Portulaca oleracea L. extract ameliorates the cisplatin-induced toxicity in chick embryonic liver

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Cisplatin, a cytotoxic agent used in treating cancer, at high doses induces hepatotoxicity. In this study, we investigated the protective role of aqueous extract of aerial parts of Portulaca oleracea L. (Po) against cisplatin-induced hepatotoxicity in chick embryonic liver. A group of 12 day old chick embryos, acclimatized to laboratory conditions were treated with a single dose of cisplatin (100 µg), while another group received Po extract at different doses (1 and 3 mg) 6 h prior to cisplatin treatment. The biochemical parameters were estimated after 24 and 72 h of incubation. A dose-dependent increase in biochemical parameters, such as alanine transaminase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase, malondialdehyde levels and a decrease in antioxidant enzymes levels like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-s-transferase and reduced glutathione were observed in cisplatin-treated animals, indicating a definite damage to the liver tissue. Pre-treatment with Po extract was found to provide significant protection against cisplatin-induced hepatotoxicity, as evident by the recovered levels of the altered changes in the measured biochemical parameters.

Keywords: Cisplatin, Liver tissue, Chick embryo, Antioxidants, Lipid peroxidation, Portulaca oleracea L., Hepatotoxicity.

Chemotherapy using cytotoxic antineoplastic agents remains as an important strategy in overall management of patients with malignant tumors. Many antineoplastics display steep dose-response curves and low therapeutic indices coupled with toxicities which can be severe and life-threatening. Cisplatin or cisdiamminedichloroplatinum (II), (CDDP), an alkylating agent is one of the regular antineoplastic agents in ovarian, testicular, bladder, head and neck and uterine cervix carcinomas. However, its clinical use is limited by severe side-effects, such as nephrotoxicity, gastrointestinal toxicity, bone marrow toxicity, ototoxicity and peripheral neuropathy. The chick embryonic system permits controlled administration of substances and direct observation of the embryonic development. The chick embryo is capable of metabolic activation of xenobiotics as easy as the initial organogenetic period.

Portulaca oleracea L., a tropical plant of cosmopolitan distribution and commonly known as purslane is used as a vegetable and as herbal medicine against several diseases for many centuries. It has been used as antiseptic, anti-scorbutic, antispasmodic, diuretic, vermifuge, in oral ulcers and urinary disorders. The fleshy succulent leaves of Po contain high concentration of essential fatty acids. In addition, Po has shown other beneficial effects, such as antibacterial, analgesic, anti-inflammatory, antidiabetic, anti-hypoxic, antioxidant and wound healing properties.

In the present study, the toxic effects of cisplatin on the liver biochemical parameters and antioxidant status and the possible protective effect of aqueous extract of aerial parts of P. oleracea L. (Po) against cisplatin-induced toxicity have been investigated in the chick embryos.

Materials and Methods

Chemicals
Cisplatin was gifted by Prof. B Nagarajan, Head, Department of Tumor Biochemistry and Microbiology, Cancer Institute, Adyar, Chennai, India. Other chemicals used were of analytical grade and obtained from Qualigen and Merck, Mumbai, India.

Preparation of Portulaca oleracea L. extract
Aerial parts of P. oleracea L. collected in and around marshy places of Tirupati and Tirumala Hills. The plant was authenticated in the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Aerial parts were cleaned, dried under shade for 1 wk and made into fine powder and plant material (100 g) was kept in double-distilled
Maintenance of eggs and experimental design

Freshly laid Bobcock strain fertilized eggs were procured from Govt. Veterinary University, Tirupati and Balaji Hatcheries, Chittoor, Andhra Pradesh. The chicken eggs, in five groups of six each, cleaned with distilled water, followed by absolute alcohol were placed in an egg incubator maintained at 37°C with 65% relative humidity. The humidity of the incubator was maintained by keeping the tray full of water inside. The day, the eggs were set was designated as ED 0. The eggs were injected with single dose of cisplatin (100 µg) and different doses of P. oleracea explant (1 mg and 3 mg) into the air-sac of chick embryo.

