Renoprotective effect of grape seeds extract in ethylene glycol induced nephrotoxic mice

M Mohanasundari, M Sabesan & S Sethupathy*
Department of Zoology, and *Division of Biochemistry, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar 608 002, India

Received 27 April 2004; revised 10 December 2004

Grape seed extract treatment in ethylene glycol (EG) induced nephrotoxic mice improved antioxidant status and significantly decreased urinary lactate dehydrogenase (LDH) and lipid peroxidation. The extract rendered antioxidant protection against oxidative stress induced by EG and may help in protecting renal tissue against EG toxicity.

Keywords: Ethylene glycol, Grape seeds, Nephrotoxic, Renoprotection, Mouse

Being a cheap substitute for alcohol, ethylene glycol (EG) is commonly used as an anti-freeze in cooling systems of automobiles, aircrafts and has wide industrial applications. Many accidental deaths due to its poisoning have been reported\(^1\)\(^3\). Toxicity from ethylene glycol is produced from the metabolites such as glycoaldehyde, glyoxylate, and oxalate, producing widespread tissue injury to the kidney. Patients die of acute renal failure due to EG toxicity\(^4\). The importance of traditional system of medicine has gained recognition all over the world and several indigenous drugs form an indispensable part of the health care\(^5\)\(^6\)\(^7\). Grape seed extract, primarily (95%) a mixture of proanthocyanidins\(^7\), has been shown to modulate a wide range of biological, pharmacological and toxicological effects, which are mainly cytoprotective\(^8\) and grape seed proanthocyanidin extract has been shown to provide multiorgan protection against drug and chemical induced assaults by its free radical scavenging ability\(^9\). Oxalates formed from EG as calcium oxalate crystals in the kidney are injurious\(^10\) and may cause the kidney to remain under excessive oxidative stress\(^11\). Therefore, the effect of grape seed extract has been explored, in ethylene glycol induced nephrotoxic male albino mice.

Materials and Methods

**Chemicals**—Thiobarbituric acid (TBA), reduced glutathione and 3,5-dithio-bis nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co USA. All other reagents were of analytical grade and obtained locally.

**Animals**—Male Albino mice (20-25 g, 10 weeks old) from the Department of Experimental Medicine, Central Animal House, Rajah Muthiah Medical College, were housed in a temperature-controlled room (27° ± 2°C) with a 2:1 hr light and dark cycle. Institute's animal ethical committee clearance was obtained before the experiments.

**Experimental design**—Mice (32) were divided into 4 groups of 8 each. The first group was control. Animals of the second group were given the grape seed extract (100 mg/kg weight; po) daily for 7 days. Third and fourth group animals were treated with 20% (v/v) EG (2 ml/kg weight; po) daily for 7 days. In the fourth group grape seed extract (100 mg/kg weight) was given after 2 hr of EG administration. The animals were killed by cervical decapitation 24 hr after the last dose.

**Preparation of extract**—Vitis vinifera (Grape) seeds were procured from local market (Chidambaram) and dried in shade. They were powdered and extracted with methanol. Extract was evaporated under low pressure by using Buchi type rotary evaporator. The concentrated extract was kept in vacuum desiccator till the constant weight of solvent free extract was attained. The extract (100 mg/kg weight) was mixed with Tween 80 and diluted with distilled water to get the final ratio of water:Tween 80 as 9:1\(^12\).

Serum urea and creatinine were analysed by auto analyzer (Smart Lab, USA) using Boehringer Mannheim kits.
The enzyme lactate dehydrogenase (LDH) catalyses the conversion of pyruvate to lactate with the oxidation of NADH. The rate of decrease in absorbance at 340 nm is proportional to LDH activity. Activities of LDH\(^1\), superoxide dismutase (SOD)\(^2\), catalase (CAT)\(^3\), glutathione peroxidase (GP)\(^4\) and reduced glutathione (GSH)\(^5\) were assayed in renal tissue.

**Lipid peroxidation**—Thiobarbituric acid reactive substances (TBARS) in renal tissue were estimated as per Ohkawa et al.\(^6\).

**Histopathological study**—Formalin fixed renal tissue was prepared for histology studies using hematoxyline eosin.

**Statistical analysis**—The data were analysed using ANOVA. All values are reported as mean±SD and statistical significance was set at 0.05.

