Chemomodulatory action of Aloe vera on the profiles of enzymes associated with carcinogen metabolism and antioxidant status regulation in mice.

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Summary

The effect of two doses (30 µl and 60 µl/day/mice daily for 14 days) of the fresh leaf pulp extract of Aloe vera was examined on carcinogen-metabolizing phase-I and phase-II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase and lipid peroxidation in the liver of mice. The modulatory effect of the pulp extract was also examined on extrahepatic organs (lung, kidney and forestomach) for the activities of glutathione S-transferase, DT-diaphorase, superoxide dismutase and catalase. The positive control mice were treated with butylated hydroxyanisole (BHA). Significant increases in the levels of acid soluble sulfhydryl (-SH) content, NADPH-cytochrome P450 reductase, NADH-cytochrome b5 reductase, glutathione S-transferase (GST), DT-diaphorase (DTD), superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX) and glutathione reductase (GR) were observed in the liver. Aloe vera significantly reduced the levels of cytochrome P450 and cytochrome b5. Thus, Aloe vera is clearly an inducer of phase-II enzyme system. Treatment with both doses of Aloe caused a decrease in malondialdehyde (MDA) formation and the activity of lactate dehydrogenase in the liver, suggesting its role in protection against prooxidant-induced membrane and cellular damage. The microsomal and cytosolic protein was significantly enhanced by Aloe vera, indicating the possibility of its involvement in the induction of protein synthesis. BHA, an antioxidant compound, provided the authenticity of our assay protocol and response of animals against modulator. The pulp extract was effective in inducing GST, DTD, SOD and catalase as measured in extrahepatic organs. Thus, besides liver, other organs (lung, kidney and forestomach) were also influenced favorably by Aloe vera in order to detoxify reactive metabolites, including chemical carcinogens and drugs.

Key words: Chemoprevention, Aloe vera, Carcinogen metabolizing enzymes, Antioxidant enzymes and xenobiotic detoxification.

Introduction

Aloe vera (family Liliaceae), is found predominantly in dry localities in most parts of the world. Aloe vera leaves reportedly have tremendous medicinal value. Its juice is commonly used on burns and minor cuts for enhancing healing of dermal wounds (Chitra et al., 1998). In addition, it is also used as an emollient, purgative, laxative, antibacterial, anaesthetic and anti­septic. Washing the eyes with Aloe vera is suggestive of protecting the eyes from ultraviolet rays. Furthermore, angiogenic activity of Aloe vera gel has recently been documented in vitro (Lee et al., 1998). Aloe vera gel, along with vitamin C supplementation, was found to be effective in reducing the incidence of chemical carcinogenesis in rats (Shamaan et al., 1998). Another independent study by Lissoni et al. (1998) found an additive protective effect of Aloe vera, along with melatonin, in terms of stabilization of disease and survival, in patients with advanced solid tumors for whom no other standard effective therapy was available. A major sugar present in Aloe vera, associated with the wound-
healing activity and anti-inflammatory property of the leaf extract, has been identified as the growth substance mannose-6-phosphate (Davis et al., 1991, 1994; Vazquez et al., 1996). It has also been reported that its hypoglycemic effect plays a role in the accelerated healing of dermal wounds in diabetic rats (Chitra et al., 1998). Its anti-inflammatory potency has also been evaluated experimentally against a wide spectrum of irritants (kaolin, albumin, dextran, gelatin and mustard). A polysaccharide fraction obtained from Aloe was found to decrease oxidative DNA damage and PMA-induced ornithine decarboxylase activity. It has also induced glutathione S-transferase activity in in vitro models (Kim et al., 1999). Acemannan, a major carbohydrate fraction isolated form Aloe vera is claimed to have several important therapeutic properties, including acceleration of wound healing, immune stimulation, anti-cancer and anti-viral effects. But, the biological mechanisms of these activities are unclear (Zhang and Tizard, 1996). Alprogen, a glycoprotein, extracted from Aloe leaf is shown to inhibit mediator release caused by the mast cell activation (Ro et al., 2000). Some lectins purified from Aloe vera leaf pulp are shown to possess haemagglutinating activity (Akev and Can, 1999).

The present investigation is designed to investigate the possible cancer chemoprevention action of the leaf pulp extract of Aloe vera as indicated by its effect on the enzymes of carcinogen metabolism and cellular antioxidant status.

