Reduction of hexachlorocyclohexane-induced oxidative stress and cytotoxicity in rat liver by *Emblica officinalis* Gaertn

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The effect of prefeeding of dehydrated *E. officinalis* (amla) powder at 5 and 10% levels on hexachlorocyclohexane (HCH)-induced changes in multicomponent antioxidant system and lipid peroxides in rat liver was studied. HCH induced significant elevation in hepatic malondialdehyde, conjugated dienes and hydroperoxides. The prefeeding of amla at 10% level could decrease the formation of these lipid peroxides significantly. The HCH abuse resulted in a significant reduction in hepatic glutathione S-transferase (GST), glucose-6-phosphate dehydrogenase (G-6-PDH) and superoxide dismutase (SOD) activities with an elevation in the activities of glutathione peroxidase and \( \gamma \)-glutamyl transpeptidase (GGT). On the other hand, the HCH-induced impairment in hepatic catalase, G-6-PDH and SOD activities were modulated by amla at the 10% level of intake. Prefeeding of amla at 5 and 10% levels appeared to reduce the HCH-induced raise in renal GGT activity. The results show the elevation of hepatic antioxidant system and reduction of cytotoxic products as a result of prefeeding of amla, which were otherwise affected by the HCH administration.

**Keywords:** Amla, Antioxidant enzymes, Hexachlorocyclohexane, Lipid peroxides, Oxidative stress, Rat liver

*Emblica officinalis* Gaertn, commonly known as amla is found all over India, Sri Lanka, Malaysia, China, Pakistan and Bangladesh and is reported to be an immunomodulator. The fruit extract was found to modify hepatotoxic and renotoxic effects of metals in mice. The fruit pulp is used against a variety of disease conditions such as liver injury, atherosclerosis and diabetes. Amla extract has antioxidant activity, which could scavenge superoxides and hydroxyl radicals, as well as inhibit lipid peroxidation *in vitro*. Ascorbic acid and other polyphenols present in the natural formulation of amla show much superior antioxidant activity compared to their equivalent amounts in pure isolated form. Dimethyl hydrazine-induced toxicity can be reduced by amla powder. The living system has inherent mechanisms to reduce free radical-induced injury by the action of enzymes such as superoxide dismutase, glutathione peroxidase, catalase etc. and by the non enzymatic means involving ascorbic acid, tocopherols, etc. Many a time these defence mechanisms may not be sufficient to detoxify the xenobiotic insult on the body.

Supplementation with chemoprotectants may be helpful in these conditions. Therefore, the present study has been undertaken to investigate the effect of dehydrated amla powder on the modulation of antioxidant enzymes and cytotoxicity in rats challenged with hexachlorocyclohexane (HCH), a hepatocarcinogen and oxidative stress inducer.

**Materials and Methods**

- **Preparation of dehydrated amla containing diet**—Good quality amla was purchased locally, washed, blanched with potassium metabisulphite (0.12%), and dried in a cross flow drier in order to get a moisture content of 13% and powdered and incorporated into the control diet. The dehydrated powder contained 61.25% carbohydrates, 3.2% protein, 0.65% fat, 3.72% ash and 18.8% fibre. Control and amla containing diets were made nearly isoenergetic by adjusting corn starch and casein.

- **Animal treatment**—Male Wistar rats (110-130 g) were allocated randomly to 6 groups of 6 rats each. The grouping of rats is as given below:
  - **Group I:** treated as control
  - **Group II:** treated with HCH (300 mg/kg body wt.)
  - **Group III:** treated with 5% amla
  - **Group IV:** treated with 5% amla + HCH (300 mg/kg body wt.)
Group V: treated with 10% amla
Group VI: treated with 10% amla + HCH (300 mg/kg body wt.)

All the rats were housed in individual stainless steel wire-bottomed cages at 27°C ± 2°C and 69% RH. They were fed ad libitum with free access to water. Weekly food intake and weight gains were monitored. Twenty-four hours prior to sacrifice, rats in groups 2, 4 and 6 were administered with HCH (300mg/kg body weight, ip) and those groups in 1, 3 and 5 received groundnut oil. At the end of the experimental period, all the rats were sacrificed under mild anaesthesia and organs were quickly excised and stored in liquid nitrogen until analysis. Clearance of experimental design by the Institutional Ethics Committee for rats was taken.

