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SESBANIA GRANDIFLORA DIMINISHES OXIDATIVE STRESS AND AMELIORATES ANTIOXIDANT CAPACITY IN LIVER AND KIDNEY OF RATS EXPOSED TO CIGARETTE SMOKE

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Cigarette smoke is a major risk factor for many chronic diseases. However, it may be possible to relieve the smoke-induced damage by increasing the defensive system. In this study, we planned to evaluate the protective mechanism of *Sesbania grandiflora* (*S. grandiflora*) leaves against cigarette smoke-induced oxidative damage in liver and kidney of rats. Adult male Wistar-Kyoto rats were exposed to cigarette smoke for a period of 90 days and consecutively treated with *S. grandiflora* aqueous suspension (SGAS, 1000 mg/kg body weight per day by oral gavage) for a period of 3 weeks. Hepatic marker enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), as well as renal markers such as urea and creatinine were analysed in serum. Lipid peroxidation marker mainly thiobarbituric acid reactive substances (TBARS) and antioxidant enzymes namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and glucose-6-phosphate dehydrogenase (G6PDH) activities and non-enzymatic antioxidants such as reduced glutathione, ascorbic acid and α-tocopherol levels were studied. In addition, micronutrients mainly copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) levels were analyzed in liver and kidney of rats exposed to cigarette smoke. The results indicated that SGAS significantly decreased the elevated hepatic, renal and lipid peroxidation markers and ameliorated the diminished antioxidant levels while restored the hepatic and renal architecture in cigarette smoke-exposed rats. This study concludes that *S. grandiflora* leaves restrain cigarette smoke-induced oxidative damage in liver and kidney of rats.

Key words: *Sesbania grandiflora, cigarette smoke, oxidative damage, lipid peroxidation, antioxidant, liver, kidney, micronutrients*

INTRODUCTION

Cigarette smoking is a preventable risk factor and introduces many chronic diseases to increase morbidity and mortality. However, it may be possible to mitigate the smoke-induced oxidative damage by ameliorating the defense mechanism. The toxicity of cigarette smoke is due to nicotine, cadmium, benzopyrene, oxidants, and inducers of reactive oxygen species (ROS) like nitric oxide (NO), nitrogen dioxide (NO_2), peroxynitrite, and nitrosamines that initiate, promote, or amplify oxidative damage (1). Free radicals or ROS induced by cigarette smoke are thought to be responsible for the induction of many diseases, including toxicity in the lung, liver and kidney.

Cigarette smoking is the leading risk factor for lung cancer, contributing to 80-90% of lung cancer cases. Several studies were depicted the potential mechanism of cigarette smoking induced lung cancer and chemoprevention. Yoshie and Ohshima (2) explained the DNA single-strand breakage when incubation of plasmid DNA with aqueous extract of cigarette smokes tar which is a NO-releasing compound by the formation of potent ROS such as peroxynitrite. This will play an important role in cigarette smoke induced lung cancer. Polycyclic aromatic hydrocarbons (PAHs) are some of the most well-known compounds of the very tumorigenic portion of cigarette smoke condensates. Bay or baylike regions of PAHs will inhibit gap junctional intercellular communication (GJIC) results in uncontrolled cellular growth leading to the development of tumors (3). Izzotti *et al*. (4) reported that environmental cigarette smoke causes remarkable proteome alterations in rat lung which contributing to the pathogenesis of a variety of lung diseases including lung cancer and also they observed modulation in proteome alteration using *N*-acetylcysteine as a chemopreventive agent.

Considerable experimental evidence supports the idea that ROS play a key role in the pathophysiological progression of hepatic and renal tissue damage (5, 6). Cigarette smoking may also cause an oxidative burst resulting from ROS at the cellular level (1). The potential harmful effects of ROS are controlled by cellular antioxidant defense mechanisms including enzymatic defense systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as non-enzymatic defense systems such as reduced glutathione (GSH),

vitamin A, vitamin C and vitamin E. The toxic effects of the free radicals are kept under control by a fragile balance between the rate of their production and the rate of their elimination by these defense systems (7). When there is an extreme accumulation of free radicals from exogenous sources added to the endogenous production, the available tissue defense system becomes overwhelmed resulting in oxidative damage to the tissues. The mandatory use of the body's reserve of antioxidants to detoxify the remarkable level of these free radicals in smokers therefore results in severe antioxidant deficiency status, thereby predisposing them to the development of life threatening diseases. Besides, this deficiency in smokers may be enhanced by their generally lower intake of both supplementary and dietary antioxidants (8). When the normal level of antioxidant defense system is inadequate for the eradication of excessive free radicals, supplementation of exogenous antioxidants has a protective role to play (9). Numerous micronutrients and antioxidants of natural origin have been experimentally showed as efficient protective agents against cigarette smoking induced oxidative stress (10-13). Besides, we are warning that cigarette smokers should not take high amount of vitamins because high dose of α-tocopherol and β-carotene supplementation increases the incidence of lung cancer (14). Duffield-Lillico and Begg (15) reported that alpha-tocopherol beta-carotene cancer prevention (ATBC) trial and carotene and retinol efficacy (CARET) trial has failed. This is due to the free radical rich environment produced by chemicals in cigarette smoke and the resultant inflammatory response in the lung combine to induce oxidation of β-carotene, resulting in a proxidant effect. Low physiologic doses of β-carotene (equivalent to the 6 mg of β-carotene per day attainable from a human diet high in fruits and vegetables) provided mild protection against cigarette smoke-induced squamous metaplasia. In this view, we planned to evaluate the protective mechanism of *S. grandiflora* against cigarette smoke induced oxidative damage.

