exposure enhances oxidative stress, thereby disturbing the tissue defense system and *S. grandiflora* protects the lung from the oxidative damage through its antioxidant potential.

Key words: Antioxidant, cigarette smoke, lipidperoxidation, lung, *Sesbania grandiflora*

INTRODUCTION

Cigarette smoke is a complex mixture which contains more than 4700 chemical constituents (Rahman and MacNee, 1996). These include various compounds which are capable of causing an increase in the generation of various reactive oxygen species like O₂^{•-}, H₂O₂, OH[•], ROO[•]. These Reactive Oxygen Species (ROS) in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation. Cigarette smoke is the major etiologic factor associated with the development of Chronic Obstructive Pulmonary Disease (COPD) as well as lung cancer. In addition, significant associations were made between smoking and a host of other diseases such as cancer of the gastrointestinal tract, bladder and cervix and cardiovascular and cerebrovascular disease (Jeffery, 1999). Cigarette smoke exposure increases the recruitment and activation of phagocytes in the lungs resulting further production of reactive oxygen species by inflammatory cells. Numerous investigators have highlighted and emphasized ROS production and inflammation in smokers and individuals with COPD (Rahman *et al.*, 2000). A number of enzymes are involved in the protection against the injurious effects of ROS. These enzymes include superoxide dismutase, catalase and glutathione peroxidase. In addition to these enzymes, antioxidants such as glutathione, ascorbate, α -tocopherol, uric acid and lipoic acid also protect against oxidants. The harmful effects of cigarette smoke generated ROS are kept under

investigation by a delicate balance between the rate of their production and elimination by the different antioxidant defense systems. If any change in this critical balance would result in an increase in the levels of ROS and may lead to cellular damage. Antioxidants have been shown to decrease smoke-induced oxidative damage (Panda *et al.*, 2000). In addition, numerous epidemiologic studies have found a positive correlation between dietary intake of antioxidants and lung function (Tabak *et al.*, 1999; Butland *et al.*, 2000). In view of this, we selected *S. grandiflora* which has antioxidant property to evaluate the protective role on lung antioxidant defense system in rats exposed to cigarette smoke.

Sesbania grandiflora L. Pers (Fabaceae), commonly known as sesbania and agathi, is used as an important dietary nutritive source in Southeast Asian countries. *S. grandiflora* leaves are the richest source of amino acids, minerals and antioxidant vitamins (The Wealth of India, 1972; Govindan and Shanmugasundaram, 1987). Various parts of this plant are used in Indian traditional medicine for the treatment of a broad spectrum of illness including leprosy, gout, rheumatism and liver disorders (Vijayakumar *et al.*, 1997; Joshi, 2000; Pari and Uma, 2003). It also has anxiolytic and anticonvulsive (Kasture *et al.*, 2002), anti-inflammatory, analgesic and antipyretic activity (Tamboli, 1996, 2000). Recently we reported that *S. grandiflora* has protective effect against cigarette smoke induced oxidative damage and hypolipidemic property on cigarette smoke exposed rats (Ramesh and Begum, 2006, 2007). However, the mechanisms underlying its beneficial effects against smoking associated diseases are yet to be fully elucidated. The present study is performed to investigate the protective role of *S. grandiflora* against oxidative stress in the lung of rats exposed to cigarette smoke by measuring the lipid peroxides, enzymatic and non-enzymatic antioxidants and trace elements.

MATERIALS AND METHODS

Chemicals and Reagents

Thiobarbituric acid, Reduced glutathione, Oxidized glutathione, NADH, NADP, ascorbic acid and α -tocopherol were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals and reagents used were of analytical grade with highest purity and obtained from Glaxo Laboratories (P) Ltd., Mumbai, India.

Plant Material

Fresh *Sesbania grandiflora* leaves were collected from a local plantation (Poovathur, Thanjavur, India). The leaves were washed for any contaminants, dried thoroughly under shade and powdered finely. The powdered leaves of *S. grandiflora* were reconstituted in distilled water as 0.1% suspension and administered 10 mL kg⁻¹ body weight. The aqueous suspension of *Sesbania grandiflora* (ASSG) leaves was prepared freshly every day prior to administration.

Experimental Animals

Male Wistar-Kyoto (WKY) rats weighing 125-150 g were obtained from Venkateshwara Animal Breeding Centre, Bangalore, India. All animal experiments and maintenance were carried out according to the ethical guidelines suggested by the Institutional Animal Ethics Committee. Animals were housed in polypropylene cages with filter tops under controlled conditions of a 12 h light/12 h dark cycle at 27±2°C. All the rats received standard pellet diet (Amrut rat feed, Pune, India) and water *ad libitum*.