Materials and experimental procedure

Chemicals
Butylated hydroxyanisole (BHA), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), oxidized glutathione (GSSG), pyrogallol, 2,6-dichlorophenol-indophenol (DCPIP), potassium ferricyanide, triton X-100, ethylenediamine tetraacetic acid (EDTA), bovine serum albumin (BSA), sodium pyruvate, thiobarbituric acid (TBA), reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were all obtained from Sigma Chemical Co. (St. Louis, MO, USA). The rest of the chemicals used were obtained from local firms (India) and were of highest purity grade.

Test material
The test material was collected from the garden of the JNU Campus. It was identified and authenticated as Aloe vera by a botanist. After peeling off the green layer of the fresh, fleshy leaves, the transparent pulp was homogenized and passed through cheese cloth. The resulting pulp extract was stored at 4 °C and used for treating the animals. A voucher specimen of Aloe vera has been preserved in the herbarium of our laboratory.

Animals
Random-bred Swiss albino male mice (6–8 weeks old) were used for the study. They were maintained in our air-conditioned animal facility (Jawaharlal Nehru University, New Delhi) under a 12-h light/dark cycle, and provided (unless otherwise stated) with standard food pellets (Hindustan Lever Ltd., India) and tap water ad libitum.

Experimental design
Experiments were designed to study the effect of Aloe vera on xenobiotic/drug metabolizing enzymes and antioxidant status in mice. Animals were assorted randomly into the following groups.

Group I (n = 8): Animals were fed a normal diet and sham-treated with distilled water through oral gavage daily, for 14 days; this group of animals served as control.

Group II (n = 8): Animals were fed a normal diet and treated with 30 µl of extract/animal (wt. ~22 g)/day through oral gavage daily, for 14 days.

Group III (n = 8): Animals were fed a normal diet and treated with 60 µl of extract/animal (wt. ~22 g)/day through oral gavage daily, for 14 days.

Group IV (n = 8): Animals were fed a diet containing 0.75 % BHA for 14 days; this group of animals served as positive control.

The rationale for selecting the above mentioned doses of Aloe vera is based on the doses usually prescribed for human beings by traditional medicine practitioners.

Biochemical studies in liver, lung, kidney and forestomach
Animals were killed by cervical dislocation and the entire liver was then perfused immediately with ice cold 0.9% NaCl and thereafter carefully removed, trimmed free of extraneous tissue and rinsed in chilled 0.15 M Tris-KCl buffer (pH 7.4). The liver was then blotted dry, weighed quickly and homogenized in ice cold 0.15 M Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. An aliquot of this homogenate (0.5 ml) was used for assaying the acid-soluble sulfhydryl group (-SH) while the remainder was centrifuged at 10,000 rpm for 20 min. The resultant supernatant was transferred into pre-cooled ultracentrifugation tubes and centrifuged at 1,05,000 x g for 60 min using a Beckman ultracentrifuge (Model-L8 70 M). The supernatant (cytosol fraction), after discarding any floating lipid layer and appropriate dilution, was used for as-
Table 1. Modulatory influence of two different doses of *Aloe vera* leaf pulp extract and BHA on body weight gain and toxicity-related parameters in mouse.

<table>
<thead>
<tr>
<th>Group (Gr)</th>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Liver wt. x 100/Final body wt.</th>
<th>LDH Microsome</th>
<th>Protein (mg/ml) Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr I</td>
<td>Control (only vehicle-d.w.)</td>
<td>21.25 ± 1.49 (1.00)</td>
<td>22.00 ± 1.85 (1.00)</td>
<td>5.28 ± 0.337 (1.00)</td>
<td>2.89 ± 0.288 (1.00)</td>
</tr>
<tr>
<td>Gr II</td>
<td><em>Aloe</em> (30 µl/animal)</td>
<td>22.00 ± 1.07 (1.04)</td>
<td>25.25 ± 1.83 (1.15)</td>
<td>5.45 ± 0.396 (1.03)</td>
<td>1.36 ± 0.223 (0.47)</td>
</tr>
<tr>
<td>Gr III</td>
<td><em>Aloe</em> (60 µl/animal)</td>
<td>22.25 ± 1.04 (1.05)</td>
<td>26.75 ± 2.12 (1.22)</td>
<td>5.79 ± 0.516 (1.10)</td>
<td>1.30 ± 0.172 (0.45)</td>
</tr>
<tr>
<td>Gr IV</td>
<td>BHA (0.75 % in diet)</td>
<td>20.48 ± 0.72 (0.96)</td>
<td>21.07 ± 0.80 (0.96)</td>
<td>5.56 ± 0.534 (1.05)</td>
<td>1.77 ± 0.122 (0.61)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 8 animals. Values in parentheses represent relative changes in parameters when compared with control. *(P < 0.05), (P < 0.01), (P < 0.005) and (P < 0.001) represent significant changes against control.*

Abbreviation d.w.-distilled water; BHA-butylated hydroxyanisole; LDH-lactate dehydrogenase.