Of the five groups of chick embryos, group I received physiological saline (served as control); group II, aqueous extract of Po at a dose of 3 mg/egg; group III, only cisplatin at a dose of 100 µg/egg; and group IV and group V received pre administration of aqueous extract of Po at a dose of 1 mg and 3 mg 6 h prior to the cisplatin 100 µg treatment. The hepatoprotective effect of Po extract was assessed after 24 and 72 h of incubation against cisplatin-induced toxicity.

Preparation of liver tissue homogenate

After the experimental period, 12th day old chick embryos were sacrificed by opening their air-sacs. The liver tissue was collected aseptically, stored in cold physiological saline at −20°C and used for further biochemical analyses.

The 10% homogenate of the liver tissue prepared by grinding it in 0.1 M Tris-HCl buffer (pH 7.4) was centrifuged at 3000 rpm in refrigerated centrifuge and the supernatant was used for biochemical analyses. The activity of marker enzymes and antioxidant enzymes, such as alkaline phosphatase (ALP), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST), and reduced glutathione (GSH) and lipid peroxidation (LPO) levels were estimated.

Statistical analysis

The results were analyzed by student’s t-test and differences between groups were considered significant, when the P value was less than 0.05.

Results and Discussion

Cisplatin-induced toxicity in liver

Table 1 and 2 represent the biochemical parameters and the levels of antioxidant enzymes in the hepatic tissue of control and experimental animals and the pre-treatment effects of Po extract after 24 and 72 h treatment with cisplatin. The cisplatin treatment significantly increased the enzyme activities of AST, ALT, ALP and LDH levels and the marked decline in the base line antioxidant enzymes, such as SOD, CAT, GPx, GR and GST levels (group III), suggesting the oxidative damage in cisplatin-induced toxicity.

Liver damage was measured using marker enzymes AST, ALT, ALP and LDH, the indicators of hepatic toxicity. The AST and ALT concentrations were high in the liver. ALP is a membrane bound enzyme located in bile canalicular pole of hepatocytes. LDH, a cytosolic enzyme involved in biochemical regulation reactions of the body tissues and fluids. During oxidative stress, all these enzymes were elevated, because cells tend to loose their internal milieu and also get damaged, thereby leaking out into the blood stream. Figure 1 depicts the levels of LPO in hepatic tissue extract of control and experimental animals. Group III chick embryos treated with cisplatin showed increased LPO levels compared to the control group.

Protective effect of Po extract against cisplatin-induced toxicity

Po is a potent antioxidant and is reported to contain omega-3-fatty acids. The decreased enzyme activities were possibly due to the presence of antioxidants in Po, which act against oxidative stress. The elevated activities of AST, ALT, ALP and LDH in liver tissue as observed in group III were brought back to near normal (p<0.01) with the pretreatment of Po extract (1 mg and 3 mg) 6 h prior to the cisplatin administration (groups IV and V) in a dose-dependent manner. Liver damage was prevented significantly by pretreatment with the extract, indicating the protective nature of Po extract (Table 1).

Many natural products are reported to influence the antioxidant systems and are good cytoprotective agents. SOD, CAT, GPx, GST, GR and GSH play
### Table 1—Pre-treatment of Po extract on hepatic enzyme activities at 12th day old chick embryonic liver

[Values are average of six sets of separate experiments (mean ± SE)]

