Effect of Sesaniba grandiflora on Membrane-bound ATPases in Cigarette Smoke Exposed Rats

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Abstract: The aim of present study was to assess the harmful effects of chronic cigarette smoking on membrane-bound ATPases and the protective effect of S. grandiflora in rat lung, liver, kidney and heart. 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 levels of lipid peroxides as marker for evaluating the extent of membrane damage, the activities of Na⁺-K⁺-ATPase, Ca²⁺-ATPase and Mg²⁺-ATPase and associated cations sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺) and magnesium (Mg²⁺) were investigated in rat lung, liver, kidney and heart. Membrane damage was evident from the increased levels of lipid peroxides, decreased activities of membrane-bound ATPases and alterations in the levels of inorganic cations were observed in cigarette smoke exposed rats. Administration of aqueous suspension of S. grandiflora (ASSG) inhibited the levels of lipid peroxides, ameliorated the activities of membrane-bound ATPases and maintained the ionic equilibrium in rats exposed to cigarette smoke. The results of our study indicate that ASSG protects the membrane-bound ATPases from cigarette smoking induced membrane damage.

Key words: Cigarette smoking, lipid peroxidation, membrane-bound ATPases, Sesbania grandiflora

INTRODUCTION

Cigarette smoking is associated with various pathological conditions including pulmonary, cardio and cerebrovascular diseases, cancers and several others (US Department, 2001; Centers for Disease, 2004). Cigarette smoke is a complex mixture of over 4000 identified constituents (Genbacev-Krtolica, 2005) that include numerous reactive substances such as a large quantity of reactive aldehydes (Park et al., 1998), free radical species, such as oxygen free radicals and nitrogen species (Pryor and Stoner, 1993) and diverse metals such as cadmium (Cd²⁺) (WHO, 1992). Free radicals and other reactive oxygen and nitrogen species (ROS and NOS, respectively) are capable of initiating or promoting oxidative damage (Cross et al., 1993; Panda et al., 1999). The membrane-bound ATPases such as Na⁺- K⁺ ATPase, Ca²⁺ ATPase and Mg²⁺ ATPase contributes the maintenance of vascular homeostasis via facilitating transport of sodium, potassium, calcium and magnesium ions across the cell membranes (Steinhorn and Bonting, 1981). It is reported that chronic exposure to cigarette smoke inhibited the activities of all these ATPases, alter the vascular homeostasis and hence contribute to development of vascular disease (Anbarasi et al., 2005). Increased oxidative stress coupled to increased production of superoxide anions may play a critical role in these processes (Pryor et al., 1983; Church and Pryor, 1985). Superoxide anions can affect the membrane lipids by abstracting hydrogen atoms to form lipid free radicals, which in turn react with an oxygen molecule.
to give a lipid perox radical (Halliwell and Gutteridge, 1984). Peroxidation of membrane lipids initiates the loss of membrane integrity and membrane-bound enzyme activities and hence leads to impairment in cellular homeostasis (Toskullar and Glinsukon, 1992). Indeed, treatment with antioxidant micronutrients and vitamins modulated cigarette smoke induced lipid peroxidation, suggesting a pathological role for the free radicals in smoking related impairment in the activities of membrane bound ATPases and in cellular homeostasis (Tiwari, 2004).

_Sesbania grandiflora_ L. pers (Fabaceae), commonly known as ‘sesbania’ and ‘agathi’, has been used as an important dietary nutritive source in Southeast Asian countries (Fernantinos, 1990-1991). _S. grandiflora_ leaves are 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 (Joshi, 2000; Vijayakumar _et al._, 1997; Parth and Uma, 2003). It also has anxiolytic and anticonvulsive (Kasture _et al._, 2002), anti-inflammatory, analgesic and antipyretic activity (Tamboli, 1996, 2000). Besides, _S. grandiflora_ is mentioned as a potent antidote for tobacco and smoking-related diseases (Munagesan, 1988). Recently our study reported that _S. grandiflora_ has hypolipidemic property on cigarette smoke exposed rats (Ramesh and Hazema Begum, 2006). However, the mechanisms underlying its beneficial effects against smoking associated diseases are to be fully elucidated. In the present study, we investigated the effects of _S. grandiflora_ on activities of membrane-bound ATPases in cigarette smoke exposed rats.