Experimental Protocol

The animals were divided into four groups of each six animals. Group I: Control. Group II: Rats administered with ASSG (1000 mg kg⁻¹ b.w./day, p.o) for a period of three weeks. Group III: Rats exposed to cigarette smoke. Group IV: Rats exposed to cigarette smoke and consecutively administered

with ASSG (1000 mg kg⁻¹ b.w./day, p.o) for a period of three weeks. Group III and Group IV rats were exposed to cigarette smoke followed by the modified method of Eun-Mi *et al.* (1998) as described below.

The rats were placed in a polypropylene cage with a lid made of polythene paper. A lighted cigarette was placed in a flask connected to the cage and air was supplied into the flask for 10 min by a small air pump. A length of 5.9 cm of each cigarette was allowed to be burned by clamping the butt when it was placed in a flask. Each rat was subjected to inhalation of cigarette smoke seven times a day at regular intervals of one hour (from 11 am to 5 pm) for a period of 90 days. Control rats were treated as similar exposed to air instead of smoke.

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Lungs were isolated and cleaned of adhering fat and connective tissues. Known weight of tissues were homogenized in 0.1 M tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and used for the biochemical estimation.

Biochemical Estimation

Malondialdehyde (MDA) was estimated by the method of Beuge and Aust (1978). Superoxide dismutase (SOD) activity was assayed by the method of Kakkar *et al.* (1984). Catalase (CAT) activity was measured by the method of Beers and Sizer (1952). The activity of glutathione peroxidase (GPx) was assayed by the method of Rotruck *et al.* (1973). Glutathione Reductase (GR) activity was measured by the method of Stall *et al.* (1969). Glutathione-S-Transferase (GST) activity was determined by the method of Habig *et al.* (1974). Activity of Glucose-6-phosphate dehydrogenase (G6PDH) was assayed by the method of Ellis and Kirkman (1961). Reduced glutathione was measured according to the procedure of Moron *et al.* (1979). Vitamin C was quantified according to the method of Omaye *et al.* (1979). Vitamin E was measured by the method of Baker *et al.* (1980). Protein was estimated by the method of Lowry *et al.* (1951). Copper (Cu), zinc (Zn) and manganese (Mn) was estimated using atomic absorption spectrophotometer after digestion with nitric acid and perchloric acid and selenium (Se) using coupled atomic emission spectrophotometer and flurometer.

Statistical Analysis

Results are expressed as mean±SD (n = 6). The observed differences were analyzed for statistical significance by one-way of the analysis of variance with Tukey's multiple comparison as post test.

RESULTS

Group III rats exposed to cigarette smoke showed a significant increase in the levels of MDA and significant decrease in the levels of GSH, vitamin C and vitamin E when compared to control rats (Group I, Table 1). A significant decrease in the levels of MDA while increase in the levels GSH, vitamin C and vitamin E was observed in Group IV rats when compared to Group III rats. Group II rats administered with ASSG alone did not show any significant changes as compared with Group I rats.

Table 1: Effect of *S. grandiflora* on MDA, GSH, vitamin C and vitamin E in the lung of control and experimental animals

Parameters	Group I	Group II	Group III	Group IV
MDA	3.75±0.14	3.71±0.12 ^{NS}	4.45±0.18*	3.94±0.17*
GSH	4.50±0.23	4.75±0.25 ^{NS}	4.17±0.19*	4.69±0.16 [†]
Vitamin C	7.09±0.46	7.03±0.42 ^{NS}	5.45±0.37*	6.74±0.41*
Vitamin E	2.75±0.27	2.99±0.26 ^{NS}	2.08±0.10*	2.43±0.17 [‡]

Values are expressed as mean±SD (n = 6). Unit of MDA: nmoles mg⁻¹ protein. Units of GSH, Vitamin C and Vitamin E: µg mg⁻¹ protein. Statistical comparisons are made between Group I vs. Group II and Group III; Group III vs. Group IV. *p<0.001, [†]p<0.01, [‡]p<0.05 (Tukey's test), NS: Non Significant

Table 2: Effect of *S. grandiflora* on SOD, CAT, GPx, GR, G6PDH and GST in the lung of control and experimental animals

Parameters	Group I	Group II	Group III	Group IV
SOD	4.68±0.41	5.01±0.35 ^{NS}	0.26±0.04*	5.91±0.45*
CAT	25.35±1.22	25.39±1.25 ^{NS}	16.28±1.07*	21.31±1.24*
GPx	7.48±0.52	7.49±0.44 ^{NS}	4.92±0.38*	5.98±0.44 [#]
GR	0.35±0.03	0.32±0.03 ^{NS}	0.19±0.02*	0.26±0.03*
G6PDH	3.69±0.18	3.52±0.14 ^{NS}	2.24±0.16*	3.41±0.17*
GST	0.47±0.05	0.45±0.03 ^{NS}	0.29±0.04*	0.41±0.04*