Sesbania grandiflora L. pers (Febaceae), commonly known as 'sesbania' and 'agathi', has been used as an important dietary nutritive source in Southeast Asian countries. *S. grandiflora* leaves contains essential amino acids, minerals, vitamins (vitamin A, vitamin E, vitamin C, thiamine, riboflavin and nicotinic acid) and other active compounds such as pectin, triterpenoid, tannin, glycosides, and grandiflorol (α-5-methyl-5-pentacosanol) and also contains a saponin which is on hydrolysis gave an acid sapogenin oleanoic acid, galactose, rhamnose and glucuronic acid (16-19). Various parts of this plant are used in Indian traditional medicine for the treatment of a broad spectrum of illness including leprosy, gout, and rheumatism and liver disorders (20, 21). *S. grandiflora* leaves also has anxiolytic, anticonvulsive, antifertility, anti-inflammatory, analgesic, antipyretic, antiurolithiatic and antioxidant activity (18, 22-25). Our previous studies reported some protective effects of *S. grandiflora* on cigarette smoke-exposed rats (26, 27). However, the mechanisms underlying its beneficial effects against smoking associated diseases are to be fully elucidated. The present study was undertaken to assess the protective mechanism of *S. grandiflora* leaves on oxidative damage in the liver and kidney of rats exposed to cigarette smoke by measuring the oxidative markers, enzymatic and non-enzymatic antioxidants and micronutrients.

MATERIALS AND METHODS

Chemicals

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 and of highest purity, and obtained from Glaxo Laboratories (P) Ltd. (Mumbai, India). Locally available brand of cigarette, Scissors Standard (W.D. & H.O. Wills), manufactured by Hyderabad Deccan Cigarette Factory was used in the present study.

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 to form a suspension. The aqueous suspension of *S. grandiflora* leaves was prepared freshly every day prior to the administration.

Experimental animals

Male Wistar-Kyoto 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, Tamil University, Thanjavur, Tamil Nadu, India. Animals were housed in polypropylene cages with filter tops under controlled conditions of a 12 hour light/ 12 hour dark cycle and 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 six animals each: group I (control), administered only vehicle (distilled water 10 ml/kg body weight per day by oral gavage); group II (*S. grandiflora* aqueous suspension (SGAS)), administered SGAS alone (1000 mg/kg body weight per day by oral gavage) for a period of three weeks; group III (CSE), cigarette smoke-exposed rats; group IV (CSE+SGAS), cigarette smoke-exposed rats administered SGAS (1000 mg/kg body weight per day by oral gavage) for a period of three weeks.

Group III and Group IV rats were exposed to cigarette smoke by modified method of Eun-Mi *et al*. (28) as follows. In this method, the rats were placed individually 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 inhale the cigarette smoke seven times a day at regular intervals of an hour (from 11 a.m. to 5 p.m.) for a period of 90 days. Similarly, control rats were exposed to air instead of smoke.

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Blood was collected and separated serum by centrifugation for enzyme analysis. Liver and kidney were isolated, cleaned of adhering fat, and connective tissues. Known weight of tissues were homogenized in 0.1M tris-HCl buffer (pH 7.4) containing 0.25M sucrose and used for the biochemical estimation.

Determination of hepatic and renal markers

The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), and the levels of blood urea nitrogen (BUN) and creatinine were assayed spectrophotometrically according to the standard procedures using commercially available diagnostic kits.

Determination of lipid peroxidation marker

Thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation, were measured by the method of Buege and Aust (29). 1,1,3,3-tetraethoxypropane was used as standard for malondialdehyde (MDA). The color reaction was measured by a spectrophotometer at wavelength of 532 nm. TBARS levels were expressed as nmol of MDA/mg protein.

Determination of enzymatic antioxidants

Superoxide dismutase (SOD, EC 1.15.1.1) was assayed by the method of Kakkar *et al*. (30) based on 50% inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium (NBT) formazan at 520 nm. One unit of the enzyme activity was taken as the amount of enzyme required for 50% inhibition of NBT reduction/min/mg protein. The activity of catalase (CAT, EC.1.11.1.6) was determined by the method of Sinha (31). The values of CAT activity are expressed as µmol of H_2O_2 utilized/min/mg protein.

The activity of glutathione peroxidase (GPx, EC.1.11.1.9) was determined by the method of Rotruck *et al*. (32) using hydrogen peroxide as substrate in the presence of reduced glutathione. Values are expressed as µmol of GSH utilized/min/mg protein. Glutathione reductase (GR, EC1.6.4.2), which utilizes NADPH to convert oxidized glutathione to the reduced form, was assayed by the method of Staal *et al*. (33). One unit of enzyme activity has been defined as nmol of NADPH consumed/min/mg protein. Glutathione S-transferase (GST, EC 2.5.1 .13) was assayed by the method of Habig *et al*. (34). The conjugation of glutathione to 1-chloro-2,4-dinitro benzene (CDNB) was measured as a non-specific substrate for GST activity. The GST activity was expressed as µmol of CDNB-GSH conjugated/min/mg protein. The activity of glucose-6-phosphate dehydrogenase (G6PDH, E.C.1.1.1.49) was assayed spectrophotometrically by the method of Ellis and Kirkman (35). The activity of G6PDH is expressed as µmol of NADPH liberated/min/mg protein.