<table>
<thead>
<tr>
<th>24 h after cisplatin treatment</th>
<th>Parameter</th>
<th>Group I (Control)</th>
<th>Group II (Po-3 mg treated)</th>
<th>Group III (Cisplatin -100 µg treated)</th>
<th>Group IV (Po-1 mg + Cisplatin -100 µg treated)</th>
<th>Group V (Po-3 mg + Cisplatin -100 µg treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST</td>
<td>12.00±0.06</td>
<td>11.62±0.13</td>
<td>41.85±0.12</td>
<td>30.90±0.10**</td>
<td>12.85±0.08**</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>7.17±0.10</td>
<td>7.28±0.08</td>
<td>25.50±0.16</td>
<td>18.00±0.16**</td>
<td>9.50±0.09**</td>
</tr>
<tr>
<td></td>
<td>ALP</td>
<td>5.70±0.05</td>
<td>5.68±0.12</td>
<td>38.60±0.17</td>
<td>25.77±0.17**</td>
<td>8.40±0.09**</td>
</tr>
<tr>
<td></td>
<td>LDH</td>
<td>9.35±0.09</td>
<td>10.23±0.11</td>
<td>25.70±0.18</td>
<td>18.45±0.15*</td>
<td>11.37±0.06**</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 h after cisplatin treatment</td>
<td>AST</td>
<td>18.76±0.16</td>
<td>16.75±0.11</td>
<td>77.00±0.33</td>
<td>56.58±0.17**</td>
<td>18.63±0.15**</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>15.33±0.30</td>
<td>14.52±0.14</td>
<td>68.33±0.30</td>
<td>51.80±0.17**</td>
<td>16.90±0.17**</td>
</tr>
<tr>
<td></td>
<td>ALP</td>
<td>12.70±0.14</td>
<td>11.40±0.13</td>
<td>71.65±0.18</td>
<td>56.05±0.19**</td>
<td>13.87±0.13**</td>
</tr>
<tr>
<td></td>
<td>LDH</td>
<td>15.58±0.17</td>
<td>14.63±0.08</td>
<td>41.46±0.19</td>
<td>33.80±0.14*</td>
<td>15.83±0.11**</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01

AST and ALT expressed as µ moles of pyruvate liberated/mg protein/min, ALP as phenol liberated/mg protein/min, LDH as µ moles of pyruvate formed/mg protein/min

### Table 2—Pre-treatment of Po extract on antioxidant enzyme levels at 12th day old chick embryonic liver

[Values are average of six sets of separate experiments (mean ± SE)]

<table>
<thead>
<tr>
<th>24 h after cisplatin treatment</th>
<th>Parameter</th>
<th>Group I (Control)</th>
<th>Group II (Po-3 mg treated)</th>
<th>Group III (Cisplatin -100 µg treated)</th>
<th>Group IV (Po-1 mg + Cisplatin -100 µg treated)</th>
<th>Group V (Po-3 mg + Cisplatin -100 µg treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GST</td>
<td>1.84±0.01</td>
<td>1.75±0.06</td>
<td>1.15±0.01</td>
<td>1.30±0.05*</td>
<td>1.60±0.06**</td>
</tr>
<tr>
<td></td>
<td>GPX</td>
<td>2.65±0.01</td>
<td>2.17±0.03</td>
<td>1.16±0.03</td>
<td>1.61±0.08*</td>
<td>2.57±0.09**</td>
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<tr>
<td></td>
<td>GR</td>
<td>9.14±0.01</td>
<td>8.81±0.15</td>
<td>4.24±0.01</td>
<td>5.02±0.15*</td>
<td>9.02±0.09**</td>
</tr>
<tr>
<td></td>
<td>SOD</td>
<td>5.81±0.10</td>
<td>5.30±0.05</td>
<td>3.87±0.01</td>
<td>4.36±0.07*</td>
<td>5.30±0.05**</td>
</tr>
<tr>
<td></td>
<td>CAT</td>
<td>11.78±0.19</td>
<td>11.08±0.08</td>
<td>4.30±0.14</td>
<td>5.40±0.10*</td>
<td>10.61±0.10**</td>
</tr>
<tr>
<td></td>
<td>GSH</td>
<td>129.35±0.46</td>
<td>130.76±0.29*</td>
<td>68.06±0.15</td>
<td>91.77±0.17**</td>
<td>125.67±0.13**</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 h after cisplatin treatment</td>
<td>GST</td>
<td>4.19±0.08</td>
<td>3.85±0.03</td>
<td>1.96±0.01</td>
<td>2.38±0.07*</td>
<td>4.03±0.04**</td>
</tr>
<tr>
<td></td>
<td>GPX</td>
<td>3.82±0.01</td>
<td>3.20±0.08</td>
<td>1.64±0.01</td>
<td>1.90±0.06*</td>
<td>2.98±0.05**</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td>11.75±0.01</td>
<td>11.05±0.11</td>
<td>6.56±0.10</td>
<td>8.13±0.18**</td>
<td>11.47±0.14**</td>
</tr>
<tr>
<td></td>
<td>SOD</td>
<td>8.76±0.09</td>
<td>8.30±0.06</td>
<td>6.18±0.02</td>
<td>6.65±0.05*</td>
<td>8.56±0.04**</td>
</tr>
<tr>
<td></td>
<td>CAT</td>
<td>14.02±0.27</td>
<td>13.40±0.09</td>
<td>6.07±0.15</td>
<td>7.31±0.06*</td>
<td>13.58±0.08**</td>
</tr>
<tr>
<td></td>
<td>GSH</td>
<td>179.66±0.22</td>
<td>185.80±0.13</td>
<td>88.23±0.19</td>
<td>115.63±0.15**</td>
<td>177.63±0.26**</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01