**MATERIALS AND METHODS**

**Chemicals**

Adenosine-Tri-Phosphate (ATP) was obtained from the Sigma chemicals company (MO, St.Louis, USA). All other chemicals and solvents utilized in this study were purchased 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 to form a suspension. 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 Venkateshwaru 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 and 27±2°C. All the rats received standard pellet diet (Anmurt rat feed, Pune, India) and water _ad libitum_.

**Experimental Protocol**

The animals were divided into four groups of six animals each. Group I: Control. Group II: Rats administered with ASSG (1000 mg kg⁻¹ and 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 and b.w/day, p.o) 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._ (1998) as follows.

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 1 h (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. Blood samples collected in plain tubes were centrifuged at 3000 x g (4°C) for 10 min to obtain serum. Lungs, liver, kidney and heart were isolated, 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.

Concentration of Conjugated Dieners (CD) and hydroperoxides were estimated as a measure of lipid peroxidation by the method of Recknagel and Ghosal (1966) and Mair and Hall (1977). The activities of Na+ - K+ ATPase (Bonting, 1970), Ca2+ ATPase (Hjerten and Pan, 1983) and Mg2+ ATPase (Ohnishi et al., 1982) were measured by the methods given previously. The amount of inorganic phosphorus was determined by the method of Fiske and Subbarow (1925). Sodium (Na+) and potassium (K+) levels were analyzed in serum by the method of Butterworth (1951) and Barry and Rowland (1953). Concentrations of calcium (Ca2+) and magnesium (Mg2+) in the serum as well as in the tissues after digestion with nitric acid and perchloric acid were measured by using atomic absorption spectrophotometer according to the method of Ballentine and Burford (1957).

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

A significant increase in the concentration of hydroperoxides and conjugated dienes were observed in cigarette smoke exposed rats (Group 3) as compared to control (Group 1) rats (Table 1). These elevated levels were significantly reversed to near control in rats exposed to cigarette smoke and treated with ASSG (Group 4) when compared with Group 3 rats. ASSG alone treated rats (Group 2) did not show any significant changes in the concentration of hydroperoxides and conjugated dienes as compared to control (Group 1) rats.

A significant decrease was observed in cigarette smoke exposed rats (Group 3) as compared to control (Group 1) rats (Table 2). Group 4 rats showed significantly increased activities of Na+ - K+ ATPase, Ca2+ ATPase and Mg2+ ATPase when compared with Group 3 rats. Significant changes were not observed in the activities of Na+ - K+ ATPase, Ca2+ ATPase and Mg2+ ATPase in Group 2 rats as compared to Group 1 rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lung</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
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<td>Conjugated dienes</td>
<td></td>
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</tr>
<tr>
<td>Group I</td>
<td>35.1±0.84</td>
<td>67.49±5.79</td>
<td>8.89±0.33</td>
<td>9.01±0.19</td>
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<td>Group II</td>
<td>34.97±0.82</td>
<td>63.61±4.42</td>
<td>8.71±0.28</td>
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<tr>
<td>Group III</td>
<td>45.50±1.12**</td>
<td>79.20±4.19**</td>
<td>9.82±0.29**</td>
<td>14.21±0.26**</td>
</tr>
<tr>
<td>Group IV</td>
<td>41.11±0.97**</td>
<td>68.69±3.33**</td>
<td>9.11±0.32*</td>
<td>9.71±0.18**</td>
</tr>
<tr>
<td>Hydroperoxides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>118.00±3.43</td>
<td>12.21±0.28</td>
<td>5.09±0.05</td>
<td>16.01±0.58</td>
</tr>
<tr>
<td>Group II</td>
<td>117.95±4.33</td>
<td>11.90±0.16</td>
<td>4.99±0.05</td>
<td>15.99±0.58</td>
</tr>
<tr>
<td>Group III</td>
<td>186.90±6.16**</td>
<td>26.40±0.44**</td>
<td>7.90±0.09**</td>
<td>26.99±0.61**</td>
</tr>
<tr>
<td>Group IV</td>
<td>122.18±3.71**</td>
<td>16.60±0.26**</td>
<td>5.60±0.05**</td>
<td>22.99±0.68**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD (n = 6). Units: Conjugated Dienes and Hydroperoxides: mmol/100 g tissue. Statistical comparison are made between Group I vs Group II and Group III; Group III vs Group IV. *p<0.001, **p<0.01

561
Table 2: Effect of *S. grandiflora* on Na⁺-K⁺ATPase, Ca²⁺ATPase and Mg²⁺ATPase activities in control and experimental group of rats