Values are expressed as mean±SD (n = 6). Unit: SOD: U min⁻¹ mg⁻¹ protein, CAT: μmoles of H₂O₂ decomposed min⁻¹ mg⁻¹ protein, GPx: μmoles of GSH oxidized min⁻¹ mg⁻¹ protein, GR: μmoles of NADPH oxidized min⁻¹ mg⁻¹ protein, G6PDH: U min⁻¹ mg⁻¹ protein and GST: μmoles of CDNB-GSH conjugated min⁻¹ mg⁻¹ protein. Statistical comparisons are made between Group I vs. Group II and Group III; Group III vs. Group IV, *p<0.001, [#]p<0.01 (Tukey's test), NS: Non Significant

Table 3: Effect of *S. grandiflora* on Copper (Cu), Zinc (Zn), Manganese (Mn) and Selenium (Se) in the lung of control and experimental animals

Parameters	Group I	Group II	Group III	Group IV
Copper	2.40±0.12	2.40±0.10 ^{NS}	4.30±0.20*	3.60±0.14*
Zinc	23.00±1.42	24.00±1.63 ^{NS}	16.00±1.01*	19.00±1.21*
Manganese	1.22±0.04	1.29±0.05 ^{NS}	0.25±0.01*	1.03±0.06*
Selenium	0.64±0.03	0.67±0.04 ^{NS}	0.46±0.02*	0.61±0.03*

Values are expressed as mean±SD (n = 6). Units of Cu, Zn, Mn and Se: μg g⁻¹ wet tissue. Statistical comparisons are made between Group I vs. Group II and Group III; Group III vs. Group IV, *p<0.001 (Tukey's test), NS: Non Significant

A significant decrease in the activities of SOD, CAT, GPx, GR, G6PDH and GST were observed in Group III rats exposed to cigarette smoke as compared to Group I rats (Table 2). Group IV rats showed increased activities of these enzymes as compared to Group III rats. ASSG alone administered Group II rats did not show any significant alterations in their activities as compared to Group I rats.

The levels of zinc, manganese and selenium were significantly decreased while the levels of copper was significantly increased in rats exposed to cigarette smoke (Group III) when compared with Group I rats (Table 3). ASSG treated with cigarette smoke exposed rats (Group IV) showed a significant decrease in the level of copper and significant increase in the levels of zinc, manganese and selenium as compared to Group III rats. Significant changes were not observed in these parameters in Group II rats as compared to Group I rats.

DISCUSSION

Cigarette smoking has been implicated as a major risk factor for pulmonary diseases. The adverse effect of the cigarette smoke is due to the presence of a large amount of reactive oxygen species. ROS have been reported to play an important role in several models of lung injury (Terrasa *et al.*, 2005). When the production of ROS exceeds the ability of the antioxidant system will be eradicated, result in oxidative stress induced peroxidative lung damage. Peroxidative destruction of cell membranes may lead to loss of functional integrity of the cell membrane and formation of lipid peroxidation product such as MDA. In the present study, we observed a significant increase in the levels of MDA in rats exposed to cigarette smoke. Similarly Koul *et al.* (2001) showed increased LPO level in the lung of cigarette smoke exposed mice. Recent studies also reported that LPO level was increased in the lung of rats exposed to cigarette smoke (Unlu *et al.*, 2006; Luchese *et al.*, 2007; Ramesh *et al.*, 2007). These findings are consistent with our report. ASSG administration decreased the levels of MDA in the lung of cigarette smoke exposed rats. This might be attributed to antioxidant vitamins like ascorbic acid and β-carotene which are present in *S. grandiflora*.

Vitamins are important for lung development, being involved in several reactions regarding alveoli growth and regeneration and lung protection (Talati *et al.*, 2006). Antioxidant vitamins such as ascorbic acid (vitamin C) and α-tocopherol (vitamin E) have been described as potent substances due to their