Treatment duration: 14 days.

Estimation of cytochrome P450 and cytochrome b5

Cytochrome P450 was determined using carbon monoxide difference spectra. Both cytochrome P450 and cytochrome b5 contents were assayed in the microsomal suspension by the method of Omura and Sato (1964), using absorption coefficients of 91 and 185 cm⁻¹/mmol, respectively.

Determination of NADPH-cytochrome P450 reductase

Assay of NADPH-cytochrome P450 reductase was performed according to the method of Omura and Take-sue (1970), with some modifications. The enzyme activity was calculated using an extinction coefficient of 6.22 mM⁻¹cm⁻¹. One unit of enzyme activity is defined as that causing the oxidation of 1 mole of NADPH per min.

Determination of NADH-cytochrome b5 reductase

Assay of NADH-cytochrome b5 reductase was performed according to the method of Mihara and Sato (1972). The enzyme activity was calculated using an extinction coefficient of 1.02 mM⁻¹cm⁻¹. One unit of enzyme activity is defined as that causing the reduction of 1 mole of ferricyanide per min.

Determination of glutathione S-transferase activity

The cytosolic glutathione S-transferase activity was determined according to the procedure of Habig et al. (1974). This specific activity of glutathione S-transferase is expressed as μmoles of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6 mM⁻¹cm⁻¹.

Determination of DT-diaphorase activity

DT-diaphorase activity was measured using the procedure described by Ernster et al. (1962). The activity was calculated using an extinction coefficient of...
**Table 2.** Modulatory influence of two different doses of *Aloe vera* leaf pulp extract and BHA on mouse hepatic phase-I and phase-II drug-metabolizing enzyme levels.

<table>
<thead>
<tr>
<th>Group (GR)</th>
<th>Treatment</th>
<th>Cyt P450</th>
<th>Cyt b5</th>
<th>Cyt P450 R</th>
<th>Cyt b5 R</th>
<th>GST</th>
<th>DTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr I</td>
<td>Control</td>
<td>0.600 ± 0.046</td>
<td>0.229 ± 0.009</td>
<td>0.242 ± 0.013</td>
<td>3.78 ± 0.191</td>
<td>1.82 ± 0.181</td>
<td>0.027 ± 0.0017</td>
</tr>
<tr>
<td></td>
<td>(only vehicle-d.w.)</td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
</tr>
<tr>
<td>Gr II</td>
<td>Aloe</td>
<td>0.522 ± 0.034a</td>
<td>0.210 ± 0.012a</td>
<td>0.263 ± 0.012a</td>
<td>4.32 ± 0.169b</td>
<td>1.84 ± 0.120</td>
<td>0.03 ± 0.0030a</td>
</tr>
<tr>
<td></td>
<td>(30 μl/animal)</td>
<td>(0.87)</td>
<td>(0.92)</td>
<td>(1.09)</td>
<td>(1.14)</td>
<td>(1.01)</td>
<td>(1.15)</td>
</tr>
<tr>
<td>Gr III</td>
<td>Aloe</td>
<td>0.373 ± 0.042c</td>
<td>0.209 ± 0.019c</td>
<td>0.296 ± 0.022c</td>
<td>4.83 ± 0.198c</td>
<td>2.17 ± 0.149c</td>
<td>0.033 ± 0.0012c</td>
</tr>
<tr>
<td></td>
<td>(60 μl/animal)</td>
<td>(0.62)</td>
<td>(0.91)</td>
<td>(1.22)</td>
<td>(1.28)</td>
<td>(1.19)</td>
<td>(1.22)</td>
</tr>
<tr>
<td>Gr IV</td>
<td>BHA</td>
<td>0.599 ± 0.032</td>
<td>0.292 ± 0.017a</td>
<td>0.267 ± 0.021</td>
<td>3.51 ± 0.282</td>
<td>5.44 ± 0.324b</td>
<td>0.060 ± 0.0073b</td>
</tr>
<tr>
<td></td>
<td>(0.75 % in diet)</td>
<td>(1.00)</td>
<td>(1.28)</td>
<td>(1.10)</td>
<td>(0.93)</td>
<td>(2.99)</td>
<td>(2.22)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6–8 animals. Values in parentheses represent relative change in parameters when compared with control.

a(P < 0.05), b(P < 0.01) and c(P < 0.005) represent significant changes against control.

