Effect of *Aloe vera* gel extract on antioxidant enzymes and azoxymethane-induced oxidative stress in rats

K R Anilakumar*, K R Sudarshanakrishna, G Chandramohan, N Ilaiyaraja, Farhath Khanum & A S Bawa
Biochemistry & Nutrition Discipline, Defence Food Research Laboratory, Mysore 570011, India

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The present work was undertaken with a view to study the effect of oral feeding of 2% *Aloe vera* gel extract (AGE) for 30 days on azoxymethane (AOM)-induced oxidative stress in rats. It was observed that AOM administration resulted in a significant increase in malondialdehyde and conjugated dienes, with reduction in hepatic glutathione (GSH), vitamin A and uric acid contents. AOM-induced reduction in hepatic GSH and uric acid was brought back to normal by AGE. There was a significant raise in hepatic catalase, superoxide dismutase and glucose-6-phosphate dehydrogenase (G-6-PD) activities as a result of feeding of the extract. Ingestion of the extract effected reduction in AOM-induced colonic GSH-peroxidase, G-6-PD and glutathione S-transferase and femur bone marrow micronuclei formation. Hence, it is suggested that *Aloe vera* gel extract possess the ability to reduce AOM-induced oxidative stress and toxicity in liver.

Keywords: *Aloe vera* gel extract, Azoxymethane, Detoxifying enzymes, Micronuclei, Rat liver

*Aloe vera* (*Aloe barbadensis* Miller) has been used medicinally for several thousands of years with a long and illustrious history. The gel of *Aloe vera* contains about 99 to 99.5% water with pH in the range of 4.4 to 4.7. The major components in the gel are glucomannans, acemannan, minerals, flavonoids, tannic acid, alprogen, c-glucosyl chromone, etc. The solid material contains about 45 different ingredients including vitamins, minerals, enzymes, sugars, anthraquinone or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid. The plant is reported to contain alprogen an anti allergic glycol protein and C-glycosyl chromone a novel anti-inflammatory compound. The plant is reported to possess numerous pharmacological properties like abortifacient effect, adjuvant activity, analgesic activity, anticlastogenic, etc.

Several works are recently reported on the effect of consumption of extracts of *Aloe vera* gel on gastric ulcer, gastric microcirculatory changes, anti-inflammatory, hepatoprotective, clinical treatment of sepsis, etc. *Aloe vera* gel has extracted for antioxidant potential and exhibits radical scavenging activity (72.2%) which is higher than that of BHT (70.5%) and α-tocopherol (65.65%)³. The human bioavailability of vitamins C and E are enhanced with *Aloe vera* preparations. However, effect of gel extract on chemically induced toxicity and oxidative stress is sparse. Azoxymethane (AOM) is reported to generate the extremely reactive hydroxyl radical inducing oxidative stress that participates in peroxidation of the membrane lipids leading to the increased MDA formation. AOM metabolized in liver to methyl azoxymethanol leads to methyl carbonium ion which is believed to be the ultimate carcinogen which binds stem cells DNA in colon. Hence, in this study, the alcoholic extract of the gel was evaluated for its modulatory effect on azoxymethane (AOM)-induced oxidative stress and toxicity in rats.

Materials and Methods

Preparation of *Aloe vera* gel extract — *Aloe vera* gel separated from locally available plant, identified by Dr. H S Prakash, Botanist and Professor, University of Mysore, India. The gel was mechanically shaken in various solvents including absolute ethanol (1 kg/l) for 48 h at room temperature. The alcoholic extract was found to contain more of antioxidants than the other solvent extracts as observed by 1-diphenyl-2-picryl hydrazyl (DPPH) radical assay. The alcoholic *Aloe vera* gel extract (AGE) was collected in a flask and concentrated in the rotary flash evaporator followed by lyophilisation. Sufficient amount of extracts were prepared for feeding the rats.

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*A Correspondent author
Telephone: 0821-2474676
E-mail: anilakumarkr@gmail.com
Experimental schedule — Male Wistar rats (110-130g) were allocated randomly into 4 groups, each consisting of six animals groups. Group I served as control; Group II was administered with azoxymethane; Group III was fed with (2.0%) AGE; and Group IV was fed with (2.0%) AGE + azoxymethane (60 mg/kg body wt).