interaction with oxidizing radicals. Vitamin C is known to scavenge aqueous reactive oxygen species by rapid electron transfer and thus inhibits lipid peroxidation and reduces the level of oxidized vitamin E. vitamin E terminates the chain reaction of lipid peroxidation in membranes and lipoproteins (Tug *et al.*, 2004; Agacdiken *et al.*, 2004). The present study showed a significant decrease in the levels of vitamin C and vitamin E in cigarette smoke exposed rats. Our result is controversial with earlier studies. Airiess *et al.* (1988) reported that vitamin E concentration was significantly increased and no alteration in vitamin C level in the lung of cigarette smoke exposed guinea pigs. According to the previous study the guinea pigs were exposed to cigarette smoke twice a day but in our present study the rats were exposed to cigarette smoke seven times per day. This might be the reason for the decreased concentration of vitamin C and E. Present study suggests that overexposure of cigarette smoke generated free radicals depletes these vitamins. Recent studies showed that cigarette smoke exposure decreases the levels of vitamin C and vitamin E in the rat lung and brain (Anbarasi *et al.*, 2006; Luchese *et al.*, 2007). Administration of ASSG ameliorated the levels of vitamin C and E in the lung of cigarette smoke exposed rats. *S. grandiflora* contains only vitamin C not vitamin E. However, vitamin C can act synergistically to preserve vitamin E either by sparing it from oxidation or by regenerating it after it is oxidized (May *et al.*, 1998).

GSH is an emerged fundamental antioxidant defense mechanism in oxidant-induced lung injury and inflammation. GSH and its redox system are important for the detoxification of toxic metabolites and lipid peroxides in lung tissue. Acute exposure of cigarette smoke to lungs *in vivo* in rats and rabbits has been shown to deplete intracellular GSH (Li *et al.*, 1994; Rahman *et al.*, 1995). However, chronic inhalation of cigarette smoke in rats was associated with a dramatic depletion of GSH in the lung (Koul *et al.*, 2001). Cigarette smoking may also affect critical detoxifying and regulatory enzymes such as GPx, GR, GST and G6PDH involved in the GSH redox system in lung (Joshi *et al.*, 1988; Rahman *et al.*, 1995). Present study also showed decreased concentration of GSH and diminished activities of GPx, GR, GST and G6PDH in the lung of cigarette smoke exposed rats. This might be due to increased utilization for the detoxification of free radicals generated by cigarette smoke. Treatment with ASSG has reversed the levels of GSH and activities of these enzymes.

SOD is the primary enzyme to defend the lung from the damaging effects of superoxide radicals. It does this by converting superoxide into hydrogen peroxide, which can then be broken down into water by antioxidants such as catalase and glutathione. In the present study, SOD and catalase activities were significantly decreased in the lung of cigarette smoke exposed rats. The decreased activities of SOD and catalase in the current study are in concord with most investigators that cigarette smoke increases oxidant and decreases antioxidant levels (Pang *et al.*, 2003; Unlu *et al.*, 2006). Administration of ASSG ameliorated the activities of SOD and catalase which could be a result of decreased lipid peroxidation and/or decreased utilization.

Transition metal complexes are known to play a major role in free radical biology and cigarette tar contains large amounts of metals, complexed to some components of tar such as *o*-diphenols (Cross *et al.*, 1987). The present study observed increase in the level of copper in the lung could be due to the mobilization of copper from copper-binding protein induced by cigarette smoke exposure, which accelerate the oxidant injury through the formation of hydroxyl radicals via Haber-Weiss/Fenton reaction (Lapenna *et al.*, 1995). The increased copper level is highly toxic to lung. Damaged lung tissue undergoes rapid lipid peroxidation, presumably because metals released by cell disruption are not safely sequestered. Treatment with ASSG has decreased the levels of copper by scavenging the free radicals.

Trace elements such as zinc, manganese and selenium mainly constitute cofactors for various antioxidant enzymes like SOD, GPx, etc., The present study showed decreased levels of zinc, manganese and selenium in the lung of rats exposed to cigarette smoke. This might be attributed to heavy metals like cadmium, arsenic and lead which are present in cigarette smoke (Smith *et al.*, 1997; Satarug *et al.*,

2004). These heavy metals could replace the trace element from antioxidant enzyme and depletes the enzyme activities (Sulochana *et al.*, 1998). These results are correlated with the decreased activities of SOD and GPx which are observed in our study. ASSG administration has restored the levels of zinc, manganese and selenium. Earlier study showed that mineral mixture Ca/P, Zn and Fe replaced the heavy metals from the body (Groten *et al.*, 1991). This report is concurrence with present research because *S. grandiflora* also contains these minerals which could replace the heavy metals from the antioxidant enzymes.

In conclusion, *S. grandiflora* has a good antioxidant property, which is proved by above experimental studies that the increased antioxidants' status and decreased lipid peroxidation, which may protects the risk of chronic obstructive pulmonary disease and lung cancer in rats administered with ASSG. Hence, it can be used as a therapeutic supplement in functional food for smoke related diseases.

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