Effect of amaranth leaves on dimethylhydrazine-induced changes in multicomponent antioxidant system of rat liver

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Effect of prefeeding dehydrated amaranth (A. gangeticus) leaves at 10 and 20% levels on a chemical toxicant, dimethylhydrazine (DMH)-induced free radical stress in rat liver was evaluated. DMH-induced rise in hepatic malondialdehyde (MDA), was diminished by AL. AL intake resulted in a significant increase in hepatic glutathione (GSH). The feeding of AL at 10% level increased the hepatic glucose-6-phosphate dehydrogenase (G-6-PDH) activity, while that at 20% level increased the hepatic glutathione reductase (GSSGR) as well, in addition to G-6-PDH. Amaranth leaves at 10 and 20% levels of feeding diminished the hepatic superoxide dismutase and glutathione peroxidase (GSH-Px) activities. DMH influenced adversely the hepatic antioxidant enzyme activities. Simultaneous administration of DMH and feeding of AL enhanced the DMH-induced decrease in hepatic GSH-Px. DMH enhanced formation of micronuclei was reverted significantly by AL intake. Hence, it was concluded that the consumption of AL at 20% level reduced DMH-induced impaired antioxidant status in rat liver.

Keywords: Amaranth leaf, Antioxidant, Liver, Multicomponent antioxidant system, Rat

IPC Code: Int CI A61K A61K

Amaranth leaves (Amaranthus gangeticus Linn.) are widely consumed as a vegetable in India and are rich in carotenoids. The carotenoids and perhaps non-nutrients like dietary fibre and other phenolic constituents influence the enzymes involved in the activation and detoxification of xenobiotics including carcinogens. This effect could primarily be mediated through alterations in levels of phase I cytochrome P-450 enzyme-dependent metabolism of carcinogens and induction of phase-II enzymes i.e. glutathione S-transferase, glutathione reductase, glutathione peroxidase, catalase etc. Therefore, vegetables are important source of antioxidant nutrient and non-nutrients and are being advocated for use in the dietary management of degenerative diseases. The antioxidant compounds have been well recognized to have effective role in reducing the oxidation stress and thereby, reduce the risk of cancer. National Research Council, therefore, has recommended an increased consumption of fruits and vegetables. There are several reports on cabbage, garlic, carrot, curry leaves, Brussels sprouts etc. on their potential beneficial effects per se as well as against chemically induced carcinogenesis. Fibre, calcium and folic acid are some of the food components, currently in the centre of research to ascertain their effects. Amaranth leaves contain dietary fibre, folic acid and perhaps other bioactive nutrients such as bioflavonoids. Flavonoids are also reported to possess antioxidant potency. Further, amaranth leaves contain Mg, an antimutagen and chlorophyllin, a proven efficient antimutagen and antioxidant. The knowledge presently available on the potential for protective capacity of dietary components like green leafy vegetables in many instances is sparse and insufficient to make dietary advice on food selection. This study was, therefore, undertaken to investigate whether the consumption of dehydrated leaves of amaranth have any beneficial effect in preventing or protecting against the toxicity of a cancer causing xenobiotic, dimethylhydrazine (DMH), which is known to induce oxidative stress.

Materials and Methods

Preparation of dehydrated amaranth leaves diet—Good quality fresh amaranth (Amaranthus gangeticus) leaves procured from local market were washed, blanched for 2 min with 0.12% potassium metabisulphite and dried in a cross flow drier till the moisture content was reduced to 9%. The dehydrated amaranth leaves (AL) thus prepared were powdered.
and found to contain 41.9% carbohydrates, 25.7% protein, 2.7% fat, 14% ash and 6.7% crude fibre. The content of carbohydrates was calculated by difference. The dehydrated AL was added at 10 and 20% levels to prepare the experimental diet. The control and experimental diets were made nearly isoenergetic by adjusting corn starch, casein and oil.