Chemical assay — For the assay of malondialdehyde (MDA), liver homogenate was used and MDA was calculated using a molar extinction coefficient of 1.56x10^5 /M/cm.10. The lipid isolated from liver was mixed with CHCl_3 and CH_3OH mixture and the amount of conjugated dienes and hydroperoxides produced were estimated.10. The content of ascorbic acid in liver was determined by the method of Roe and Keuther.12 Hepatic glutathione (GSH) content was determined by the method of Ellman.13 For the assay of catalase, liver was homogenized in phosphate buffer and assayed using hydrogen peroxide as a substrate.14 Glutathione reductase (GSSGR) and glutathione peroxidase (GSH-Px) activities were determined by the method of Weiss et al.15 Hepatic glutathione S-transferase (GST) activity was determined by the procedure of Habig et al16 using 1-chloro-2, 4-dinitrobenzene as substrate. Superoxide dismutase (SOD) was measured by the prescribed procedure and one unit of SOD was defined as the amount required inhibiting the reduction of cytochrome C by 50%.17 The estimations of hepatic glucose-6-phosphate dehydrogenase (G-6-PDH)18 and γ-glutamyltranspeptidase (GGT) were carried out by the prescribed methods.19 Protein in tissues was determined according to Lowry et al20. The significance of differences among mean values was calculated according to Student’s ’t’ test

Results and Discussion

Effect of amla and HCH treatments on food intake, weight gain and organ weights of rats — Amla either at 5 or 10% did not result in any change in the food intake pattern and the corresponding weight gain of rats. Figure 1 shows the effect of amla consumption and the chemical abuse on liver and kidney weights of rats. The data clearly showed that HCH administration increases the liver weight significantly. However, the kidney weight was not altered by the treatment of HCH.

Effect of amla and HCH treatments on hepatic lipid peroxides and antioxidants in rats — Feeding of amla at 5 and 10% levels increased the hepatic vitamin C and GSH contents (Table 1). However, the conjugated dienes in liver was significantly reduced by the feeding of amla at both the levels. At the same time the levels of hydroperoxides in liver were not changed by amla. It may also be noted that the amla consumption per se did not influence MDA content.

The administration of HCH elevated the hepatic MDA, conjugated dienes and hydroperoxides significantly showing the possibility of the rat liver having been exposed to the oxidative attack. Probably on a preventive measure the hepatic vitamin C content
is also enhanced significantly. The interactive effects of HCH and amla at 10% level lead to decrease the MDA, conjugated dienes and lipid hydroperoxides significantly as compared to HCH treated group of rats. This may be due to the presence of the reported phytochemicals and reductive substances viz. ascorbic acid, flavonoids, ellagic acid, phylllemblic acid\(^{21, 22}\) present in amla.

**Effect of amla and HCH treatments on hepatic antioxidant/ detoxifying enzymes and renal GGT activity in rats** —The effects of pre-feeding of 5 and 10% amla to HCH injected rats on antioxidant/detoxifying enzymes are shown in the Table 2. The consumption of amla *per se* increased the hepatic catalase, GSH-Px, G-6-PDH and SOD (at 10% level only).

The pesticide abuse resulted in a significant reduction in hepatic GST, catalase, and G-6-PDH and SOD activities with an elevation in the activities of GSH-Px and GGT, as observed earlier\(^{23}\). The HCH-

### Table 1 — Effect of amla on HCH-induced hepatic lipid peroxides and antioxidants in rat liver
[Values are mean ± SD from 6 rats]

<table>
<thead>
<tr>
<th>Rat group</th>
<th>MDA (n moles/g)</th>
<th>Vitamin C (mg/g)</th>
<th>Conjugated dienes x10^5 (moles/g)</th>
<th>Hydroperoxides x10^5 (moles/g)</th>
<th>GSH (m moles/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.2 ± 2.11 (^{ac})</td>
<td>0.28 ± 0.031 (^{a})</td>
<td>76.9 ± 8.12 (^{a})</td>
<td>18.0 ± 2.13 (^{a})</td>
<td>8.4 ± 0.91 (^{a})</td>
</tr>
<tr>
<td>HCH (300 mg/kg b.wt.)</td>
<td>16.1 ± 2.09 (^{b})</td>
<td>0.40 ± 0.062 (^{b})</td>
<td>88.2 ± 9.10 (^{b})</td>
<td>23.9 ± 2.18 (^{b})</td>
<td>8.9 ± 0.92 (^{a})</td>
</tr>
<tr>
<td>5% amla</td>
<td>10.0 ± 2.33 (^{ac})</td>
<td>0.39 ± 0.048 (^{b})</td>
<td>61.3 ± 9.35 (^{c})</td>
<td>19.8 ± 2.06 (^{a})</td>
<td>12.3 ± 1.02 (^{b})</td>
</tr>
<tr>
<td>5% amla + HCH (300 mg/kg b.wt.)</td>
<td>15.8 ± 1.74 (^{b})</td>
<td>0.37 ± 0.041 (^{b})</td>
<td>77.2 ± 8.02 (^{a})</td>
<td>20.1 ± 3.12 (^{a})</td>
<td>9.0 ± 0.95 (^{a})</td>
</tr>
<tr>
<td>10% amla</td>
<td>9.9 ± 1.18 (^{a})</td>
<td>0.38 ± 0.050 (^{b})</td>
<td>64.3 ± 9.23 (^{c})</td>
<td>18.9 ± 2.81 (^{a})</td>
<td>14.0 ± 1.18 (^{b})</td>
</tr>
<tr>
<td>10% amla + HCH (300 mg/kg b.wt.)</td>
<td>12.1 ± 2.14 (^{c})</td>
<td>0.39 ± 0.062 (^{b})</td>
<td>71.6 ± 8.30 (^{a})</td>
<td>20.8 ± 3.09 (^{a})</td>
<td>12.4 ± 1.19 (^{b})</td>
</tr>
</tbody>
</table>

