Cigarette smoking 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
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 Bengt (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 prooxidant 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.

**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.
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 NADPH-phenazine methosulfate-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 nmol of H₂O₂ 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, EC.1.6.4.2), 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 sulfhydrys 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 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 decreased in the liver 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.

**Table 1**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control Rats</th>
<th>SGAS Treated Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td></td>
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<tr>
<td>GR</td>
<td></td>
<td></td>
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<tr>
<td>GST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6PDH</td>
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</tr>
</tbody>
</table>

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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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CAT&lt;sup&gt;b&lt;/sup&gt;</th>
<th>GPX&lt;sup&gt;c&lt;/sup&gt;</th>
<th>GR&lt;sup&gt;d&lt;/sup&gt;</th>
<th>GST&lt;sup&gt;e&lt;/sup&gt;</th>
<th>G6PDH&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.22±0.31</td>
<td>85.02±4.51</td>
<td>4.59±0.44</td>
<td>0.28±0.03</td>
<td>2.03±0.13</td>
<td>2.14±0.10</td>
</tr>
<tr>
<td>SGAS&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>5.12±0.31</td>
<td>84.69±4.44&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>4.47±0.40&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.25±0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.09±0.17&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.16±0.09&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>CSE</td>
<td>3.69±0.27</td>
<td>69.99±4.47&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.24±0.34&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.18±0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.12±0.10&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.54±0.08</td>
</tr>
<tr>
<td>CSE+SGAS</td>
<td>4.70±0.30</td>
<td>81.42±4.33&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>4.80±0.46&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.27±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.97±0.14&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.03±0.08</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.04±0.15</td>
<td>45.02±2.25</td>
<td>5.88±0.66</td>
<td>0.25±0.02</td>
<td>0.67±0.05</td>
<td>1.46±0.07</td>
</tr>
<tr>
<td>SGAS&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.34±0.21</td>
<td>45.15±2.31&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>6.05±0.60&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.24±0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.63±0.05&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.45±0.07</td>
</tr>
<tr>
<td>CSE</td>
<td>0.63±0.09</td>
<td>34.53±2.07</td>
<td>9.53±0.87</td>
<td>0.20±0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.41±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.03±0.06</td>
</tr>
<tr>
<td>CSE+SGAS</td>
<td>2.18±0.15</td>
<td>41.24±2.61&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>5.82±0.68&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.24±0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.59±0.06&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.42±0.08</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.D. (n=6). a: 50% inhibition of NBT reduction/min/mg protein, b: µmol of H<sub>2</sub>O<sub>2</sub> 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH</th>
<th>Ascorbic acid</th>
<th>α-Tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.34±0.60</td>
<td>4.42±0.20</td>
<td>1.67±0.12</td>
</tr>
<tr>
<td>SGAS&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>7.03±0.52</td>
<td>4.70±0.17&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.76±0.11&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>CSE</td>
<td>4.29±0.44</td>
<td>2.41±0.14&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.03±0.08&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>CSE+SGAS</td>
<td>6.03±0.35&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>4.85±0.25&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.83±0.12&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.26±0.39</td>
<td>2.15±0.06</td>
<td>2.06±0.15</td>
</tr>
<tr>
<td>SGAS&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>5.39±0.49&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.21±0.07&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.15±0.18&lt;sup&gt;NS&lt;/sup&gt;</td>
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<td>CSE</td>
<td>4.03±0.33&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.74±0.04&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.73±0.12&lt;sup&gt;NS&lt;/sup&gt;</td>
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<tr>
<td>CSE+SGAS</td>
<td>5.16±0.49&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.98±0.06&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.03±0.19&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.20±0.13</td>
<td>29.00±1.79</td>
<td>1.80±0.09</td>
<td>0.65±0.03</td>
</tr>
<tr>
<td>SGAS&lt;sup&gt;NS&lt;/sup&gt;</td>
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<td>30.00±1.89&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.90±0.09&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.67±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>CSE</td>
<td>4.18±0.16&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>21.00±1.43&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.24±0.06&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.31±0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>CSE+SGAS</td>
<td>3.43±0.25&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>26.00±1.78&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.61±0.08&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.53±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.14±0.26</td>
<td>22.00±1.38</td>
<td>1.30±0.06</td>
<td>0.54±0.02</td>
</tr>
<tr>
<td>SGAS&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>6.30±0.32</td>
<td>22.00±1.36&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.23±0.04&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.55±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
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<tr>
<td>CSE</td>
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<td>14.00±0.95&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.71±0.02&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.41±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>CSE+SGAS</td>
<td>6.41±0.24&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>19.00±1.36&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.03±0.04&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.51±0.03&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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; #p<0.01; @p<0.05; 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. 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 hepatocellular 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 hepatocellular 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, interstitial edema (mild), eosinophilic cytoplasm and hydropic 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 smokers, leukotriene B4 (LTB4) which is enzymatically formed prostaglandin from arachidonic acid by 5-lipoxygenase and 8-iso-prostaglandin (8-iso-PGF2a) belongs to the group of F2-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, grandifloro (*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 (O2−) and hydroxyl ions (OH) in biological systems. CAT prevents oxidative hazards by catalyzing the formation of H2O and O2 from H2O2. 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 H2O2 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 H2O2 and organic hydroperoxides. GR is a glutathione regenerating enzyme that permits the conversion of oxidized glutathione (GSGG) 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 stability 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).

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 induced by decrease in glutathione in the liver as the cause of 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.

REFERENCES

37. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods
35. Ellis HA, Kirkman HN. A colorimetric method for assay of
34. Habig WH, Pabst MJ, Jakpoby WB. Glutathione transferase:
33. Staal GE, Visser J, Veeger C. Purification and properties of
28. Eun-Mi P, Young-Mee P, Young-Seob G. Oxidative damage
24. Tamboli SA. Analgesic and antipyretic activity of Sesbania
23. Tamboli SA. Anti-inflammatory activity of Sesbania
22. Shrivastav N, Janin SK. Plants bearing antifertility
17. Ching LS, Mohamed S. Alpha-tocopherol content in 62 edible
and liver.
1974; 249: 7130-7139.
Chem
DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Biophys Acta
Beuge JA, Aust SD. The thiobarbituric acid assay. Method
Kakkar B, Das PN, Viswanathan A. Modified spectrophotometric assay of SOD. Ind J Biochem Biophys
Sinha AK. Colorimetric assay of catalase. Anal Biochem
Moron MS, de Pierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione-S-transferase in rat lung and liver. Biochim Biophys Acta 1979; 82: 67-70.

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