Animal treatment—Thirty-six male Wistar rats (110-130 g) were allocated randomly to 6 groups of each. Groups 1 and 2 were fed the control diet, groups 3 and 4 received 10% AL incorporated control diet and groups 5 and 6 received AL at 20% level in control diet for 12 weeks. Groups 2, 4 and 6 were administered 10 weekly injections of DMH (60 mg/kg body weight; sc) and groups 1, 3 and 5 received saline. All the rats were housed in individual stainless steel wire-bottomed cages at 27±2°C. They were fed ad libitum with free access to water. Weekly food intake and weight gain were monitored. At the end of 12 weeks, all the rats were sacrificed under mild anaesthesia (sodium pentobarbitone) and organs/tissues were quickly excised and stored in liquid nitrogen until analyses.

Chemical assay—For the assay of MDA18, liver homogenate (0.5 g) was precipitated with trichloroacetic acid (10%) and reacted with thiobarbituric acid reaction mixture 0.35% consisting of sodium dodecyl sulphate, ferrie chloride and butylated hydroxy toluene in 0.1 M of glycine- HCl buffer. After boiling and cooling, OD was taken at $\lambda_{max}$ 532 and MDA was calculated using a molar extinction coefficient of 1.56 x $10^5$/M/cm. Hepatic glutathione (GSH) content was determined by the method of Ellman19 using 5,5'-dithiobis-2-nitrobenzoic acid reagent. For the assay of catalase20, liver (0.5 g) was homogenized in phosphate buffer (5 M pH 7.4) and the homogenates were centrifuged at 700 x g. The supernatant was assayed using hydrogen peroxide as a substrate. Glutathione reductase (GSSGR) and glutathione peroxidase (GSH-Px) activities were determined by the method of Weiss et al21 in the supernatant of liver homogenate prepared in phosphate buffer (0.5 M pH 7.0) using H$_2$O$_2$ and NADPH as substrates. Hepatic glutathione S-transferase (GST) activity was determined by the procedure of Habig et al22 in the supernatant of liver homogenate in phosphate buffer (0.1 M pH 6.5), centrifuged at 700xg using 1-chloro-2,4-dinitrobenzene as substrate. Superoxide dismutase (SOD) was measured by the inhibition of cytochrome C reduction mediated via superoxide anions generated by xanthine-xanthine oxidase and monitored at 550 nm. One unit of SOD was defined23 as the amount required to inhibit the reduction of cytochrome C by 50%. The assay mixture for the estimation of hepatic glucose-6-phosphate dehydrogenase (G-6-PDH) consisted of the enzyme source prepared in 0.1 mM of Tris buffer, 2 mM of glucose-6-phosphate, 0.3 mM of NADP and ΔA was monitored at 340 nm24. Gamma glutamyltranspeptidase (GGT) was estimated in kidney by the method of Meister et al25 using L-$\gamma$-glutamyl-p-nitroanilide as the substrate in a homogenate prepared with 0.1 M of Tris- HCl buffer. Micronuclei in femur bone marrow was estimated essentially according to Countryman and Heddle26. Protein in tissues was determined according to Lowry et al27. The significance of differences among mean values was calculated according to Student’s t test28.

Results

Effect on daily food intake, weight gain and liver weight of rats—The effect of consumption of AL for 12 weeks with or without DMH injection on daily food intake and weight gain of rats are given in Fig. 1A and

![Fig 1](image-url)

Fig 1—Effect of AL with or without the treatment of DMH on (A) food intake; and (B) weight gain of rats. [Values bearing different superscripts in the figure are significantly different ($P<0.05$); Values are Mean±SD for 6 rats].
Dehydrated amaranth leaves were fed to rats at 10 and 20% DMH injection did not appear to influence the food intake and weight gain pattern of rats. The consumption of AL containing diet was found to be reduced significantly both at 10 and 20% levels as compared to control irrespective of DMH administration.

Effect on hepatic lipid peroxides and antioxidants in liver—The data for effect of AL ingestion and DMH treatment on cytotoxic product viz., MDA and tissue antioxidant (GSH) are given in Table 1. DMH induced a significant increase in MDA with a concomitant decrease in GSH as compared to control. An increase in the hepatic GSH level was seen in the group fed AL at 10 and 20% levels. Dimethylhydrazine did not influence the hepatic lipid peroxides and the combination of AL intake and DMH injection did not bring about any change in these parameters.

Effect on hepatic antioxidant / detoxifying enzymes in liver—Table 2 presents the effect of feeding of dehydrated AL on DMH-induced changes in hepatic antioxidant/detoxifying enzymes. DMH influenced adversely the hepatic enzyme activities viz., GSH-Px, catalase and SOD activities as compared to control.