(1) nmole/mg protein, (2) μmole of NADPH oxidised/min/mg protein, (3) μmole of NADH oxidised/min/mg protein, (4) μmole CDNB-GSH conjugate formed/min/mg protein and (5) μmole of CDPIP reduced/min/mg protein.

Abbreviation: Cyt P450 – cytochrome P450; Cyt b5 – cytochrome b5; Cyt P450 R – cytochrome P450 reductase; Cyt b5 R – cytochrome b5 reductase; GST – glutathione S-transferase; DTD – DT-diaphorase.

21 mM⁻¹ cm⁻¹. One unit of enzyme activity is defined as the amount of enzyme required to reduce on μmole of DCPIP per min.

**Estimation of the acid-soluble sulfhydryl (-SH) group**

The level of acid-soluble sulfhydryl group was estimated as total non-protein sulfhydryl group by the method described by Moron et al. (1979). Reduced glutathione was used as a standard to calculate nmole of -SH content/g tissue.

**Determination of glutathione reductase**

Glutathione reductase was determined using the procedure described by Carlberg and Mannervik (1985). One unit of enzyme activity is defined as nmole of NADPH consumed/min/mg protein, based on an extinction coefficient of 6.22 mM⁻¹ cm⁻¹.

**Determination of glutathione peroxidase**

Glutathione peroxidase activity was measured by the coupled-assay method as described by Paglia and Valentine (1967). One unit of enzyme activity has been defined as nmole of NADPH consumed/min/mg protein based on an extinction coefficient of 6.22 mM⁻¹ cm⁻¹.

**Determination of catalase**

Catalase was estimated as described by Aebi (1984). The specific activity of catalase has been expressed as moles of H₂O₂ reduced/min/mg protein.

**Determination of superoxide dismutase**

Superoxide dismutase was assayed using the method of Marklund and Marklund (1974). A single unit of enzyme is defined as the quantity of superoxide dismutase required to produce 50% inhibition of autoxidation.

**Estimation of lipid peroxidation**

Lipid peroxidation was estimated using the thiobarbituric acid-reactive substances (TBARS) method as described by Varshney and Kale (1990) and is expressed in terms of malondialdehyde (MDA) formed per mg protein.

**Determination of lactate dehydrogenase**

Lactate dehydrogenase (LDH) was assayed according to the method of Bergmeyer and Bernt (1974). The enzyme activity was calculated using an extinction coefficient 6.22 mM⁻¹ cm⁻¹. One unit of enzyme activity is defined as that causing the oxidation of 1 μmole of NADH per min.

**Estimation of protein**

Protein was determined using the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard, at 660 nm.

**Statistical analysis of the data**

Results are presented as mean ± SD. Statistical analysis was performed using ANOVA following a Mann-Whitney U-test. A value of < 0.05 was considered to indicate a significant difference between groups.
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Table 3. Modulatory influence of two different doses of *Aloe vera* leaf pulp extract and BHA on mouse hepatic antioxidant-related parameters and lipid peroxidation.

<table>
<thead>
<tr>
<th>Groups (GR)</th>
<th>Treatment</th>
<th>GSH 1</th>
<th>GPX 2</th>
<th>GR 3</th>
<th>SOD 4</th>
<th>CAT 5</th>
<th>LPO 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr I</td>
<td>Control</td>
<td>25.10 ± 2.83</td>
<td>61.02 ± 2.94</td>
<td>47.4 ± 3.77</td>
<td>6.13 ± 0.377</td>
<td>76.10 ± 5.76</td>
<td>0.521 ± 0.037</td>
</tr>
<tr>
<td></td>
<td>(only vehicle-d.w.)</td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(1.00)</td>
</tr>
<tr>
<td>Gr II</td>
<td>Aloe (30 pl/animal)</td>
<td>34.30 ± 3.94</td>
<td>61.26 ± 4.31</td>
<td>47.7 ± 2.56</td>
<td>6.66 ± 0.445</td>
<td>145.50 ± 12.64</td>
<td>0.479 ± 0.030</td>
</tr>
<tr>
<td></td>
<td>(1.37)</td>
<td>(1.00)</td>
<td>(1.01)</td>
<td>(1.09)</td>
<td>(1.91)</td>
<td>(0.92)</td>
<td></td>
</tr>
<tr>
<td>Gr III</td>
<td>Aloe (60 pl/animal)</td>
<td>35.70 ± 4.88</td>
<td>74.61 ± 5.07</td>
<td>56.8 ± 3.03</td>
<td>8.83 ± 0.475</td>
<td>161.9 ± 13.61</td>
<td>0.415 ± 0.035</td>
</tr>
<tr>
<td></td>
<td>(1.42)</td>
<td>(1.25)</td>
<td>(1.20)</td>
<td>(1.44)</td>
<td>(2.12)</td>
<td>(0.80)</td>
<td></td>
</tr>
<tr>
<td>Gr IV</td>
<td>BHA (0.75 % in diet)</td>
<td>54.55 ± 4.39</td>
<td>58.69 ± 4.68</td>
<td>69.24 ± 1.33</td>
<td>6.01 ± 3.67</td>
<td>63.28 ± 3.45</td>
<td>0.317 ± 0.024</td>
</tr>
<tr>
<td></td>
<td>(2.17)</td>
<td>(0.96)</td>
<td>(1.46)</td>
<td>(0.98)</td>
<td>(0.83)</td>
<td>(0.61)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of 6–8 animals. Values in parentheses represent relative change in parameters when compared with control. *P < 0.05*, *b(P < 0.01)* and *c(P < 0.005)* represent significant changes against control. GSH – reduced glutathione; GPX – glutathione peroxidase; GR – glutathione reductase; SOD – superoxide dismutase; CAT – catalase; LPO – lipid peroxidation.

