Smokeless Tobacco Impairs the Antioxidant Defense in Liver, Lung, and Kidney of Rats

Pramod Kumar Avti, Surender Kumar, Chander Mohan Pathak, Kim Vaiphei,* and Krishan Lal Khanduja

Departments of Biophysics and *Histopathology, Postgraduate Institute of Medical Education and Research, Chandigarh–160012, India

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INTRODUCTION

Use of smokeless tobacco (ST) is quite popular in countries of the Far East and Middle East and Europe (Bates et al., 2003), and it is increasing in the USA (Changrani and Gany, 2005). The main types of ST in Western and Asian countries are chewing tobacco and oral snuff. Chewing tobacco is predominantly used in USA and snuff (Snus) in Sweden. In developing countries like India, the chewing tobacco is mixed with betel leaves, areca nut, lime, and catechu and is sold as legally commercial products termed “gutkha.” The product is basically a flavored and sweetened dry mixture of areca nut, catechu, and slaked lime with tobacco (Richter and Spierto, 2003). The mixture is chewed slowly, and in this process the aqueous extract due to saliva is not only absorbed locally but also ingested to enter into the systemic circulation. The use of gutkha has been classified as carcinogenic to humans and may be associated with oral disease (Merchant et al., 2000; Boucher, 2001; Stepanov et al., 2005a). Smokeless tobacco use can be addictive, leading to oral leukoplaikias (oral mucosal lesions) and gingival recession, and it may play a contributory role in the development of cardiovascular disease, peripheral vascular disease, hypertension, peptic ulcers, and fetal morbidity and mortality (Crichtley and Unal, 2003). Although interest is growing in the patterns, distribution, consumption, and compositions of tobacco and its use in various parts of the world (Subramanian et al., 2004), the precise health effects of ST use are uncertain but are not necessarily limited to oral cancers (Jorenby et al., 1998). The epidemiological analyses available to date on the cancer rates from Western countries have been inconclusive. Sweden has a low rate of oral and pharyngeal cancers despite high ST use. West Virginia, the state in USA with the highest ST consumption, does not have high rates of oral cancers (Bouquot and Meckstroth, 1998). In Asia, the majority of ST studies have been carried out in India, where both smoking and habits of ST use are common and the outcomes, mainly oral cancers, are most prevalent (Avon, 2004; Nair et al., 2004; Rodu and Jansson, 2004). A strong dose-dependent association has been found between the patterns of tobacco use and oral cancer in Asians (Hirayama, 1966; Sankaranarayanan et al., 1991).

So far very few systematic investigations have been done to reveal the molecular mechanisms involved in different types of ST-related health effects. Although cigarette smoking and generation of free radicals is well established (Aoshiba and Nagai, 2003), recent investigations into cell culture studies have demonstrated that long-term use of ST could also generate...
free radicals (Lam et al., 2003; Kilinc et al., 2004; Sener et al., 2005). The highly reactive radicals can act as initiators and/or promoters of carcinogenesis, cause DNA damage, activate procarcinogens, and alter the cellular antioxidant defense system. The effective detoxification mechanism (the antioxidant defense system), comprises SOD and catalase, which work in a sequential manner in the disposal of superoxide radical and the conversion of hydrogen peroxide to water. Changes in GSH homeostasis have also been implicated in the etiology and progression of a number of pathological diseases. Hence in the present study, the detrimental effects of long-term administration of AEST on the plasma vitamins (A, C, and E) levels, histological and antioxidant defense changes in liver, lungs and kidney of male rats were evaluated.

MATERIALS AND METHODS

Materials
Nicotinamide adenine dinucleotide phosphate reduced (NADPH), glutathione reductase, α-tocopherol succinate, thiobarbituric acid (TBA), tert-butyl hydroperoxide (BHP), quinine hemisulfate and α,α′-dipyridyl were purchased from Sigma Chemical (St. Louis, MO, USA). Phosphate buffered saline (PBS), Tris-HCl buffer, ethylenediamine tetrachloroacetic acid (EDTA), were purchased from M/s HiMedia Chemicals (Mumbai, India). Reduced glutathione, hydroxylamine hydrochloride, trichloroacetic acid, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), nitroblue tetrazolium (NBT), ferrous sulfate (FeSO4), potassium hydroxide were procured from M/s Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). The pellet diet duly approved by the Institute’s Animal Ethics Committee was obtained from M/s Ashirwad Industries (Punjab, India).