Determination of non-enzymatic antioxidants

Reduced glutathione (GSH) was assayed by the method of Moron *et al*. (36). On the basis of the reaction of 5,5'-dithiobis-(2-nitrobenzoic acid) which is readily reduced by sulfhydryls forming a yellow substance which is measured at 412 nm. GSH concentration was expressed as µg/mg protein. Ascorbic acid and α-tocopherol content were estimated by the methods of Omaye *et al*. (37) and Baker *et al*. (38), respectively. The content of ascorbic acid and α-tocopherol are expressed as µg/mg protein.

Determination of micronutrients

Copper (Cu), zinc (Zn), and manganese (Mn) were analyzed using an atomic absorption spectrophotometer and selenium (Se) was estimated by coupled atomic emission spectrophotometer and fluorometer after digestion of tissue with nitric acid and perchloric acid. The content of Cu, Zn, Mn and Se are expressed as µg/g tissue.

Protein assay

Protein content was determined by the method of Lowry *et al*. (39) using bovine serum albumin as reference standard.

Histopathological investigation

The liver and kidney samples fixed for 48 hours in 10% formal saline were dehydrated by passing them successively in different mixtures of ethyl alcohol-water, cleaned in xylene, and embedded in paraffin. Sections of liver and kidney (5 µm thickness) were prepared, stained with hematoxylin and eosin (H-E), and mounted using neutral deparaffinated xylene (DPX) medium for microscopic observation.

Statistical analysis

Results are expressed as mean ± S.D. (n=6). The observed differences were analyzed for statistical significance by One-way of the analysis of variance with Tukey's multiple comparison as a post hoc test. A p-value <0.05 was considered significant.

RESULTS

Effect of SGAS on hepatic marker enzymes in cigarette smoke-exposed rats

Activities of serum AST, ALT and ALP were significantly increased ($p<0.001$) in cigarette smoke-exposed rats when compared with the control rats. Administration of SGAS to cigarette smoke-exposed rats showed significant decrease ($p<0.001$) in the activities of these enzymes when compared with the untreated cigarette smoke-exposed rats. Significant changes were not observed in the activities of these enzymes in SGAS alone treated rats (*Fig. 1A-C*).

Effect of SGAS on renal markers in cigarette smoke-exposed rats

As shown in *Fig. 2A* and *2B*, serum BUN and creatinine concentrations were significantly increased ($p<0.001$) in cigarette smoke-exposed rats when compared with the control rats. SGAS administration in the cigarette smoke-exposed rats significantly attenuated ($p<0.001$) the levels of BUN and creatinine when compared with the untreated cigarette smoke-exposed rats. SGAS alone treated rats did not show significant changes in these levels.

Effect of SGAS on lipid peroxidation in cigarette smoke-exposed rats

Cigarette smoke produced a significant elevation in hepatic and renal TBARS (an index of lipid peroxidation) levels in cigarette smoke-exposed rats ($p<0.001$) as compared with control rats. Administration of SGAS significantly decreased ($p<0.001$) the value of TBARS production in cigarette smoke-exposed rats as compared with untreated cigarette smoke-exposed rats. SGAS alone treated rats showed no significant changes on TBARS levels when compared with control rats (*Fig. 3A-B*).

Effect of SGAS on enzymatic antioxidants in cigarette smoke-exposed rats

Table 1 depicts the enzymatic antioxidants activities in the liver and kidney of control and experimental rats. The activities of SOD, CAT, GR, GST and G6PDH were significantly decreased but GPx activity was markedly increased in the kidney and decreased in the liver ($p<0.001$, 0.05, respectively) of rats exposed to cigarette smoke as compared with control rats. The decreased activities of SOD, CAT, GR, GST and G6PDH were significantly increased ($p<0.001$, 0.01, 0.05, respectively) while; the GPx activity was reverted to near normal in the liver and kidney by SGAS administration as compared with untreated cigarette smoke-exposed rats. Significant changes were not observed in the activities of these enzymatic antioxidants in SGAS alone treated rats when compared with control rats.