<table>
<thead>
<tr>
<th>Rat group</th>
<th>GSH (mmole/g)</th>
<th>MDA (mmole/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.0 ± 1.01</td>
<td>7.9 ± 0.81</td>
</tr>
<tr>
<td>DMH</td>
<td>7.1 ± 0.89</td>
<td>10.6 ± 1.00</td>
</tr>
<tr>
<td>AL 10%</td>
<td>10.4 ± 1.46</td>
<td>7.6 ± 0.91</td>
</tr>
<tr>
<td>AL 10%+DMH</td>
<td>7.9 ± 0.91</td>
<td>7.8 ± 0.74</td>
</tr>
<tr>
<td>AL 20%</td>
<td>12.4 ± 1.05</td>
<td>6.5 ± 0.82</td>
</tr>
<tr>
<td>AL 20%+DMH</td>
<td>8.2 ± 0.93</td>
<td>8.0 ± 0.75</td>
</tr>
</tbody>
</table>

Values bearing different superscripts in the same column are significantly different at p<0.05.

Table 2—Effect of AL with or without DMH on hepatic antioxidant/detoxifying enzymes

<table>
<thead>
<tr>
<th>Rat group</th>
<th>GSH-Px (x10^4)</th>
<th>GSSGR (x10^4)</th>
<th>Catalase (x10^4)</th>
<th>G-6-PDH @</th>
<th>SOD x10^5</th>
<th>GSTx10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.3 ± 0.30</td>
<td>3.4 ± 0.45</td>
<td>0.91 ± 0.124</td>
<td>66.4 ± 5.52</td>
<td>1.61 ± 0.131</td>
<td>2.0 ± 0.29</td>
</tr>
<tr>
<td>DMH</td>
<td>1.61 ± 0.16</td>
<td>2.3 ± 0.30</td>
<td>0.50 ± 0.105</td>
<td>89.5 ± 4.91</td>
<td>0.92 ± 0.139</td>
<td>1.9 ± 0.23</td>
</tr>
<tr>
<td>AL 10%</td>
<td>1.90 ± 0.20</td>
<td>3.2 ± 0.44</td>
<td>0.96 ± 0.180</td>
<td>73.9 ± 9.84</td>
<td>1.29 ± 0.252</td>
<td>2.3 ± 0.28</td>
</tr>
<tr>
<td>AL 10%+DMH</td>
<td>1.41 ± 0.18</td>
<td>3.5 ± 0.54</td>
<td>0.54 ± 0.115</td>
<td>84.4 ± 5.38</td>
<td>1.02 ± 0.287</td>
<td>2.2 ± 0.31</td>
</tr>
<tr>
<td>AL 20%</td>
<td>1.94 ± 0.34</td>
<td>5.1 ± 0.78</td>
<td>1.00 ± 0.125</td>
<td>89.2 ± 8.35</td>
<td>1.35 ± 0.256</td>
<td>2.0 ± 0.33</td>
</tr>
<tr>
<td>AL 20%+DMH</td>
<td>1.94 ± 0.19</td>
<td>5.2 ± 0.83</td>
<td>0.70 ± 0.112</td>
<td>86.3 ± 6.42</td>
<td>1.04 ± 0.233</td>
<td>2.4 ± 0.39</td>
</tr>
</tbody>
</table>

Values bearing different superscripts in the same column are significantly different at p<0.05.

Effect of AL at 20% level enhanced the hepatic GSSGR activity as compared to control. At the same time the simultaneous administration of DMH and feeding of AL did not alter the DMH-induced decrease in hepatic GSH-Px and SOD activities. The activity of GSSGR in 20% of AL fed rats was significantly higher than control and DMH administration had no effect.

Gamma glutamyltranspeptidase in kidney was increased significantly by DMH, and AL at both the levels of feeding reduced it (Fig. 2A).