All the rats were housed in individual stainless steel, wire-bottomed cages at 27° ± 2° C, and fed ad libitum with free access to the laboratory stock diet and water. The Aloe vera gel extract was fed to groups III and IV for 30 days, whereas groups II and IV were administered with four weekly injections (sc), azoxymethane (60 mg/kg body wt). Weekly food intake and weight gain were monitored. After 24h of the last treatment, all the rats were sacrificed under mild anesthesia (sodium pentobarbitone, 50 mg/kg body wt; ip). Blood was removed through cardiac puncture. The organs were quickly excised and stored in liquid nitrogen until analysis. Clearance of experimental design by Institutional Ethical Committee for rats was taken.

Chemical analysis — Malondialdehyde (MDA) in liver homogenate (0.5 g) was estimated by precipitation with trichloroacetic acid (10%) and was assayed as per reported method. Lipid isolated from liver was used to assay the amount of conjugated dienes and the upper layer of the same assay was utilized for estimation of hydroperoxides. The content of ascorbic acid in liver was determined by the method of Roe and Keuther. Lipids isolated from liver were used for estimation of tocopherols. Hepatic vitamin A and uric acid were estimated by the methods reported elsewhere. Hepatic and colonic glutathione (GSH) contents were determined by the method of Ellman. Hepatic bilirubin content was determined according to Lowry. Gamma glutamyltranspeptidase (GGT) was estimated by the method of Meister et al. Protein in tissues was determined according to Lowry et al. Bone marrow micronuclei were counted as per Countryman and Heddle.

Statistical analysis — Data was subjected to statistical analysis using analysis of variance (ANOVA). Significance or non-significance of differences between mean values was determined at 5% level of significance.

Results

Preliminary study using 1% extract was not found to induce the key enzymes viz. GST, GGT, etc. in liver and colon. On this basis, the detailed study was conducted at 2% level of feeding of the extract. Table 1 presents the food intake, weight gain pattern and organ weights of rats fed with AGE. It was observed that feeding of extract or administration of AOM did not affect the daily food intake and weight gain of rats. The organ (liver, colon, kidney, heart and brain) weight was also not influenced by the intake of AGE or with the AOM injection.

Assessment of oxidative status and antioxidant status — The results showed that the AOM administration resulted in a significant increase in MDA and conjugated dienes without altering the hydroperoxide levels (Table 2). Feeding of AGE per se did not change MDA level, conjugated dienes and hydroperoxides. Prefeeding of AGE brought down the AOM-induced raise in hepatic MDA and conjugated dienes. There was no change in hepatic ascorbic acid levels with AOM treatment or with AGE feeding. At the same time, there was a significant reduction in hepatic GSH, vitamin A and uric acid content, as a result of administration of azoxymethane. However there was no change in hepatic levels of tocopherols.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Food intake (g/day)</th>
<th>Weight gain (g/day)</th>
<th>Organ wt (g/100g bodywt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colon</td>
</tr>
<tr>
<td>Control</td>
<td>14.23±0.39</td>
<td>5.42±0.54</td>
<td>3.62±0.34 0.29±0.031</td>
</tr>
<tr>
<td>AOM (60 mg/kg body wt.)</td>
<td>12.82±0.89</td>
<td>5.80±0.41</td>
<td>3.80±0.35 0.32±0.023</td>
</tr>
<tr>
<td>AGE (2%)</td>
<td>13.82±1.01</td>
<td>5.72±0.53</td>
<td>3.50±0.34 0.30±0.029</td>
</tr>
<tr>
<td>AGE (2%)</td>
<td>14.13±0.88</td>
<td>5.30±0.52</td>
<td>3.71±0.30 0.28±0.040</td>
</tr>
</tbody>
</table>

Table 1—Effect of A. vera gel extract azoxymethane–induced changes on food intake, weight gain and organ weight of rats
[Values are Mean ± SD for 6 rats]
and bilirubin due to AOM. Feeding of AGE did not influence GSH, tocopherols, bilirubin, vitamin A and uric acid in liver. It was observed that AOM-induced reduction in hepatic GSH and uric acid was recovered to normal level by AGE feeding (Table 2).

Assessment of hepatic antioxidant enzymes —

Effect of feeding of AGE was studied on AOM-induced changes in hepatic catalase, GSH-Px, GSSG-reductase, and SOD, G-6-PD, GST and GGT activities (Table 3). Administration of AOM reduced the hepatic catalase, GSH-Px, SOD and G-6-PD activities and increased the hepatic GST and GGT activity significantly. However, a recovery in hepatic catalase, SOD and G-6-PD was observed in AOM affected animals after feeding AGE (20%) and brought back to the normal levels (Table 3). Decreased GSH-Px in liver due to the administration of the AOM was not altered by AGE feeding. Significant increase in hepatic GST and GGT activities was observed due to administration of AOM which were reduced significantly due to the feeding of AGE. Feeding of AGE resulted in significant increase in hepatic GSH-Px, GSSG-reductase and SOD activities.