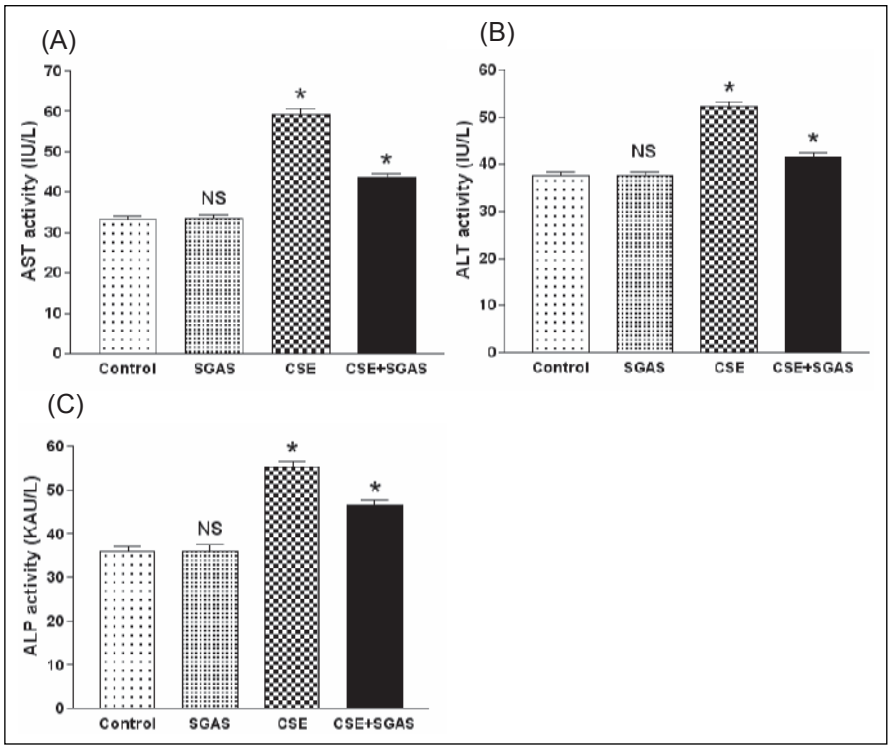

Fig. 1. Effect of SGAS on serum AST (A), ALT (B) and ALP (C) activities in control and cigarette smoke-exposed rats. Values are expressed as mean±S.D. (n=6). Statistical comparisons are made between Control *vs*. SGAS and CSE; CSE *vs*. CSE+SGAS. *p<0.001, NS-non significant.

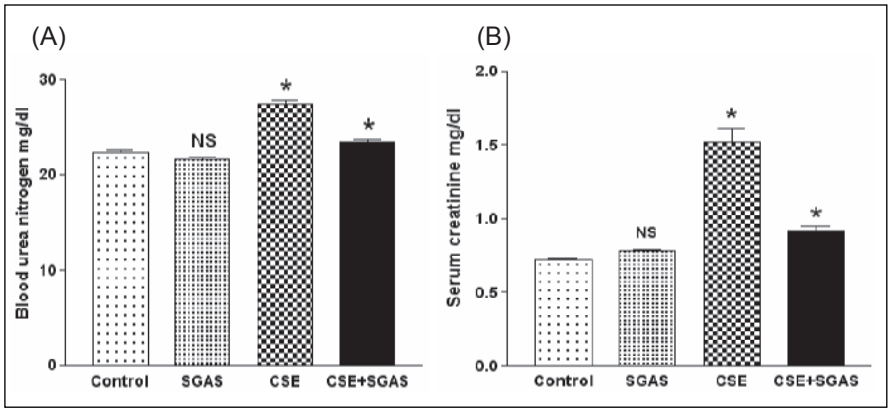

Fig. 2. Effect of SGAS on BUN (A) and creatinine (B) in control and cigarette smoke-exposed rats. Values are expressed as mean±S.D. (n=6). Statistical comparisons are made between Control *vs*. SGAS and CSE; CSE *vs*. CSE+SGAS. *p<0.001, NS-non significant.

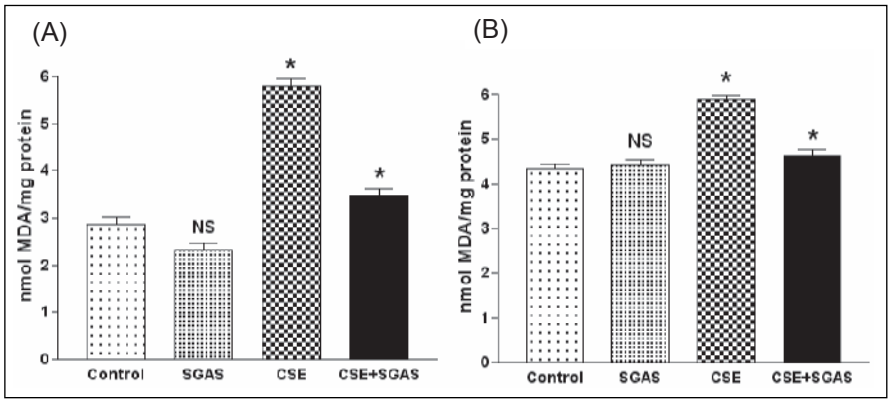

Fig. 3. Effect of SGAS on TBARS in liver (A) and kidney (B) of control and cigarette smoke- exposed rats. Values are expressed as mean±S.D. (n=6). Statistical comparisons are made between Control *vs*. SGAS and CSE; CSE *vs.* CSE+SGAS. *p<0.001, NS-non significant.

Effect of SGAS on non-enzymatic antioxidants in cigarette smoke-exposed rats

Table 2 represents the concentrations of non-enzymic antioxidants in the liver and kidney of control and experimental rats. Cigarette smoke-exposed rats showed significant reduction ($p<0.001$, 0.05) in GSH, ascorbic acid and α-tocopherol levels as compared with control rats. Administration of SGAS restored ($p<0.001$, 0.01) the concentration of GSH, ascorbic acid and α-tocopherol in cigarette smoke-exposed rats when compared with untreated cigarette smoke-exposed rats. These levels were not significantly altered in SGAS alone treated rats as compared with control rats.

Table 1. Effect of SGAS on enzymatic antioxidants in control and experimental rats.