### Results

The findings of the present study are presented in Tables 1–6. The experimental groups of mice treated with *Aloe* leaf extract showed significant increase in body weight and body weight gain at both the dose levels of treatment (Table 1).

#### Hepatic studies

*Aloe*-treated groups of mice showed a slight increase in the liver-somatic index as compared to that of the control group. Protein levels were increased significantly in microsomal as well as cytosolic fractions in groups treated with extract and BHA. In the lower-dose-treated group, protein levels were increased by 1.13 (P < 0.05) and 1.37 (P < 0.001) folds while in the higher-dose-treated group, the increases in protein levels were 1.10 (P < 0.05) and 1.20 (P < 0.01) fold in microsome and cytosol, respectively (Table 1).

#### Cytochrome P450 system

The estimated levels of cytochrome P450 and cytochrome b5 presented statistically significant decreases in both *Aloe*-treated groups as compared to their controls, except Group III of Cyt b5 in which the decrease (9 %) in the level was not significant. The maximum reduction of 38 % (P < 0.005) was evident in Cyt P450 at the high-dose level of treatment. In the lower-dose-treated group of mice, levels of Cyt P450 and Cyt b5 were reduced by 13 % (P < 0.05) and 8 % (P < 0.05), respectively. A dose-dependent significant increase was observed in specific activities of NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase at both the dose levels of treatment, relative to their control values. The percentages of elevation in Cyt P450 reductase and Cyt b5 reductase were 9 % and 14 % in the low-dose-treated group and 22 % and 28 % in the high-dose-treated group, respectively. BHA could increase only the level of Cyt b5 (P < 0.05) (Table 2).

#### Phase II enzymes

In phase II enzymes, GST and DTD were assayed in the cytosol of liver homogenate. Hepatic glutathione S-transferase showed a significant increase of 1.19 fold (P < 0.005) in its specific activity in the higher-dose-treated group of mice, while in the lower-dose-treated group, it was comparable to that of the control. Hepatic DT-diaphorase showed a dose-dependent induction in its specific activity following *Aloe* treatment. The activity of DTD was elevated by 1.15 (P < 0.05) and 1.22 (P < 0.005) folds, respectively, at the lower- and higher-dose levels of treatment, as compared to the values in the control group. BHA induced the activities of both GST and DTD remarkably (P < 0.01) (Table 2).

#### Antioxidative parameters

The antioxidant parameters studied were GSH, GPX, GR, SOD and catalase. The level of reduced glutathione (GSH) measured as acid-soluble sulfhydryl group (-SH) was significantly elevated in a dose-de-
dependent manner following Aloe treatment. The magnitude of elevation in the -SH level was 1.37 fold (P < 0.005) for Group II and 1.42 fold (P < 0.005) for Group III as compared to that of Group I. Only a higher dose of Aloe treatment was effective in significantly elevating the specific activities of glutathione peroxidase (GPX) and glutathione reductase (GR), while it remained comparable to control values following treatment with the lower dose. The activities of GPX and GR were elevated, respectively, by 1.25 fold (P < 0.05) and 1.30 (P < 0.005) fold, in the higher-dose-treated group of mice. Superoxide dismutase and catalase presented a statistically significant, as well as dose-dependent induction in their activities at both dose levels of treatment. The magnitude of induction of SOD and catalase was 1.09 fold (P < 0.05) and 1.91 fold (P < 0.01) for Group II; 1.44 fold (P < 0.005) and 2.12 fold (P < 0.005) for Group III, respectively, over that of their control values. BHA induced the level of sulfhydryl group (P < 0.005) and activity of GR (P < 0.005) (Table 3).