Assessment of colonic antioxidant enzymes —

Effect of feeding of AGE was also observed on AOM-induced changes on colonic antioxidant/detoxifying enzymes (Table 4). Administration of AOM resulted in significant reduction in colonic GSH with an associated increase in MDA content. Feeding of AGE per se did not result in any change in colonic GSH and MDA content. Change in colonic MDA produced by AOM did not alter by feeding of AGE. The result showed a significant decrease in colonic GSH-Px with an associated decrease in G-6 PD and GST. AGE feeding did not result in any change in colonic enzyme activity except in G-6 PD, wherein a significant raise was observed (Table 4). Combined effect of AOM and AGE showed that the AOM-induced decline in colonic GSH-Px, G-6 PD and GST were brought back to normalcy, as a result of the feeding of the extract. However, colonic catalase was not influenced either by AOM treatment or by feeding of AGE.

Micronuclei (%) formed in femur bone marrow was found to be 1.0±0.02, 6.5±0.61, 1.3±0.19 and 2.9±0.40 for control, AOM, 2%AGE and 2%AGE+AOM groups of rats, respectively. It showed that the function of cell properties with micronuclei was significantly increased on treatment with AOM.

### Table 2 — Effect of Aloe vera gel extract on azoxymethane-induced changes in hepatic antioxidants and lipid peroxides

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA (nmole/g)</th>
<th>CDx10⁻⁵ (mole/g)</th>
<th>HP (μmole/g)</th>
<th>Ascorbic acid (mg/g)</th>
<th>GSH (μmole/g)</th>
<th>Tocopherols (mg/g)</th>
<th>Bilirubin (mg/dl)</th>
<th>Vitamin A (RE/g)</th>
<th>Uric acid (μmole/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.08±0.00²</td>
<td>0.08±0.00²</td>
<td>0.03±0.00²</td>
<td>0.03±0.00²</td>
<td>0.03±0.00²</td>
<td>0.03±0.00²</td>
<td>0.03±0.00²</td>
<td>0.03±0.00²</td>
<td>0.03±0.00²</td>
</tr>
<tr>
<td>AOM (60 mg/kg body wt.)</td>
<td>0.14±0.00²</td>
<td>0.14±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
</tr>
<tr>
<td>AGE (2%)</td>
<td>0.06±0.00²</td>
<td>0.06±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
</tr>
<tr>
<td>AGE (2%)+AOM (60 mg/kg body wt.)</td>
<td>0.10±0.00²</td>
<td>0.10±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
<td>0.04±0.00²</td>
</tr>
</tbody>
</table>

Values bearing different superscripts in the same column are significantly different (P<0.05).

### Table 3 — Effect of Aloe vera gel extract on azoxymethane-induced changes in hepatic antioxidant/detoxifying enzymes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>* Catalase (x10⁻¹⁵)</th>
<th>** GSH-Px (x10⁻³)</th>
<th>#GSSG R (x10⁻³)</th>
<th>SOD (x10⁻³)</th>
<th>* G-6-PD</th>
<th>° GST (x10⁻⁵)</th>
<th>* GGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.91±0.01²</td>
<td>2.29±0.11²</td>
<td>3.03±0.11²</td>
<td>2.31±0.08²</td>
<td>67.72±6.25²</td>
<td>2.72±0.12²</td>
<td>1.38±0.03²</td>
</tr>
<tr>
<td>AOM (60 mg/kg body wt.)</td>
<td>0.72±0.02³</td>
<td>1.69±0.12³</td>
<td>3.36±0.08³</td>
<td>1.68±0.09³</td>
<td>55.55±4.32³</td>
<td>3.08±0.12³</td>
<td>2.61±0.16³</td>
</tr>
<tr>
<td>2%AGE</td>
<td>0.92±0.01³</td>
<td>2.92±0.12³</td>
<td>3.97±0.09³</td>
<td>2.78±0.11³</td>
<td>63.88±1.70³</td>
<td>2.63±0.05³</td>
<td>1.39±0.02³</td>
</tr>
<tr>
<td>2%AGE+AOM (60 mg/kg body wt.)</td>
<td>0.91±0.01³</td>
<td>2.07±0.10³</td>
<td>3.00±0.28³</td>
<td>2.74±0.11³</td>
<td>66.12±3.20³</td>
<td>2.65±0.13³</td>
<td>1.31±0.06³</td>
</tr>
</tbody>
</table>

Values are represented as * ΔA/min/mg protein x10⁴; ** μ mole NADP produced min/mg protein; # units/min/mg protein; @ μmole NADP reduced/min/mg protein; $ mole conjugate formed/min/mg protein; + nmole p-nitroanilide released/min/mg protein.