Groups	SOD[a]	CAT[b]	GPx[c]	GR[d]	GST[e]	G6PDH[f]
Liver						
Control	5.22±0.31	85.02±4.51	4.59±0.44	0.28±0.03	2.03±0.13	2.14±0.10
SGAS	5.12±0.31^{NS}	84.69±4.44^{NS}	4.47±0.40^{NS}	0.25±0.02^{NS}	2.09±0.17^{NS}	2.16±0.09^{NS}
CSE	3.69±0.27*	69.99±4.47*	3.24±0.34*	0.18±0.02*	1.12±0.10*	1.42±0.08*
CSE+SGAS	4.70±0.30*	81.42±4.33$^{\#}$	4.80±0.46*	0.27±0.03*	1.97±0.14*	2.03±0.08*
Kidney						
Control	2.04±0.15	45.02±2.25	5.88±0.66	0.25±0.02	0.67±0.05	1.46±0.07
SGAS	2.34±0.21^{NS}	45.15±2.31^{NS}	6.05±0.60^{NS}	0.24±0.02^{NS}	0.63±0.05^{NS}	1.45±0.07^{NS}
CSE	0.63±0.09*	34.53±2.07*	9.53±0.87*	0.20±0.02$^{@}$	0.41±0.03*	1.03±0.06*
CSE+SGAS	2.18±0.15*	41.24±2.61*	5.82±0.68*	0.24±0.02$^{@}$	0.59±0.06*	1.42±0.08*

Values are expressed as mean±S.D. (n=6). a: 50% inhibition of NBT reduction/min/mg protein, b: µmol of H_2O_2 utilized/min/mg protein, c: µmol of GSH consumed/min/mg protein, d: µmol of NADPH consumed/min/mg protein, e: µmol of CDNB-GSH conjugated/min/mg protein, f: µmol of NADPH liberated/min/mg protein. Statistical comparisons are made between Control *vs*. SGAS and CSE; CSE *vs*. CSE+SGAS. **p*<0.001; $^{\#}p$<0.01; $^{@}p$<0.05; NS-non significant.

Table 2. Effect of SGAS on non-enzymatic antioxidants in control and experimental rats.

Groups	GSH	Ascorbic acid	α-Tocopherol
Liver			
Control	7.34±0.60	4.42±0.20	1.67±0.12
SGAS	7.03±0.52^{NS}	4.70±0.17^{NS}	1.76±0.11^{NS}
CSE	4.29±0.44*	2.41±0.14*	1.03±0.08*
CSE+SGAS	6.03±0.35*	4.85±0.25*	1.83±0.12*
Kidney			
Control	5.26±0.39	2.15±0.06	2.06±0.15
SGAS	5.39±0.49^{NS}	2.21±0.07^{NS}	2.15±0.18^{NS}
CSE	4.03±0.33*	1.74±0.04*	1.73±0.12$^{@}$
CSE+SGAS	5.16±0.49*	1.98±0.06*	2.03±0.19$^{\#}$

Values are expressed as mean±S.D. (n=6). Unit of GSH, ascorbic acid and α-tocopherol are expressed as µg/mg protein. Statistical comparisons are made between Control *vs*. SGAS and CSE; CSE *vs*. CSE+SGAS. * *p*<0.001; $^{\#}p$<0.01; $^{@}p$<0.05; NS-non significant.

Table 3. Effect of SGAS on micronutrients in control and experimental rats.

Parameters	Cu	Zn	Mn	Se
Liver				
Control	3.20±0.13	29.00± 1.79	1.80±0.09	0.65±0.03
SGAS	3.23±0.14^{NS}	30.00±1.89^{NS}	1.90±0.09^{NS}	0.67±0.03^{NS}
CSE	4.18±0.16*	21.00±1.43*	1.24±0.06*	0.31±0.02*
CSE+SGAS	3.43± .25*	26.00±1.78*	1.61±0.08*	0.53±0.03*
Kidney				
Control	6.14±0.26	22.00±1.38	1.30±0.06	0.54±0.02
SGAS	6.30±0.32^{NS}	22.00±1.36^{NS}	1.23±0.04^{NS}	0.55±0.03^{NS}
CSE	7.53±0.33*	14.00±0.95*	0.71±0.02*	0.41±0.03*
CSE+SGAS	6.41±0.24*	19.00±1.36*	1.03±0.04*	0.51±0.03*

Values are expressed as mean±S.D. (n=6). Units of Cu, Zn, Mn and Se: µg/g of wet tissue. Statistical comparisons are made between Control *vs*. SGAS and CSE; CSE *vs*. CSE+SGAS. * *p*<0.001; NS-non significant.

Effect of SGAS on micronutrients in cigarette smoke-exposed rats

Table 3 shows the concentrations of micronutrients in the liver and kidney of control and experimental rats. Cu level was significantly elevated in cigarette smoke-exposed rats ($p<0.001$) as compared with that of the control rats. SGAS treatment with cigarette smoke-exposed rats caused a significant reduction ($p<0.001$) in Cu level as compared with untreated cigarette smoke-exposed rats. Zn, Mn and Se levels were reduced markedly ($p<0.001$) in cigarette smoke-exposed rats when compared with that of the control rats. SGAS treatment showed significant amelioration ($p<0.001$) of Zn, Mn and Se concentrations in cigarette smoke-exposed rats as compared with untreated cigarette smoke-exposed rats. Significant changes were not observed in the concentrations of Cu, Zn, Mn and Se in SGAS alone treated rats when compared with control rats.