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Kidney</th>
<th>Heart</th>
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<td></td>
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<tr>
<td>Group I</td>
<td>66.6±2.24</td>
<td>61.7±3.55</td>
<td>76.2±4.72</td>
<td>20.5±1.21</td>
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<td>Group II</td>
<td>67.1±3.86</td>
<td>60.9±4.25</td>
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<td>20.6±1.28</td>
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<td>Group III</td>
<td>41.7±2.72**</td>
<td>45.2±2.58**</td>
<td>52.4±3.26**</td>
<td>12.6±1.01**</td>
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<td>Group IV</td>
<td>54.9±2.18**</td>
<td>53.4±3.01*</td>
<td>65.7±4.06**</td>
<td>18.1±1.22**</td>
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<td>Ca²⁺ATPase</td>
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<tr>
<td>Group I</td>
<td>49.3±2.64</td>
<td>56.6±3.05</td>
<td>62.1±3.18</td>
<td>51.5±2.78</td>
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<td>Group II</td>
<td>49.2±2.68</td>
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<td>61.9±3.33</td>
<td>51.6±2.79</td>
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<td>37.1±2.06**</td>
<td>42.2±2.04**</td>
<td>49.2±2.04**</td>
<td>43.6±2.48**</td>
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<tr>
<td>Group IV</td>
<td>43.2±2.53*</td>
<td>49.7±2.48**</td>
<td>57.6±3.02**</td>
<td>49.3±2.36**</td>
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<td>Mg²⁺ATPase</td>
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<tr>
<td>Group I</td>
<td>9.4±0.51</td>
<td>7.2±0.39</td>
<td>5.1±0.28</td>
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<tr>
<td>Group II</td>
<td>9.5±0.54</td>
<td>7.3±0.38</td>
<td>5.3±0.26</td>
<td>5.2±0.25</td>
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<tr>
<td>Group III</td>
<td>4.4±0.24**</td>
<td>4.1±0.24**</td>
<td>3.0±0.18**</td>
<td>3.1±0.19**</td>
</tr>
<tr>
<td>Group IV</td>
<td>7.2±0.35**</td>
<td>6.3±0.37**</td>
<td>4.2±0.27**</td>
<td>4.0±0.27**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD (n = 6). Units: Na⁺K⁺ATPase, Ca²⁺ATPase and Mg²⁺ATPase μmoles of inorganic phosphorus liberated/hour/mg protein. Statistical comparison are made between Group I vs Group II and Group III, Group III vs Group IV. **p<0.001, *p<0.01

Fig. 1: Effect of *S. grandiflora* on serum Na⁺ and K⁺ in control and experimental group of rats. Values are expressed as mean±SD (n = 6). Unit: Sodium and Potassium: mg dL⁻¹ serum. Statistical comparison are made between Group I vs Group II and Group III, Group III vs Group IV. **p<0.001, *p<0.01

Na⁺ concentration was significantly decreased and K⁺ concentration was significantly increased in Group 3 rats as compared to Group 1 rats Fig. 1. The levels of Na⁺ and K⁺ in Group 4 remains

562
similar to that observed in Group 1, suggesting that ASSG treatment restored the levels of Na' and K' in cigarette smoke exposed rats. In addition, there were no significant differences observed in the levels of Na' and K' in Group 2 rats when compared with Group 1 rats.

A significant decrease in the level of Ca and increase in the level of Mg in serum with concomitant increase of Ca and decrease of Mg in all the tissues was observed in Group 3 rats (Table 3). These alterations were reversed to near control in rats exposed to cigarette smoke and treated with ASSG (Group 4) when compared with Group 3 rats. Group 2 rats didn't show any significant differences in the concentrations of Ca and Mg as compared to Group 1 rats.

**DISCUSSION**

Cigarette smoke is a complex milieu possessing an array of free radicals and reactive oxygen species (Pryor, 1997). Cell membranes being primarily composed of lipids especially polyunsaturated fatty acids are particularly susceptible to attack by these free radicals from cigarette smoke, leading to increased permeability and altered fluidity of the membrane and thereby causing cellular damage (Cross et al., 1987). The sustained release of free radicals from cigarette smoke imposes an oxidant stress, promotes lipid peroxidation and consequently perturbs the antioxidant defense systems in blood and tissues of smokers (Pryor and Stone, 1993). In the present study, the lipid peroxide levels (hydroperoxides and conjugated dienes) were significantly increased in lung, liver, kidney and heart of cigarette smoke exposed rats. These results are consistent with earlier studies (Solak et al., 2005; Tanriverdi et al., 2006). They reported that cigarette smoke generated free radicals enhanced the lipidperoxide levels in cigarette smoke exposed subjects. It is also reported that increased production of superoxide anions and/or its metabolites may play a critical role in lipidperoxidation process (Pryor et al., 1983; Church and Pryor, 1985).