Methods

Preparation of Smokeless Tobacco Extract

Aqueous extract of gutkha (AEST) was prepared as described in the literature (Lam et al., 2003) with slight modification. Commercially available gutkha (Pan Parag) was finely powered, and 20 g was dissolved in 50 ml of PBS (pH 7.4) and incubated at 37°C for 30 min with thorough shaking. The dissolved contents were filtered twice through filter paper and quickly frozen at −80°C before lyophilization. The dried yield of AEST was found to be around 1 mg/10 mg of gutkha containing about 24% salt concentration (NaCl, KCl, and phosphates). The required amount of lyophilized extract was reconstituted in 300 µl distilled water and was orally administered through gavage to the rats at the desired doses for different time periods.

Smokeless Tobacco Administration

The experiments were performed on pathogen-free young male Wistar rats weighing 100–120 g after the study was cleared by the Institute’s Animal Ethics Committee. The animals were obtained from the Central Animal House of the Postgraduate Institute of Medical Education and Research, Chandigarh, India. Animals were allowed free access to water and a normal pellet diet. They were housed in polypropylene cages bedded with sterilized rice husks under 12 h cycles of light and dark. The aqueous extract of gutkha containing 24% salts was administered orally in 300 µl of distilled water by gavage twice a day at two different doses, the low dose (96 mg/kg body weight per day) for 2 or 32 weeks and the high dose (960 mg/kg body weight per day) for 2 weeks only. The animals in the sham control group were given an equivalent amount of lyophilized PBS in 300 µl of distilled water. The weight of the animals was recorded weekly. Animals were sacrificed at various time intervals under pentobarbital anesthesia. Liver, lung, and kidneys were excised and perfused with ice-cold perfusion solution (0.15 M KCl, 2 mM EDTA, pH 7.4). Tissues were homogenized in Tris-HCl buffer (50 mM, pH 7.4), and the homogenates were centrifuged at 10,000 × g at 4°C for 30 min to obtain post-mitochondrial supernatant (PMS). The PMS was used for the estimation of the antioxidant defense system.

Reduced Glutathione (GSH)

Reduced glutathione estimation in the tissue homogenate was performed by the method of Moron et al. (1979). The required amount of the tissue homogenate was mixed with 25% of trichloroacetic acid and centrifuged at 2000 × g for 15 min to settle the precipitated proteins. The supernatant was aspirated and diluted to 1 ml with 0.2 M sodium phosphate buffer (pH 8.0). Later, 2 ml of 0.6 mM DTNB was added. After 10 minutes the optical density of the yellow-colored complex formed by the reaction of GSH and DTNB (Ellman’s reagent) was measured at 405 nm. A standard curve was obtained with standard reduced glutathione. The levels of GSH were expressed as μg of GSH/mg protein.

Superoxide dismutase (SOD)

Superoxide dismutase was estimated according to the method of Kono et al. (1978). Briefly, the reaction mixture containing solution A (50 mM sodium carbonate, 0.1 mM EDTA, pH 10.0), solution B (96 μM nitroblue tetrazolium [NBT] in solution A), and solution C (0.6% Triton X-100 in solution A) were incubated at 37°C for 10 min. Reaction was initiated by adding 100 μl of solution D (20 mM hydroxylamine hydrochloride, pH 6.0). The rate of NBT dye reduction by O2− anion generated due to photoactivation of hydroxylamine hydrochloride was recorded at 560 nm in the absence of PMS. Later, small aliquots of PMS were added to the reaction mixture and 50% inhibition in the rate of NBT reduction by SOD present in the enzyme source was recorded. One unit of enzyme activity was defined by the 50% inhibition of NBT. The levels of SOD were expressed in terms of IU/mg protein.

Catalase (CAT)

Catalase activity was measured in the PMS by the method of Luck (1963). The final reaction volume of 3 ml contained 0.05 M Tris-buffer, 5 mM EDTA (pH 7.0), and 10 mM H2O2 (in 0.1 M potassium phosphate buffer, pH 7.0). About 50 or 100 μl aliquots of the tissue PMS were added to the above mixture. The rate of change of absorbance per min at 240 nm was recorded. Catalase activity was calculated by using the molar extinction coefficient of 43.6 M−1 cm−1 for H2O2. The level of CAT was expressed in terms of μmoles H2O2 consumed/min per milligram of protein.