Effect of SGAS on histopathologic changes in cigarette smoke-exposed rats

Fig. 4c showed cigarette smoke induced pathological changes in liver mainly nuclear disintegration, portal

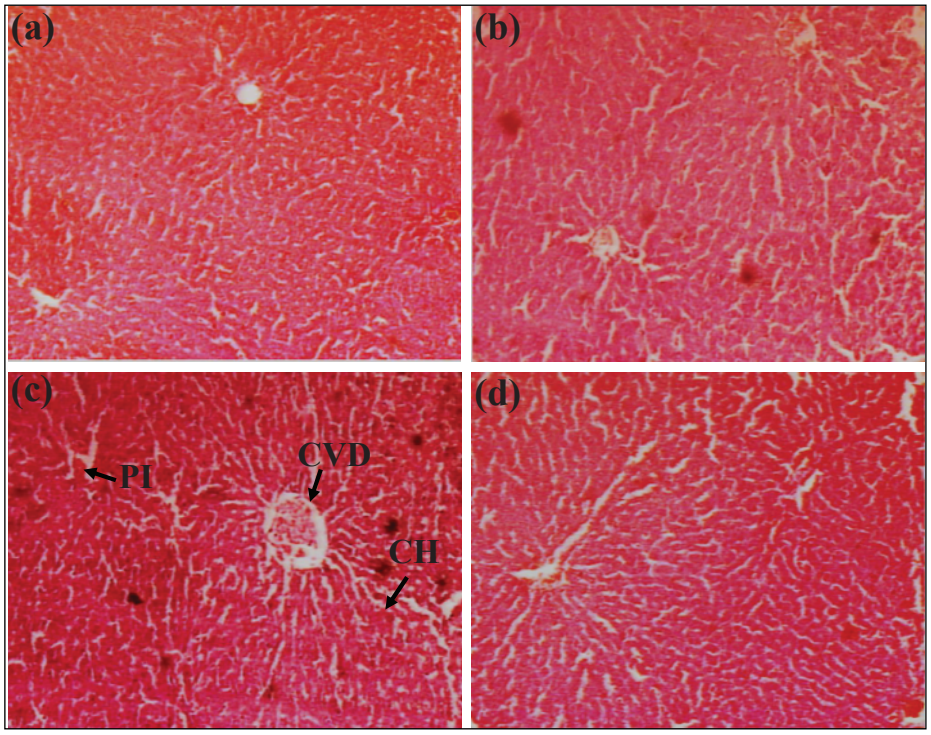

Fig. 4. Histopathological examination of haematoxylin-eosin stained liver section of normal and experimental rats with magnification ×100. (a) Liver section of control rat demonstrated the normal hepato-cellular structure. (b) Section of the liver of SGAS alone treated rat showed normal hepatic architecture. (c) Hepatic section of CSE rat depicted central venous dilation (CVD), portal inflammation (PI), congestion and hemorrhage (CH). (d) Section of the liver of CSE+SGAS treated rat reverted to almost normal hepato cellular architecture.

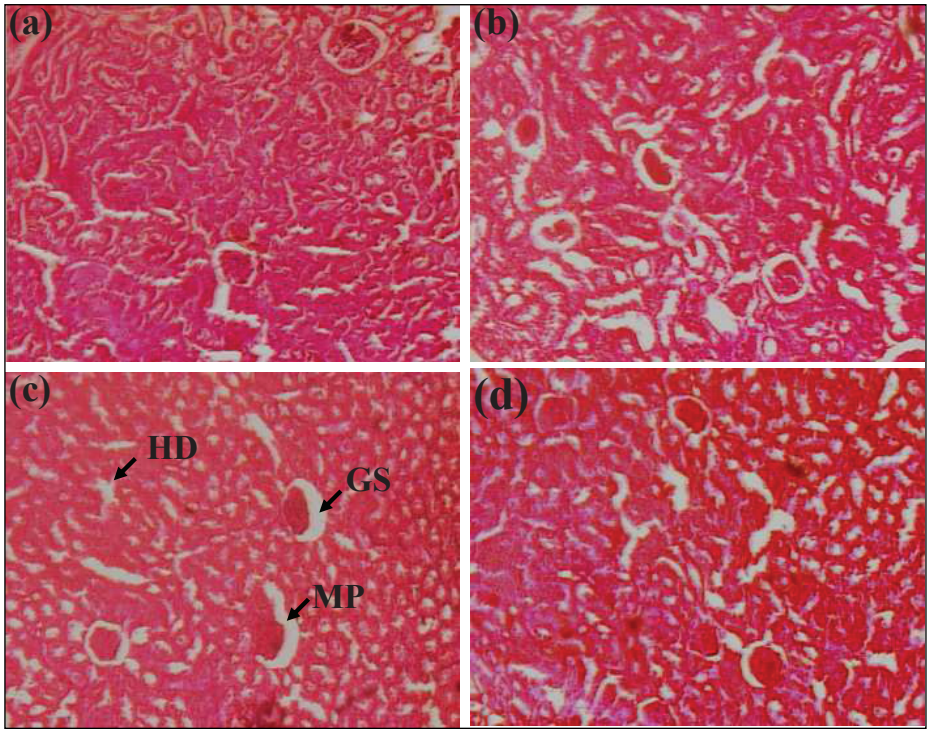

Fig. 5. Histopathological examination of haematoxylin-eosin stained kidney section of normal and experimental rats with magnification ×100. (a) Kidney section of control rat demonstrated the normal cellular structure. (b) Section of the kidney of SGAS alone treated rat showed normal renal architecture. (c) Kidney section of CSE rat depicted mesangial proliferation (MP), glomerular sclerosis (GS), and hydrophic degeneration (HD). (d) Kidney section of CSE+SGAS treated rat improved to near normal renal cellular architecture.

inflammation, sinusoidal dilation, central venous dilation, congestion and hemorrhage. Cigarette smoke-exposed rat hepatic architecture was reverted to near normal after administration with SGAS (*Fig. 4d*). Control (*Fig. 4a*) rats and rats treated with SGAS alone (*Fig 4b*) showed normal liver histology without any alterations.