Values bearing different superscripts in the same column are significantly different (P<0.05).
Rats treated with AOM and AGE, the micronuclei fraction reduced significantly as compared to AOM administered rats.

**Discussion**

Generation within the membrane and lipoprotein of peroxyl and alkoxyl radicals, aldehyde and other products of lipid oxidation affects the liver to a great extent, causing the formation of high molecular mass protein aggregate within the membrane. Hence increased level of MDA and associated products viz. conjugated dienes is a factual indicator of lipid peroxidation which highlight the toxic effect of AOM in liver.

Thiols are thought to play a vital role in protecting cells against lipid oxidation. AGE feeding was found to be effective in increasing the GSH content rendering the protection against AOM-induced hepatic and colonic lipid peroxidation. Ames et al. have outlined the potential of uric acid as a biological antioxidant in mitigating cellular damage caused by oxygen free radical. Hence, it is relevant to note the increase in uric acid by the treatment of AOM. Possible reason for raise in bilirubin and uric acid levels by AGE warrants further investigation.

During oxidative stress, catalase activity decreases, hydrogen peroxide accumulate and thereby more peroxidation of lipids is favoured. An accelerated lipid peroxidation and drastic fall in hepatic GSH contents by toxicants has been demonstrated. GSH exhibits its antioxidant effect by reducing with superoxide radical and hydroxyl radical following the formation of oxidized glutathione and reduces peroxides in the non-enzymatic reaction. GSH is a major mechanism for regulating intracellular free radical concentration. Activity of GSH and GSH-dependent enzymes, GSH-Px and GSSG-reductase was reduced significantly in rats administered AGE. Glushkov et al. have reported decline in activities of GSH, GSSG-reductase, G-6PD and GSH-Px on administering the rats with xenobiotics, which is in consonance with the present observation. GSH - dependant enzymes have been demonstrated to be involved in elimination of peroxides and with restoration of altered GSH/GSSG balance of the cell. The ingestion of AGE resulted in increased availability of reducing equivalents in liver, possibly due to improved G-6-PD, GSH-Px and also SOD activities with subsequent regeneration of the GSH pool. GSH is regarded as an indigenous protective agent against drugs and GST is a soluble protein located in cytosol which plays an important role in detoxification and excretion of xenobiotics. Increased colonic GSH associated with G-6-PD and SOD might help in reducing the formation of peroxidative stress as observed by the decrease in MDA by AGE.

Abnormally high levels of GGT have often been observed in tumors in a variety of tissues including hepatocellular carcinomas. Elevated levels of GGT reduce the stress on GSH in cell and enable the cell to respond to proliferation and other stimuli of GGT positive foci facilitating transformation and tumor progression. The concomitant feeding of Aloe vera gel extract at 2% level resulted in a significant reduction of GGT activity.

Evaluation of preventive effect of AGE using the technique of bone marrow micronucleus assay was also carried out. The micronuclei test conducted on bone marrow cells is considered to be a highly reliable test of pre-cancerous changes in tissues. Ingestion of AGE resulted in reduction in AOM - induced micronuclei showing its capability to reduce genotoxicity. The results showed that AGE had the potency to counter the chromosomal aberration caused by AOM.

Biochemical parameters in liver and colon were...
estimated in the present work, because AOM is metabolized in the liver to methyl azoxymethanol leading to methyl carbonium ion which is believed to be the ultimate carcinogen binds to stem cells DNA in colon. Antioxidant potency of Aloe vera is already known\(^\text{[3]}\) and the possible mechanism of protective actions of AGE has been attributed to its antioxidant activity as indicated by protection against increased lipid peroxidation\(^\text{[40]}\). Aloe-emodin present in the gel has been reported to exhibit protective effect against chemically induced hepatotoxicity\(^\text{[41]}\). The synergy of a number of components viz. barbaloin, glucomannans, acemannan, minerals, flavonoids, tannic acid, c-glucosyl chromone, etc. present in the AGE may be responsible for the observed reduction in AOM-induced oxidative stress and toxicity in this study. Hence it is suggested that Aloe vera gel extract possesses the ability to reduce the AOM-induced oxidative stress and toxicity in liver.

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