Effect on DMH-induced alterations in colonic antioxidant status in rats—Table 3 shows the effect of feeding of dehydrated AL with or without DMH treatment in colonic GSH, MDA and antioxidant enzymes. An increased GSH content was observed in colon by AL at both the levels of feeding. The superoxide dismutase activity was decreased with AL (at both the levels) with a simultaneous increase in GSH-Px and GSSGR activities (at 20% level only). As seen earlier, DMH produced significant decrease in colonic GSH and GSH-Px, GST and SOD activities. At the same time, the colonic catalase activity was enhanced by DMH. The AL feeding per se increased colonic GSH and the co-administration of AL at 10 and 20% levels in DMH treated rats improved the DMH-induced reduction in GSH. The colonic MDA showed a different pattern. AL feeding increased the colonic MDA on co-administration with DMH. Here, the MDA values remained higher than the control. Catalase was increased after DMH insult which remained unaltered after inclusion of AL in the diet.

Effect on bone marrow micronuclei—Fig. 2B presents the effect of AL on DMH-induced alterations in micronuclei formation in femur bone marrow. The results showed the DMH-induced
enhanced formation of micronuclei and the reversion by AL at both levels.

Discussion

Effect of amaranth leaves on biomarkers of oxidative stress was studied in order to demonstrate antioxidative potentials of phytochemicals, if any, in vivo. Our earlier work and the present paper describe the onset of oxidative stress by the treatment of DMH as evidenced by enhanced MDA and decreased GSH and activities of GSH-Px, catalase and SOD in rat liver. The concomitant feeding of AL and administration of DMH reversed the DMH-induced elevation of MDA and the DMH-induced reduction of GSH in liver. The reduced levels of GSH might allow peroxides to accumulate in erythrocytes and haemolysis can occur due to their oxidative effect on lipids of the red cell membrane. Formation of MDA might result in oxidative stress in vivo. Oxidative stress is a state of imbalance between generation of reactive oxygen species like hydroxyl and superoxide radicals and the level of antioxidant defence system.

The increased GSH levels in rats fed with amaranth leaves might be helpful in reducing the DMH-induced elevated levels of oxidative radicals. Peroxidation in vivo is influenced by the activity of SOD, catalase and GSH-Px. The interactive effects of AL and DMH treatments, which led to decrease in lipid peroxidation in liver, may be ascribed to the protective phytochemicals. Carotenoids are thought to scavenge free radicals and other oxidants involved in disease process. Decrease in levels of GSH-Px and SOD in liver after feeding of amaranth leaves as observed in the present study warrant further studies.

Gamma glutamyltranspeptidase is important in transport of amino acids required for the synthesis of GSH in cells. The elevated levels of GGT in kidney of rats treated with DMH might reduce the stress on GSH in the cell and enable the cells to respond to proliferative and other stimuli. GGT has been shown by WHO to be a very sensitive and reliable index of pre-cancerous changes in tissues. Amaranth leaves produced remarkable change in the DMH-induced increase in GGT activity.

Amaranth leaves modulated the significant reduction produced by DMH in colonic GSH-Px. At the same time GSSGR activity was significantly increased by 20% of AL. The increased GSH-Px and

![Graph showing effect of AL with or without the treatment of DMH on GGT activity and micronuclei formation.](image)

**Fig. 2.** Effect of AL with or without the treatment of DMH on (A) GGT activity (nmole p-nitroaniline released/min/mg protein) of kidney; and (B) femur bone marrow micronuclei of rats [Values bearing different superscripts in the figure are significantly different (P<0.05); Values are Mean±SD for 6 rats].