The renal architecture of cigarette smoke exposed rats showed glomerular mesangial proliferation, proximal tubular cell swelling, glomerular sclerosis, interstial edema (mild), eosinophilic cytoplasm and hydrophic degeneration with stellate lumen (*Fig. 5c*). These changes were modulated by SGAS administration to the cigarette smoke-exposed rats (*Fig. 5d*). Control (*Fig. 5a*) rats and rats treated with SGAS alone depicted normal renal architecture (*Fig. 5b*).

DISCUSSION

Cigarette smoke has been identified as a major risk factor for liver and kidney-related diseases. It has the capacity to produce a highly diffusible ROS which cause oxidative damage in vital organs. ROS and reactive nitrogen species (RNS) have several effects on bronchial airways, which may enhance the inflammatory response. These effects might be mediated by direct actions of ROS/RNS in the airways, or indirectly *via* activation of signal transduction pathways and transcription factors (40). The damage to the organs by cigarette smoke is evidenced by the elevation of biomarkers in serum (41). These markers are the important indices for the diagnosis of hepatic and renal dysfunction and these indicates the damage of cells, cellular leakage and loss of functional integrity of cell membrane in the liver and kidney. In this study, we observed significant elevation in serum AST, ALT and ALP activities, BUN and creatinine concentrations in cigarette smoke-exposed rats. The elevations of these markers proved that cigarette smoke induce oxidative damages in liver and kidney. The damage caused by cigarette smoke is generally associated with free radicals. Administration of SGAS normalized the hepatic and renal markers in cigarette smoke-exposed rats. These observations are correlated with earlier studies (21, 42).

Cigarette smoke has been reported to generate lipid peroxidation in tissues (43). The increase in the level of lipid peroxidation product mainly MDA is reported the indices of lipid peroxidation. The damage to the tissues is the site of lipid peroxidation, which subsequently leads to cell injury and cell death that disrupts membrane structure and function. In cigarette smokers, leukotriene B_4 (LTB_4) which is enzymatically formed from arachidonic acid by 5-lipooxygenase and 8-iso-prostaglandin $F_{2\alpha}$ (8-iso- $PGF_{2\alpha}$) belongs to the group of F_2-isoprostanes and is formed by free radical-catalyzed peroxidation of arachidonic acid, reflecting oxidative stress and lipid peroxidation (44). In the present study, the level of MDA was found to be significantly elevated in liver and kidney of cigarette smoke-exposed rats. SGAS treatment with cigarette smoke-exposed rats showed a significant decrease in the level of MDA in liver and kidney. This result represent as *S. grandiflora* can reduce the toxicity of cigarette smoke induced free radicals and maintained normal cell function. This effect is due to its antioxidant property (vitamin A, vitamin E, vitamin C and other active compounds such as pectin, triterpenoid, tannin, glycosides, grandiflorol (α-5-methyl-5-pentacosanol) and saponin) and shows that *S. grandiflora* acts as a good scavenger against the free radical generation and thereby inhibits lipid peroxidation (16-19).

SOD and CAT are two important enzymatic antioxidants that act against toxic oxygen free radicals such as superoxide ($O^{-\cdot}_2$) and hydroxyl ions ($^{\cdot}OH$) in biological systems. CAT prevents oxidative hazards by catalyzing the formation of H_2O and O_2 from H_2O_2. A previous study by Anbarasi *et al*. (11) has shown that cigarette smoke exposure usually decreases the activities of SOD and CAT. Our earlier laboratory results have also shown similar results in heart and lung (27, 45). In the present study also we observed a similar trend in the SOD and CAT activities of the liver and kidney tissues on cigarette smoke exposure. SGAS administration to cigarette smoke-exposed rats significantly ameliorated the SOD and CAT activities. This may be due to the free radical scavenging and antioxidant property of *S. grandiflora* (16-19).

GPx is an enzyme containing four selenium cofactors that catalyzes the breakdown of H_2O_2 and organic hydroperoxides. It also plays a significant role in protecting cells against cytotoxic and carcinogenic chemicals by scavenging ROS. In this study GPx activity was significantly elevated in the kidney and decreased in the liver of rats exposed to cigarette smoke. The elevated GPx activity may serve as a protective measure against further peroxidative damage in the kidney. The decreased GPx activity might be attributed to increased utilization for elimination of H_2O_2 and organic hydroperoxides. GR is a glutathione regenerating enzyme that permits the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the oxidation of NADPH to $NADP^+$. GST plays an important role in the detoxification of toxic electrophiles by conjugating them with glutathione. G6PDH is involved in GSH synthesis by donating NADPH for GR to reduce oxidized glutathione to reduced glutathione. In the present study GR, GST and G6PDH activities were significantly decreased in the liver and kidney of rats exposed to cigarette smoke as compared to control rats. The decline in the activities of GR, GST and G6PDH on cigarette smoke exposure may be due to the involvement of these enzymes in the detoxification and possibly repair mechanism in liver and kidney. SGAS administration to the cigarette smoke-exposed rats normalized the activities of GPx, GR, GST and G6PDH. Induction of these enzymes has been evaluated as a means for determining the potency of many antioxidant substances (46). In this context SGAS is known to suppress reactive oxygen species and enhance these enzymes activities. Thus the ameliorated activities of GPx, GR, GST and G6PDH in cigarette smoke-exposed rats on SGAS supplementation may be due to the antioxidant constituents which can scavenge free radicals (16-19).