### Results and Discussion

The results are presented in Tables 1 and 2 and Fig.1. TBARS, an indirect measure of lipid peroxidation, were significantly increased in EG treated mice which is suggestive of oxidative stress due to EG. Hyperoxaluria induced renal epithelial cell injury is associated with lipid peroxidation, which is suggestive of free radical involvement\(^7\). The damage starts with hyperoxaluria and intensifies with crystal deposition of renal tubules\(^8\). Ethylene glycol is metabolized via glycolic acid, glyoxylic acid and finally oxalate and excreted in the urine as calcium oxalate\(^9\). Calcium oxalate is nephrotoxic and its toxicity could be attributed to enhanced free radical generation as it is evident from elevated TBARS in renal tissue\(^10\). Antioxidant enzymes and GSH were significantly decreased in EG treated mice, which is suggestive of free radical involvement in the causation of tissue injury\(^11\). Urinary excretion of LDH has been used as a marker of renal cellular injury\(^12\). Urinary LDH was significantly elevated in Group III suggesting renal tubular injury, which is also evident from dilated tubules with thinner epithelium (Fig. 1c), and the increase of serum urea and creatinine. Hyperoxaluria is injurious to renal tubular cells as it induces apoptic changes\(^13\). Group IV showed significant reduction of urinary LDH, blood urea, creatinine and dilated tubules lined by normal intact epithelium indicating recovery (Fig. 1d). This suggests that the grape seed extract has renal protective effect and its effect could be most probably due to improvement in the antioxidant status, as it is evident from decrease of TBARS and the restoration of antioxidant enzyme activities. Therefore, it can be concluded that grape seed extract by its antioxidant

### Table 1—Effect of grape seed extract on body weight, serum urea, creatinine, urinary LDH activity in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight changes (g)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>LDH (units/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>+0.67 ± 0.14</td>
<td>37.5 ± 3.22</td>
<td>0.84 ± 0.06</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>II</td>
<td>+1.05 ± 0.21*</td>
<td>38.25 ± 3.64</td>
<td>0.81 ± 0.09*</td>
<td>0.31 ± 0.06*</td>
</tr>
<tr>
<td>III</td>
<td>-1.84 ± 0.51*</td>
<td>49.3 ± 4.7**</td>
<td>1.42 ± 0.24**</td>
<td>0.96 ± 0.13**</td>
</tr>
<tr>
<td>IV</td>
<td>-1.15 ± 0.24</td>
<td>40.4 ± 3.9**</td>
<td>0.94 ± 0.14**</td>
<td>0.42 ± 0.12**</td>
</tr>
</tbody>
</table>

*Group II vs Group I; **Group III vs Group I; *Group IV vs Group III; NS Non significant

### Table 2—Effect of grape seed extract on renal antioxidant status & lipid peroxidation in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase (µ moles of H(_2)O(_2) utilized/min/mg of protein)</th>
<th>SOD (units/mg of protein)</th>
<th>GPX (µ moles of GSH consumed/min/mg of protein)</th>
<th>GSH (µ moles/mg of protein)</th>
<th>TBARS (n moles/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>49.125 ± 4.086</td>
<td>5.58 ± 0.80</td>
<td>0.87 ± 0.16</td>
<td>1.38 ± 0.36</td>
<td>1.32 ± 0.30</td>
</tr>
<tr>
<td>II</td>
<td>55.125 ± 5.489</td>
<td>6.41 ± 0.42</td>
<td>1.04 ± 0.15</td>
<td>1.58 ± 0.35</td>
<td>1.18 ± 0.50</td>
</tr>
<tr>
<td>III</td>
<td>39 ± 6.655</td>
<td>4.3 ± 0.97</td>
<td>0.64 ± 0.19</td>
<td>0.88 ± 0.26</td>
<td>1.93 ± 0.388</td>
</tr>
<tr>
<td>IV</td>
<td>47.375 ± 3.852</td>
<td>5.63 ± 0.038</td>
<td>0.84 ± 0.27</td>
<td>1.54 ± 0.42</td>
<td>1.15 ± 0.418</td>
</tr>
</tbody>
</table>

*Group II vs Group I; **Group III vs Group I; *Group IV vs Group III; NS Non significant

**P values:** *<0.05; **<0.02; *<0.01
activity may have prevented renal tubular damage due to EG toxicity.

References