<table>
<thead>
<tr>
<th>Rat group</th>
<th>GSH μmol/g</th>
<th>MDA nmol/g</th>
<th>Catalase **</th>
<th>GSH-Px x10⁻³</th>
<th>GSSGR x10⁻³</th>
<th>G-6-PDH *</th>
<th>GST *</th>
<th>SOD $</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.1 ± 7.19*</td>
<td>1.39 ± 0.21*</td>
<td>0.14 ± 0.018*</td>
<td>0.21 ± 0.033*</td>
<td>0.41 ± 0.056*</td>
<td>11.28 ± 1.39*</td>
<td>46.2 ± 4.90*</td>
<td>3.7 ± 0.39*</td>
</tr>
<tr>
<td>DMH</td>
<td>40.2 ± 5.29*</td>
<td>1.40 ± 0.24*</td>
<td>0.25 ± 0.024*</td>
<td>0.11 ± 0.021*</td>
<td>0.39 ± 0.042*</td>
<td>11.11 ± 1.48*</td>
<td>37.1 ± 4.23*</td>
<td>1.6 ± 0.18*</td>
</tr>
<tr>
<td>AL10%</td>
<td>87.1 ± 9.12*</td>
<td>2.40 ± 0.23*</td>
<td>0.12 ± 0.020*</td>
<td>0.32 ± 0.042*</td>
<td>0.45 ± 0.059*</td>
<td>12.39 ± 1.24*</td>
<td>46.9 ± 5.29*</td>
<td>1.8 ± 0.41*</td>
</tr>
<tr>
<td>AL10% + DMH</td>
<td>58.2 ± 6.22*</td>
<td>2.07 ± 0.19*</td>
<td>0.25 ± 0.031*</td>
<td>0.25 ± 0.028*</td>
<td>0.38 ± 0.043*</td>
<td>12.02 ± 1.40*</td>
<td>34.1 ± 2.91*</td>
<td>1.8 ± 0.23*</td>
</tr>
<tr>
<td>AL20%</td>
<td>85.2 ± 9.56*</td>
<td>2.39 ± 0.14*</td>
<td>0.12 ± 0.019*</td>
<td>0.30 ± 0.042*</td>
<td>0.52 ± 0.063*</td>
<td>12.01 ± 1.38*</td>
<td>45.2 ± 4.68*</td>
<td>1.6 ± 0.24*</td>
</tr>
<tr>
<td>AL 20% + DMH</td>
<td>59.1 ± 6.81*</td>
<td>2.01 ± 0.16*</td>
<td>0.22 ± 0.038*</td>
<td>0.24 ± 0.032*</td>
<td>0.40 ± 0.051*</td>
<td>11.29 ± 1.51*</td>
<td>36.8 ± 3.91*</td>
<td>1.9 ± 0.21*</td>
</tr>
</tbody>
</table>

Values bearing different superscripts in the same column are significantly different at P<0.05

*nmole conjugate formed/min/mg protein; **nmole NADP formed/min/mg protein; ***AA of 0.1/min/mg protein; $ units/min/mg protein.

*μmol NADP reduced/min/mg protein.
GSSGR activities seen in 20% of group were associated with a significant enhancement in colonic GSH level, because the proper GSH status is achieved by the activities of GSH-Px and GSSGR activities. The dehydrated amaranth leaves did not affect the colonic detoxifying enzymes like GST and catalase, but did produce increase in GSH levels.

It is interesting to note the onset of oxidative stress by AL as evidenced by increase in MDA with an associated decrease in SOD activity in the colon. At the same time liver was found to be effective in mitigating the DMH-induced peroxidative stress by reducing MDA and increasing the antioxidant/detoxifying enzymes. This dramatic contrast might depend on the pro-oxidant action of carotenoids in AL, which is present in large amount. The antioxidant activity of carotenoids may shift into pro-oxidant activity depending on their redox potential of the carotenoid molecules as well as on the biological environment in which they act. The oxidative stress induced by DMH in conjunction with high carotenoids concentration in AL might have contributed towards MDA formation in the co-administered group.

Evaluation of preventive effect of AL using the technique of bone marrow micronuclei assay was also carried out. The decrease in micronuclei by AL was interesting because, studies reporting the preventive role of AL against the chemically induced genotoxicity are limited and are of considerable importance with respect to nutrition and cancer.

From the foregoing discussion, it was concluded that the supplementation with AL at 20% level produced significant changes in DMH impaired antioxidant status. Measurement of cytotoxicity was carried out by assaying lipid peroxidation product viz., MDA. Positive effects of AL may be ascribed to the high amount of carotenoids present in them or the synergistic effects of polyphenolic compounds and other phytochemicals. Effect of β-carotene and other carotenoids on human chronic diseases is known. Reports are there that people who ingest more dietary carotenoids exhibit less risk for several diseases and some cancers. Thus evaluation of antioxidants as suppressors of chemically induced lipid peroxidation provides a scope to select natural free radical scavengers which on co-administration in vivo, may reduce toxic effects of chemicals. At the same time, the amaranth leaves affect the hepatic GSH-Px and SOD activities adversely. Further studies are warranted to elucidate this phenomenon.

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References