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

### Table 2 — Effect of amla on HCH-induced changes in antioxidant/detoxifying enzymes in rat liver
[Values are mean ± SD from 6 rats]

<table>
<thead>
<tr>
<th>Rat group</th>
<th>GST x 10^7 (@)</th>
<th>GSH-Px x10^3*</th>
<th>GSSGR x10^-3*</th>
<th>Catalase x10^4***</th>
<th>G-6-PDH **</th>
<th>SOD x 10^2 $</th>
<th>GGT#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.07 ± 0.282 (^{a})</td>
<td>1.9 ± 0.15 (^{a})</td>
<td>3.96 ± 0.67 (^{a})</td>
<td>0.87 ± 0.062 (^{a})</td>
<td>62.8 ± 7.9 (^{a})</td>
<td>1.54 ± 0.22 (^{a})</td>
<td>5.7 ± 0.60 (^{a})</td>
</tr>
<tr>
<td>HCH (300 mg/kg b.wt.)</td>
<td>1.70 ± 0.189 (^{b})</td>
<td>2.4 ± 0.38 (^{b})</td>
<td>3.38 ± 0.33 (^{a})</td>
<td>0.31 ± 0.051 (^{b})</td>
<td>50.1 ± 6.2 (^{b})</td>
<td>0.71 ± 0.12 (^{b})</td>
<td>22.8 ± 2.91 (^{b})</td>
</tr>
<tr>
<td>5% amla</td>
<td>2.08 ± 0.202 (^{a})</td>
<td>2.9 ± 0.19 (^{b})</td>
<td>3.91 ± 0.41 (^{a})</td>
<td>1.05 ± 0.092 (^{c})</td>
<td>93.8 ± 9.9 (^{c})</td>
<td>1.42 ± 0.16 (^{a})</td>
<td>6.1 ± 0.70 (^{a})</td>
</tr>
<tr>
<td>5% amla + HCH (300 mg/kg b.wt.)</td>
<td>1.82 ± 0.101 (^{b})</td>
<td>2.8 ± 0.21 (^{a})</td>
<td>4.00 ± 0.45 (^{a})</td>
<td>1.04 ± 0.117 (^{c})</td>
<td>65.1 ± 7.2 (^{c})</td>
<td>0.91 ± 0.11 (^{b})</td>
<td>10.3 ± 1.64 (^{c})</td>
</tr>
<tr>
<td>10% amla</td>
<td>2.15 ± 0.142 (^{a})</td>
<td>2.7 ± 0.24 (^{b})</td>
<td>4.04 ± 0.38 (^{a})</td>
<td>0.07 ± 0.120 (^{c})</td>
<td>90.8 ± 10.2 (^{c})</td>
<td>2.1 ± 0.34 (^{c})</td>
<td>5.9 ± 0.92 (^{a})</td>
</tr>
<tr>
<td>10% amla + HCH (300 mg/kg b.wt.)</td>
<td>1.79 ± 0.182 (^{b})</td>
<td>2.6 ± 0.35 (^{b})</td>
<td>4.03 ± 0.30 (^{a})</td>
<td>1.08 ± 0.112 (^{c})</td>
<td>60.3 ± 6.8 (^{a})</td>
<td>1.9 ± 0.28 (^{c})</td>
<td>9.1 ± 1.88 (^{c})</td>
</tr>
</tbody>
</table>

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

@ m moles conjugate formed/min/mg protein

* m moles NADP formed/min/mg protein

** μ moles NADP* reduced/min/mg protein

*** ΔA of 0.1/min/mg protein

$ units /min/mg protein

# n moles p-nitroanilide released/min/mg protein.
induced reduction in GST activity in liver was found to be not influenced by amla; at the same time the HCH-induced impairment in enzyme activities viz. catalase, G-6-PDH and SOD in liver was modulated by amla feeding at 10% level. The hepatic GSH-Px activity was increased by amla whereas the co-administration with HCH did not alter the HCH-induced rise in the activity. At the same time, amla did not influence the GSSGR activity. The abnormally high levels of GGT in kidney were noted to mitigate the chemically induced stress effects, tending to normalize the catalase, G-6-PDH, SOD and GGT activities. These effects will, therefore lead to decreased peroxides and attenuation of the adverse effects of HCH-induced stress.

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