Moreover our study demonstrated that the activities of Na-K ATPase, Ca ATPase and Mg ATPase were significantly diminished in lung, liver, kidney and heart of cigarette smoke exposed rats. This might be attributed to peroxidation of membrane lipids induced by cigarette smoke may lead to the reduced activities of membrane-bound ATPases and hence to impairment in the cellular homeostasis (Toskullar and Günsel, 1992). Indeed, cigarette smoke consists of heavy metals like cadmium, nickel and chromium then nitric oxide (NO) and other reactive oxidants, which could directly affect the activities of Na-K ATPase, Ca ATPase and Mg ATPase (Pryor and Stone, 1993; Smith et al., 1997). The decreased activities of these ATPases can not regulate the inorganic cations like Na, K, Ca and Mg. In this study, the serum Na concentration was significantly decreased and K concentration was significantly increased in cigarette smoke exposed rats. This result is indicated that Na-K ATPase failure to regulate the Na, K, levels. In addition, the intracellular concentration of Ca was significantly increased and Mg was decreased while the serum Ca was significantly decreased and Mg was increased in rats exposed to cigarette smoke. These alterations
might be due to decreased activities of Ca\(^{2+}\)ATPase and Mg\(^{2+}\)ATPase. Besides, previous studies showed that cigarette smoke could increase the influx of Ca\(^{2+}\) via the voltage gated channels (Anbarasi et al., 2005; Dajas-Bailador et al., 2002) and increased intracellular Ca\(^{2+}\) displaced the Mg\(^{2+}\) from its binding sites and thus affects the functional availability of Mg\(^{2+}\) (Ravikumar 2000).

The treatment with ASSG decreased and normalized the levels of hydroperoxides and conjugated diens in cigarette smoke exposed rats, suggesting that ASSG can prevent the formation of free radicals and hence reduce extent of lipid peroxidation in cigarette smoke exposed rats. These observations stands in the same line with previous report of *S. grandiflora* offered protection against erythromycin estolate-induced hepatotoxicity through inhibition of free radicals (Pari and Uma, 2003). We also observed that ASSG administration ameliorated the activities of Na\(^{-}/K^{+}\) ATPase, Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase in lung, liver, kidney and heart of cigarette smoke exposed rats. In addition, the altered levels of inorganic cations such as Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\) and Mg\(^{2+}\) were reverted to near physiological values in ASSG treated cigarette smoke exposed rats. The present study granted that cigarette smoke-induced alterations in the activities of membrane bound ATPases and thus concentrations of inorganic cations is a result of peroxidation of membrane lipids. ASSG were reverted these alterations by inhibition of the peroxidation of membrane lipids. This might be due to antioxidant potential components such as vitamin-A, vitamin-C and sulfur containing amino acids which are present in *S. grandiflora* (The wealth of India, 1972; Govindan and Shannugasundaram, 1987). The sulfur containing amino acids like cystine and methionine are used to synthesis taurine. Taurine aids the movement of sodium, potassium, calcium and magnesium in and out of cells and maintaining the cell membrane integrity (Anitha Nandhini and Anuradha, 2003). In addition the sulfur containing amino acids are essential to synthesis antioxidants such as glutathione, thioredoxin and metallothionein (Mudd, 2001). Further, the sulfur containing amino acids binds with heavy metals which are derived from cigarette smoke and thus can protect the ATPases enzyme.

In conclusion, the present findings suggest that *S. grandiflora* ameliorated the activities of membrane bound ATPases and restored the levels of inorganic cations in rats exposed to cigarette smoke. These ameliorative and restorative properties of *S. grandiflora* may be due to its ability to seavange free radicals and inhibit lipid peroxidation by the antioxidant components which are present in *S. grandiflora*. These results provide pharmacological support to the traditional use of *S. grandiflora* as an antidote for tobacco and tobacco smoke related diseases.

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