Lipid peroxidation and lactate dehydrogenase

The extent of lipid peroxidation estimated as MDA formation was inhibited significantly by 20% (P < 0.01) at the higher-dose level of treatment. In the lower-dose-treated mice, it was reduced by 8% but was not significant as compared to the control value (Table 3). The specific activity of LDH was reduced in a dose-dependent manner following Aloe treatment. The magnitude of reduction was 53% (P < 0.01) and 55% (P < 0.005) in Groups II and III, respectively, as compared to that in control group (Table 1). BHA effectively inhibited both the level of lipid peroxidation (P < 0.01) and the activity of LDH (P < 0.005).

Extrahepatic studies

There were no significant alterations in the relative weights and protein contents of extrahepatic organs (except lung) examined, following Aloe and BHA treatment. In lung, a significant increase (P < 0.01) in protein content was evident at the lower-dose level of Aloe treatment (Tables 4–6).

Glutathione S-transferase

The extrahepatic organs examined revealed a dose-dependent increase in the constitutive basal level of specific activity of glutathione S-transferase, following the administration of Aloe. The magnitude of change was in the order of lung > kidney > forestomach at both the dose levels of treatment (Groups II and III) as compared to those in their controls. In the lower-dose-treated group, the elevations in the GST activity were 1.25 (P < 0.001), 1.15 fold (P < 0.05) and 1.11 fold (P < 0.005) induced the activity of GST in lung, kidney and forestomach (Tables 4–6).
DT-diaphorase

Aloe-treated groups of mice presented an increase in the specific activity of DT-diaphorase in all the extrahepatic organs examined, but the increases were significant only in the forestomach of Group II (P < 0.001), and in the forestomach and kidney of Group III (P < 0.005 and P < 0.05, respectively). BHA induced the activity of DTD in the lung (P < 0.005), kidney (P < 0.01) and forestomach (P < 0.01) (Tables 4–6).

Superoxide dismutase

The specific activity of superoxide dismutase in lung, kidney and forestomach was elevated significantly in all the Aloe-treated groups of mice. However, the maximal inductions observed in the lower-dose-treated group were 1.37, 1.55 and 1.21 folds in the lung, kidney and forestomach, respectively, as compared to their control values. The higher-dose-treated group showed increases in SOD activity of 1.31, 1.14 and 1.16 folds in the lung, kidney and forestomach, respectively. BHA induced the activity of SOD in the kidney (P < 0.01) and forestomach (P < 0.005) (Tables 4–6).

Catalase

Catalase activity was detectable only in lung and kidney supernatants under our present assay conditions. It presented significant alterations following Aloe treatment. In the lower-dose-treated group, specific activity of catalase was increased by 1.35 fold (P < 0.005) in the lung and 1.29 fold (P < 0.001) in the kidney; and in the higher-dose-treated group, it was increased by 1.18 fold (P < 0.005) in the lung and 1.40 fold (P < 0.001) in the kidney. BHA induced the activity of catalase in the lung (P < 0.01) and kidney (P < 0.005) (Tables 4–6).

Discussion

The use of medicinal plants is based on the experience of many generations of physicians and traditional ethnic systems of medicines. The use of medicinal plants in modern medicine suffers from the fact that although hundreds of plants are used in the world to prevent or to cure diseases, scientific evidence in terms of modern medicine is lacking in most cases. However, today it is necessary to provide scientific proof as to whether it is justified to use a plant or its active principles (Ammon and Wahl, 1991). As far as modern drugs are concerned, they must be further characterized after their pharmacological screening, i.e., for pharmacokinetic and pharmacodynamic properties, including toxicity (Kelloff et al., 1994). A recent upsurge in identifying non-dietary natural products associated with a high degree of safety margin as cancer chemopreventive agents has been hailed by many investigators to be practically beneficial, especially when the carcinogenic effect is mild to moderate. Our present knowledge on chemoprevention of cancer has revealed the presence of a diverse array of naturally occurring bioactive compounds that inhibit carcinogenesis at almost every site (Tanaka, 1994; Morse and Stoner, 1996; Pezzuto, 1997). Aloe leaves reportedly have tremendous medicinal value. They are used commonly as an emollient, purgative, laxative, antibacterial, anaesthetic, antiseptic and for healing burns and wounds (Chitra et al., 1998). Aloe has also been reported to have angiogenic and anticancerogenic activity (Lee et al., 1998; Shamaan et al., 1998).