GSH is a major non-protein thiol in living organisms, which plays a central role in coordinating the body's antioxidant defense process. Perturbation of GSH status of a biological system has been reported to lead to serious consequences. In our study the GSH level was significantly lowered in the liver and kidney of rats exposed to cigarette smoke. GSH depletion is due to the destruction of free radicals and enhanced utilization during detoxification of toxic components released from cigarette smoke. This observation is consistent with earlier reports (5, 11, 27, 43, 45). Treatment with SGAS brought the GSH level to near normal. Hence, the protective effect of SGAS might be due to its free radical scavenging property (16-19) and improvement in the endogenous antioxidant level.

Ascorbic acid and α-tocopherol concentrations were found to be significantly decreased in the liver and kidney of rats exposed to cigarette smoke. Ascorbic acid is a naturally occurring free radical scavenger which decreases free radical ability and lipid peroxidation sequence. It regenerates membrane-bound α-tocopherol radical and removes the radical from the lipid to the aqueous phase (47). α-Tocopherol, the major constituent in the membrane is viewed as a last line defense against membrane lipid peroxidation (47). Thus its protection is by terminating the lipid peroxidation side chain

rather than scavenging extracellular non-lipid radicals that initiate lipid peroxidation. In this study, increased lipid peroxidation in the liver and kidney of cigarette smoke-exposed rats was associated with the decreased ascorbic acid and α-tocopherol levels. SGAS administration to the cigarette smoke-exposed rats increased the concentrations of ascorbic acid and α-tocopherol. The enhanced level might be due to vitamin C and vitamin E which is present in *S. grandiflora* (16, 17).

Cu level was significantly increased in the liver and kidney of rats exposed to cigarette smoke. This might be attributed to the mobilization of Cu from Cu-binding protein induced by cigarette smoke exposure, which accelerate the oxidant injury through the formation of hydroxyl radicals *via* Haber-Weiss/Fenton reaction (48). The increased Cu level is highly toxic to liver and kidney. Damaged hepatic and renal tissue undergoes rapid lipid peroxidation, presumably because metals released by cell disruption are not safely sequestered (5, 43). SGAS treatment was reduced the levels of Cu by scavenging the free radicals in liver and kidney of rats exposed to cigarette smoke.

Zn, Mn and Se mainly constitute cofactors for various antioxidant enzymes like SOD and GPx. In the present study Zn, Mn and Se levels were significantly decreased in the liver and kidney of rats exposed to cigarette smoke. This might be due to increased utilization by antioxidant enzymes. In addition, heavy metals like cadmium, arsenic and lead which are present in cigarette smoke (49, 50). The heavy metals from cigarette smoke might be replaced the Zn, Mn and Se which are present in antioxidant enzymes and decrease the enzyme activities (51). Treatment with SGAS restored the levels of Zn, Mn and Se in the liver and kidney of cigarette smoke exposed rats. Earlier report showed that mineral mixture of Ca/P, Zn and Fe replaced the heavy metals from the body (52). This report is consistent with our present results because *S. grandiflora* also contains these minerals which could replace the heavy metals from the antioxidant enzymes.

Significant pathomorphological alterations including nuclear disintegration, portal inflammation, sinusoidal dilation, central venous dilation, congestion and hemorrhage in the liver were observed in cigarette smoke-exposed rats. This may occur due to leukocytes which may enter the liver tissue mainly through the portal tract, where the inflammation mainly initiates (53). In addition, enhanced lipid peroxidation induced by free radicals and heavy metals which are generated from cigarette smoke. The covalent binding of heavy metals, to sulphydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatocellular injury (54). Rats exposed to cigarette smoke showed focal necrosis. Focal necrosis of liver is not much of consequence sine the function of the liver is not affected. But if it continues, the condition may progress to fatty degeneration or necrosis in which cells lost its architectural and cellular outlines. Treatment with SGAS to cigarette smoke exposed rats reduced the pathomorphological alterations and ameliorated the histomorphology of the liver.

Kidney of rats exposed to cigarette smoke showed glomerular mesangial proliferation, proximal tubular cell swelling, glomerular sclerosis, interstial edema (mild), eosinophilic cytoplasm and hydrophic degeneration with stellate lumen. These may be attributed to heavy metals which are present in cigarette smoke. Kidney is a target organ for heavy metal accumulation (55). Earlier study reported that heavy metals caused widespread congestion in the cortical region along with prominent tubular necrosis, degeneration and sloughing of tubular epithelial cells of proximal and distal tubules in kidney (56). SGAS administration restored the renal architecture in cigarette smoke-exposed rats.

In conclusion, the above results showed that cigarette smoke induced oxidative damage on the liver and kidney by enhancing lipid peroxidation and diminishing both enzymatic and non-enzymatic antioxidant status. SGAS diminished cigarette smoke induced oxidative stress probably through its free radical scavenging, anti-lipid peroxidative and antioxidant activities in the liver and kidney. Thus, the results of our investigation suggest that *S. grandiflora* has protective effects on oxidative stress induced by cigarette smoke. *S. grandiflora* can be a potent antioxidant in the liver and kidney, these organs are highly prone to oxidative stress against cigarette smoke induced toxicity and hence may have useful properties as a natural antioxidant supplement, capable of preventing hepatic and renal damage caused by oxidative stress. However, further studies pertaining to the precise mechanism of action of SGAS are warranted.

Conflict of interests: None declared.

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