Glutathione Peroxidase (GPx)

The GPx activity was measured by the method of Paglia and Valentine (1967). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide, 0.2 mM NADPH, 1 U glutathione reductase, and 1 mM NADPH. The sample, after its addition, was allowed to equilibrate for 5 min at 25°C. The reaction was initiated by adding 0.1 ml of 2.5 mM H2O2. Absorbance at 340 nm was recorded for 5 min. Values were expressed as nanomoles of NADPH oxidized to NAPD by using the extinction coefficient of 6.2 × 103 M−1 cm−1 at 340 nm. The levels of GPx were expressed in terms of μmoles NAPD consumed/min per milligram of protein.

Lipid Peroxidation

Lipid peroxidation was estimated by the method of Okhawa et al. (1979) in tissue homogenates. Briefly, the reaction mixture contained Tris-HCl buffer (50 mM, pH 7.4), tert-butyl hydroperoxide (BHP) (500 μM in ethanol) and 1 mM FeSO4. After incubating the samples at 37°C for 90 min, the reaction was stopped by adding 0.2 ml of 8% sodium dodecyl sulfate (SDS) followed by 1.5 ml of 20% acetic acid (pH 3.5). The amount of malondialdehyde (MDA) formed during incubation was estimated by adding 1.5 ml of 0.8% TBA and...
further heating the mixture at 95°C for 45 min. After cooling, samples were centrifuged, and the TBA reactive substances (TBARS) were measured in supernatants at 532 nm by using 1.53 × 10⁻³ M⁻¹ cm⁻¹ as extinction coefficient. The levels of lipid peroxidation were expressed in terms of nmole of TBARS per 90 min/mg protein.

Vitamin Analysis in Plasma

**Vitamin A.** Plasma vitamin A analysis was performed as described earlier (Khanduja et al., 1984). Briefly, plasma was obtained from heparinized blood that was centrifuged at 10,000 × g for 15 min at 4°C. Plasma was mixed thoroughly with alcoholic KOH (11 moles/l) and incubated at 60°C for 15 min. Samples were then cooled on ice and 1 ml of 16 μmoles/l benzoyl peroxide was added, vortexed thoroughly, and centrifuged at 2500 × g for 10 min. The upper organic phase was used for ultraviolet (UV) absorption at 328 nm before further heating the mixture at 95°C for 45 min. After cooling, samples were centrifuged, and the TBA reactive substances (TBARS) were measured in supernatants at 532 nm by using 1.53 × 10⁻³ M⁻¹ cm⁻¹ as extinction coefficient.

**Vitamin C.** Plasma vitamin C was measured by the method of Khanduja and Kaul (1988). The plasma with 1 mM EDTA was deproteinized with ice-cold 40% trichloroacetic acid (TCA). After 10 min the samples were centrifuged at 15,000 × g for 15 min at 4°C and the supernatant was used for vitamin C estimation. The reaction mixture contained 10 μl of 85% H₂PO₄, 80 μl of 1% α,α′-dipyridyl, and 10 μl of 3% aqueous ferric chloride and was incubated at room temperature for 15 min. The color so developed was read at 525 nm on a plate reader (Merck MIOS Junior, USA). Concentration of vitamin C in plasma was expressed as ng/ml plasma.

**Vitamin E.** Saponification and extraction of vitamin E from plasma was performed as described earlier by Desai (1984) and Taylor et al. (1976). Briefly, 2 ml of 1% ascorbic acid was added to the required amount of plasma. After thorough mixing, the tubes were heated at 70°C for 2 min, 300 μl of saturated KOH was added, and the tubes were incubated for 30 min at 70°C. The tubes were then cooled on ice and 1 ml of distilled water and 4 ml of hexane were added. After thorough mixing, the samples were centrifuged at 1600 × g for 10 min. The upper layer of hexane was removed and treated with 60% H₂SO₄ for 30 s to oxidize vitamin A. Vitamin E was estimated spectrophotometrically (Hitachi 650–40, Japan), using 286 nm and 330 nm as excitation and emission wavelengths, respectively. A standard curve was obtained by using 1–10 μg α-tocopherol/ml in absolute ethanol. Stability of the instrument was checked by recording the fluorescence of quinine hemisulfate (1 μg/ml of 0.1 N H₂SO₄)

Concentration of vitamin E in plasma was expressed as μg/ml. Protein content of the samples was measured by the method Lowry et al. (1951).

**Histopathological Examination**

Portions of the tissue from liver, lung, and kidney were used for histopathological examinations. Tissues were fixed in 10% buffered formalin (pH 7.2) and dehydrated through a series of ethanol solutions, embedded in paraffin, and routinely processed for histological analysis. Sections of 2 μm thickness were cut and stained with hematoxylin-eosin for examination. The stained tissues were observed through an Olympus microscope (BX-51) and photographed by a charge-couple device (CCD) camera.