GST values are expressed as µ moles/min/mg protein; GPX as µ moles NADPH oxidized/min/mg protein; GR, µ moles NADPH consumed/mg protein; SOD, as units/mg protein/min; CAT as units/mg protein/min; GSH as µg of GSH/mg protein.
an important role in biological systems to act against oxidative stress. Oxidative stress generated by cisplatin reduced the SOD and CAT levels in liver tissue. However, pretreatment with Po extract normalized these levels (Table 2), possibly by preventing lipid peroxide, superoxide and hydroxyl radicals’ formation. Capsaicin, a pungent ingredient of red pepper is reported to increase the SOD level, and the dried black grape extract supplementation (25 g/kg/day) enhances the CAT level against cisplatin treatment in rats.

GPx plays a pivotal role in \( \text{H}_2\text{O}_2 \) catabolism and catalyzes the reduction of \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \) and \( \text{O}_2 \) at the expense of GSH. GST is a group of multi-functional proteins encoded by a multi-gene family that perform functions ranging from catalyzing the detoxification of electrophilic compounds to protect against peroxidative damage. GR plays a main role in regenerating endogenous GSH from GSSG, thus maintaining the balance between redox couple. It requires the reducing equivalent (NADPH) for its activity which is provided by the action of glucose-6-phosphatase dehydrogenase (G6PD). In the present study, decreased levels of GPx, GST and GR on cisplatin treatment (group III) were reversed to normal (groups IV and V), indicating the protective nature of the Po extract (Table 2).

Glutathione (GSH), a multi-functional intracellular non-enzymatic antioxidant is considered to be the main thiol-disulphide redox buffer of the cell. The antioxidant property of Po could be attributed to the endogenous GSH antioxidant balance against cisplatin-mediated cellular oxidation, which protects the GSH related enzymes, and in turn inhibits the oxidation of GSH. Depleted levels of GSH by cisplatin in rat cortical slice leads to lipid peroxidation. The present study revealed that the decreased GSH level in liver tissue after cisplatin treatment was reversed to almost normal, when pretreated with Po extract. Similarly, pre-administration of Po extract reversed the LPO levels to near normal in groups IV and V animals after 24 and 72 h treatment in a dose-dependent manner, indicating the protective effect of Po in quenching free radicals (Fig. 1). Po extract is found to be rich in vitamin C, E and carotenoids. Vitamins C and E are good antioxidants capable of preventing lipid peroxidation in both plasma and tissues. In conclusion, the study demonstrated the protective nature of the aqueous extract of Po against cisplatin-induced hepatotoxicity.

Acknowledgement

The authors thank Prof. B Nagarajan, Head, Department of Tumor Biochemistry and Microbiology, Cancer Institute, Adyar, Chennai, India for gifting cisplatin.

References
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Fig. 1—Effect of pre-treatment of Po extract on lipid peroxidation levels in 12th day old chick embryonic liver after 24 and 72 h cisplatin treatment. [Values are average of six sets of separate experiments (mean ± SE)]
NOTES

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