The present investigation demonstrates the efficacy of Aloe vera in inducing drug detoxification/conjugating enzymes as well as in blocking activation enzymes, and thereby predicting hepatoprotective and cancer chemopreventive potential of the plant in the murine model system. Since inducibility of drug-metabolizing enzymes is one of the reliable markers for evaluating the chemopreventive potential of the test materials in the murine model system, extrapolating the results obtained would highlight a mechanistic insight into chemopreventive and hepatoprotective activity associated with the plant.

The chemoprevention strategy to prevent cancer owes its origin to epidemiological observation in which high consumption of fruits and vegetables has been linked to reduced cancer risk. Cancer chemoprevention is an exciting pharmaceutical cancer research involving the use of either natural or synthetic components to delay, inhibit or reverse the development of cancer in normal or preneoplastic conditions. Oxidative stress is an important pathogenic factor in the development of many human diseases, including cancer. Thus, additionally, the role of Aloe in affording protection, if any, against oxidative damage as evaluated by determining the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and main non-enzymatic antioxidant-GSH quantification has been pursued. BHA is a known antioxidant compound, which is being used as food additive. It bears no cancer hazards and has proved to be an anticarcinogenic compound (Hocman, 1988; Williamson et al., 1999). It is reported to be an effective inducer of phase II enzymes (Prochaska and Fernandes, 1993). BHA has been shown to reduce the risk of cancer in many tumor model systems (Jones et al., 1984; Iverson, 1999). It has also proved to be a chemopreventive agent against DMBA-induced transplacental and transmammary carcinogenesis in mice (Rao, 1982; 1989; Rao et al., 1989). Based on these reports, BHA was used as posi-
tive control in our experiment to validate the authenticity of assay protocols and to compare the response to modulator in our animal model system; it can be deduced from the fact that there was neither any increase in the rate of mortality nor any decrease in the body weight gain of animals following Aloe treatment. Also, at the cellular level there is no indication of damage, as observed by the measurement of lactate dehydrogenase activity as an index of cell damage. The decrease in its activity indicated the role of Aloe in protection against cellular damage.

The microsomal cytochrome P450 system is a product of the CYP super family of genes, which constitutes a major electron transport chain in the membrane of endoplasmic reticulum. It plays a key role in oxidative activation, inactivation and promotion of excretion of most xenobiotic compounds, and also in modulating the duration and intensity of their toxicity (Gengerich, 1988; Miller, 1988). Cytochrome P450 catalyzes the oxidation of lipophilic chemicals through the insertion of one atom of molecular oxygen into the substrate, thereby converting the latter to either less harmful or totally harmless hydrophilic metabolites for their ultimate removal from the system. During oxidative metabolism, in the microsomal microenvironment involving the cytochrome P450 system, the electron flows from NADPH or NADH through a flavoprotein cytochrome P450 reductase or cytochrome b5 reductase to different isomeric forms of cytochrome P450 and cytochrome b5 (Gibson and Skett, 1994). Cytochrome P450 is the terminal oxidase component of an electron transport system present in the endoplasmic reticulum responsible for many endobiotic and xenobiotic oxidation reactions. Aloe vera inhibited the levels of both cytochrome P450 and b5 but induced the activities of cytochrome P450 and b5 reductases. This observation may be indicative of inhibition of oxidative metabolic processes and thereby a blocking of the activation of xenobiotic compounds including carcinogens.

The action of phase-II enzymes on the substrates generated by the action of phase I enzymes on innocuous and/or hazardous chemicals leads to their solubilization and excretion (Gibson and Skett, 1994). Glutathione S-transferase is a critical detoxification enzyme that functions primarily in conjugating "functionalized P450 metabolites" with endogenous ligands (reduced glutathione) favoring their elimination from the body of the organism (Hartman and Shankel, 1990). There is persuasive evidence to support the induction of glutathione S-transferase and protection against a wide spectrum of cytotoxic, mutagenic and carcinogenic chemicals (De Flora and Ramel, 1989; Ketterer, 1988; Reed, 1990). We used CDNB as the non-specific substrate in our assay for glutathione S-transferase. Thus, the specific activity of the enzyme measured was the sum of all of its isoforms. The protective effect of many naturally occurring chemopreventive agents against carcinogenesis has been ascribed to decreased bioavailability of potential DNA damaging entities and their destruction into excretable metabolites facilitated through induction of glutathione S-transferase (Coles and Ketterer, 1990).