**Statistics**

Values are expressed as mean ± SEM. Statistical analysis was performed by the unpaired Student’s t-test; a p value < 0.05 was considered significant.

**RESULTS**

Oral administration of AEST at either of the doses and time intervals did not cause any change in the animal growth. AEST at low doses for 2 weeks did not alter GSH levels and the activities of SOD, CAT, GPx, and Lpx in liver, lung, and kidney. However, administration of AEST at low dose for 32 weeks significantly decreased (p < 0.05) the hepatic GSH, SOD, CAT, and GPx by 34.6%, 29%, 17.1%, and 17.4%, respectively (Table 1). Low-dose administration of AEST for 32 weeks decreased the lung GSH, SOD, CAT, GPx by 43%, 28.5%, 37%, and 40%, respectively (Table 2). Long-term administration of low dose AEST decreased the kidney GSH, SOD, CAT, and GPx by 26.6%, 23%, 33%, and 18%, respectively (Table 3). However, administration of AEST at low dose for 32 weeks significantly increased (p < 0.05) lipid peroxidation in liver, lung, and kidney by 64%, 24%, and 65%, respectively. Administration of AEST at high dose for

<table>
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<th>TABLE 1</th>
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<td><strong>Effect of Aqueous Extract of Smokeless Tobacco on Antioxidant Defense Status in Liver of Rats</strong></td>
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<th>Parametera</th>
<th>2 weeks</th>
<th>32 weeks</th>
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<tr>
<td></td>
<td>Control</td>
<td>Low doseb</td>
</tr>
<tr>
<td>GSH</td>
<td>29.3 ± 1.56</td>
<td>29.20 ± 1.64</td>
</tr>
<tr>
<td>LPx</td>
<td>3.60 ± 0.23</td>
<td>4.19 ± 0.27</td>
</tr>
<tr>
<td>SOD</td>
<td>6.53 ± 0.48</td>
<td>6.57 ± 0.55</td>
</tr>
<tr>
<td>CAT</td>
<td>21.53 ± 1.06</td>
<td>20.61 ± 1.30</td>
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<tr>
<td>GPx</td>
<td>58.83 ± 4.9</td>
<td>51.93 ± 3.53</td>
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Values are mean ± SEM; n = 7.

*p < 0.05 significant as compared to control.

aGSH (glutathione): μg/mg protein; LPx (lipid peroxidation), nmole TBARS (thiobarbituric acid reactive substances) per 90 min/mg protein; SOD (superoxide dismutase), IU/mg protein; CAT (catalase), μmoles H₂O₂ consumed per min/mg protein; GPx (glutathione peroxidase), nmole NADPH consumed per min/mg protein.

bLow dose = 96 mg/kg body weight per day.

cHigh dose = 960 mg/kg body weight per day.
2 weeks did not cause any change in the activities of antioxidant enzymes and lipid peroxidation in lung and kidney (Tables 2 and 3), but it significantly decreased the liver GSH levels and GPx activity and increased the lipid peroxidation by 17%, 19%, and 20%, respectively (Table 1).

Table 4 shows that AEST administration for 32 weeks at low doses did not affect the plasma levels of vitamin A, C, and E. Figure 1 shows mild to moderate inflammatory changes in liver and lung induced by AEST, as made evident by the infiltration of phagocytic cells. The high dose of AEST for 2 weeks caused mild portal triaditis in liver (Fig. 1B) whereas, treatment with the low dose for 32 weeks caused a moderate degree of inflammation (Fig. 1C). In the lungs, the high dose of AEST for 2 weeks caused a mild degree of interstitial inflammation, whereas long-term administration of the low dose of AEST for 32 weeks caused a moderate degree of interstitial inflammation. Surprisingly, AEST did not cause any change in the histology of the kidney.

**DISCUSSION**

In the present study, the profile of oxidative/antioxidative status in various organs after AEST administration revealed marked alterations in antioxidant enzyme activities and lipid peroxidation. Low activities of antioxidant enzymes such as SOD, CAT, and GPx might be due to the overwhelming effects of free radicals, as evidenced by the elevated levels of lipid peroxidation. The variable effects of AEST on different organs could be due to the differential load of metabolites of AEST in

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<tr>
<td>GSH</td>
<td>Control</td>
<td>Low dose</td>
</tr>
<tr>
<td>LPx</td>
<td>11.92 ± 0.82</td>
<td>11.43 ± 1.33</td>
</tr>
<tr>
<td>SOD</td>
<td>1.70 ± 0.12</td>
<td>1.92 ± 0.10</td>
</tr>
<tr>
<td>CAT</td>
<td>1.28 ± 0.07</td>
<td>1.34 ± 0.08</td>
</tr>
<tr>
<td>GPx</td>
<td>3.01 ± 0.30</td>
<td>3.18 ± 0.18</td>
</tr>
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Values are mean ± SEM; n = 7.
* p < 0.05 significant as compared to control.
**GSH, µg/mg protein; LPx, nmoles TBARS per 90 min/mg protein; SOD, IU/mg protein; CAT, µmoles H2O2 consumed per min/mg protein; GPx, nmoles NADPH consumed per min/mg protein.
*Low dose = 96 mg/kg body weight per day.
**High dose = 960 mg/kg body weight per day.

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<th>Parameter</th>
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<th>32 weeks</th>
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<tr>
<td>GSH</td>
<td>Control</td>
<td>Low dose</td>
</tr>
<tr>
<td>LPx</td>
<td>18.59 ± 2.33</td>
<td>17.99 ± 2.36</td>
</tr>
<tr>
<td>SOD</td>
<td>1.86 ± 0.12</td>
<td>1.752 ± 0.01</td>
</tr>
<tr>
<td>CAT</td>
<td>3.46 ± 0.25</td>
<td>3.42 ± 0.14</td>
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<tr>
<td>GPx</td>
<td>8.40 ± 0.60</td>
<td>7.89 ± 0.39</td>
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Values are mean ± SEM; n = 7.
* p < 0.05 significant as compared to control.
**GSH, µg/mg protein; LPx, nmoles TBARS per 90 min/mg protein; SOD, IU/mg protein; CAT, µmoles H2O2 consumed per min/mg protein; GPx, nmoles NADPH consumed per min/mg protein.
*Low dose = 96 mg/kg body weight per day.
**High dose = 960 mg/kg body weight/day.

**TABLE 2**

Effect of Aqueous Extract of Smokeless Tobacco on Antioxidant Defense Status in Lungs of Rat

**TABLE 3**

Effect of Aqueous Extract of Smokeless Tobacco on Antioxidant Defense Status in Kidney of Rats
these organs (Hecht, 2002; Ebbert et al., 2004; Stepanov and Hecht, 2005b). These might have altered the antioxidant defense system in such a way that the oxidative stress had a variable effect on the different organs.

Cellular antioxidant enzymes such as SOD, CAT, and GPx and free radical scavengers like GSH and vitamins A, C, and E protect cells and tissues against noxious radicals. An imbalance between cellular pro-oxidant and antioxidant levels results in the oxidative stress that leads to tissue damage. The antioxidant enzymes react directly with reactive oxygen species (ROS) to yield non-radical products. Superoxide dismutase, a mitochondrial as well as cytosolic enzyme, dismutates $O_2^-$ to $H_2O_2$, which is decomposed by CAT to $H_2O$. In the present study, the

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<th>Vitamin</th>
<th>Control</th>
<th>Aqueous extract of smokeless tobacco</th>
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<tr>
<td>A (ng/ml)</td>
<td>168.12 ± 6.6</td>
<td>153.12 ± 8.07</td>
</tr>
<tr>
<td>C (µg/ml)</td>
<td>10.91 ± 1.99</td>
<td>9.7 ± 1.14</td>
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<tr>
<td>E (µg/ml)</td>
<td>3.51 ± 0.37</td>
<td>2.98 ± 0.43</td>
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Values are mean ± SEM; $n = 5$.

No significant change in plasma vitamin levels was observed in aqueous extract of smokeless tobacco–treated group as compared to control.