DT-diaphorase is generally induced coordinately with other phase-II detoxifying enzymes (Talalay, 1989). Induction of DT-diaphorase has been evaluated as a means for determining the potency of many anti-carcinogenic substances (Benson et al., 1980; De Long et al., 1986). This enzyme protects against the toxicity of quinones and their metabolic precursors (polycyclic aromatic hydrocarbon, benzene, etc.) (Smart and Zinnoni, 1984; Karczewski et al., 1999). Induction of DT-diaphorase facilitates metabolism of quinones by two-electron oxido-reduction to hydroquinone, obliterating semiquinone radical and subsequent oxygen radical production. The stable hydroquinone resulted from two electron oxido-reduction of quinone by DT-diaphorase can be conjugated by glucurinide or sulfate and excreted and thus affords protection from reactive intermediates (Talalay, 1989; De Long et al., 1986; Lind et al., 1982).

The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also detoxifies reactive oxygen species directly and/or neutralizes reactive intermediate species generated from exposure to xenobiotics including chemical carcinogens (Ketterer, 1998). The increased glutathione reductase level plays a significant role in the reduction of oxidized glutathione to reduced glutathione at the expense of NADPH and regulates GSH-GSSG cycle in the cell (Gonzales et al., 1984). GSH has been endowed with an important function in maintaining the reduced state of cellular environment, in addition to its conjugating ability owing to nucleophile center and is involved in detoxification of xenobiotics that cause toxicity and carcinogenicity.

The antioxidant responsiveness mediated by Aloe as evaluated by its efficacy to modulate basal level activities of glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase may be anticipated to have biological significance in eliminating reactive free radicals that may otherwise affect the normal cell functioning. The dysfunctioning of these antioxidant enzymes has been implicated in several disorders including rheumatoid arthritis, reperfusion injury, cardiovascular diseases and immune injury as well as cancer (Vanoni et al., 1991; Guemori et al., 1991; Flagg et al., 1993; Saydem et al., 1997). These enzymes have been suggested as playing an important role in maintaining physiological levels of oxygen and hydrogen.
peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated form inadvertent exposure to xenobiotics and drugs.

It has been proposed that glutathione peroxidase is responsible for the detoxification of hydrogen peroxide in low concentration, while catalase comes into play when glutathione peroxidase pathway is reaching saturation with the substrate (Gaetani et al., 1989). Furthermore, the decreased lipid peroxidation is correlated well with the induction of antioxidant enzymes above basal level. In addition, the induction of superoxide dismutase activity may attribute to inhibition of the generation of active oxygen species from autooxidation of quinones generated from the action of DT-diaphorase. The augmented activity of metalloenzyme superoxide dismutase accelerates dismutation of superoxide radicals to hydrogen peroxide which is removed by catalase (Aebi, 1984). Thus, induced superoxide dismutase activity in conjunction with catalase and decrease in lipid peroxidation by Aloe antagonize any cellular injury induced by reactive oxygen species.

Aloe reduced the levels of cytochrome P450 and b5 and may thus decrease the activation of xenobiotic compounds. Furthermore, the decreased activated electrophilic species will mean reduced risk of damage to the cell’s genetic machinery. At the same time, the activities of glutathione S-transferase and DT-diaphorase have increased, indicating an increase in the detoxification ability of cells. Aloe has increased levels of -SH groups and antioxidant enzymes, glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase; and shows decreased malondialdehyde formation, indicating that it can impart anticarcinogenic property by favorably elevating the host’s antioxidant defense mechanisms.

The present investigation has clearly shown that Aloe could be used as a potential cancer chemopreventive agent which may possibly help to enhance the detoxification reaction of the cell and may thus be effective as a blocking agent against carcinogenesis (Wattenberg, 1985; 1992; 1997). The chemopreventive property of Aloe vera may be attributed to the various glycoproteins, polysaccharides or lectins present in it. The next step is to substantiate its chemopreventive potential by isolating and testing the different bioactive components of Aloe, using different chemical carcinogenesis models. This is a very important study, which would surely tell us whether the chemopreventive potential of Aloe could be used in humans, after proper trials. Our laboratory has already commenced the study in this direction employing skin cancer, stomach cancer, cervical cancer and oral cancer model systems in appropriate animals.

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References


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