![FIG. 1. Effect of high-dose (960 mg/kg body weight per day) administration for 2 weeks and low-dose administration (96 mg/kg body weight per day) for 2 and 32 weeks of aqueous extract of smokeless tobacco (AEST) on tissue histology (H & E). The arrows show the infiltration of phagocytic cells indicating inflammation. A. Normal morphology (H & E, ×275) of liver, lung, and kidney of control animals. B. The low dose of AEST induced (1) mild portal tract inflammation in liver (H & E, ×275), (2) normal kidney morphology (H & E, ×275), (3) focal interstitial inflammation in lung (H & E, ×275). C. The high dose of AEST induced (1) a moderate degree of portal tract inflammation in liver (H & E, ×550), (2) normal kidney morphology (H & E, ×550), and (3) interstitial inflammation comprised of lymphocytes and plasma cells in lung (H & E, ×550).](https://academic.oup.com/toxsci/article-abstract/89/2/547/1685929)
activity of SOD was decreased significantly in liver, lung, and kidney with long-term administration of a low dose of AEST for 32 weeks, which might have led to the inefficient removal of \( \text{O}_2^- \) radicals from the cellular milieu, resulting in the ROS burden. Overproduction of these radicals has an inhibitory effect on the enzymes responsible for removal of ROS such as CAT and GPx. It has been reported that superoxide radicals inhibit CAT activity and that \( \text{H}_2\text{O}_2 \) suppresses SOD activity (Hassan and Fridovich, 1978), which might explain the inhibition of these enzymes after the long-term administration of AEST in the present study. On the other hand, GPx removes \( \text{H}_2\text{O}_2 \) and lipid peroxides using GSH. This prevents \( \text{H}_2\text{O}_2 \)-mediated damage, which is thought to be a prerequisite for inflammation and a recognized risk factor for carcinogenesis (Mates et al., 1999). Reduced glutathione is only one among many potential antioxidant defenses involved in the protection of various organs against oxidant-induced injury in inflammation (Meister and Anderson, 1983; Rahman and MacNee, 1999).

It is a strong nuclophilic and often inactivates electrophilic reactive compounds by either direct non-enzymatic conjugation or enzymatic catalysis. Glutathione has been implicated in various cellular events, such as inflammatory response, modulation of redox-regulated signal transduction, regulation of cell proliferation, remodeling of extracellular matrix, apoptosis, immune modulation, and mitochondrial respiration (Rahman and MacNee, 2000). Glutathione synthesis is regulated by oxidants, antioxidants, growth factors, and inflammatory and anti-inflammatory agents (Rahman and MacNee, 1999, 2000).

In the present study, decreased levels of antioxidant enzymes in general and of GSH in particular may be important with regard to AEST-induced oxidative damage of tissue-mediated repair responses. Thus, the toxicity of AEST in various organs like liver, lung, and kidney might be due to the formation of the radical species. The various components of ST like tobacco, betel quid, areca nut, and catechu, among others, have also been reported to be toxic in experimental animals (Kumar et al., 2000; Jeng et al., 2001; Hung, 2004). Also, during the metabolism of smoked tobacco many electrophiles are generated which are detoxified by the use of GSH (Cotgreave et al., 1987). The decreased GSH levels increase the free radical burden due to ineffective removal of ROS from the tissues, which results in increased lipid peroxidation. In addition, enhanced lipid peroxidation with concomitant decrease in reduced GSH is indicative of oxidative stress that provides evidence of the relationship between lipid peroxidation, tissue damage, and inflammation (Geetha et al., 2001). It is also evident from the histopathological changes where we found mild to moderate infiltration of phagocytic cells in the portal tract of the liver and the interstitial spaces of the lung, indicating inflammation in these organs due to AEST-induced oxidative burden (Fig. 1). Also, oxidative stress–induced lipid peroxidation has been implicated in malignant transformation (Gonzalez, 1999) and other systemic disorders. Surprisingly, despite similar effects of AEST on the hepatic, pulmonary, and kidney antioxidant defense, it had more deleterious effects on liver and lung as compared to kidney.

Vitamins constitute one of the most important micronutrients that modulate the defense mechanism. Vitamins such as vitamin A, C, and E are the chief non-enzymatic antioxidants present in the body to scavenge free radicals. A strong synergy exists between GSH, vitamin C, and vitamin E (Zaidi et al., 2005). Vitamin E is an important antioxidant in the lipid domain. It is present in cell membrane and plasma. It is readily exchanged between cells and plasma with a balance in favor of plasma. Vitamin C is an important extracellular antioxidant that disappears faster than other antioxidants when exposed to ROS. The unchanged plasma levels of these vitamins indicate that blood pool of vitamins A, C, and E does not get affected easily at the used dose and time of AEST.

In conclusion, long-term administration of AEST impairs the enzymatic antioxidant defense system and reduces glutathione levels in liver, lung, and kidney. These alterations may be one of the responsible factors for AEST-induced inflammation in these organs. The understanding of mechanisms for AEST-induced cellular damage may offer therapeutic opportunities and ways to modify the adverse effects of ST.

REFERENCES


