Amelioration of alcohol-induced oxidative stress by *Emblica officinalis* (Amla) in rats

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The present study was aimed at investigating the ameliorative effect of *Emblica* (Phyllanthus Emblica. L) fruit extract (EFE) against alcohol-induced oxidative changes in plasma biochemical profile in rats. Alcohol administration (5 g/kg body wt/day) for 60 days resulted in significantly \((P<0.05)\) higher levels of plasma nitrite/nitrate (NOx), total bilirubin, creatinine, and abnormalities in lipid and lipoproteins. Moreover, alcohol receiving rats showed significantly \((P<0.05)\) lowered plasma total protein, albumin/globulin (A/G) ratio and uric acid, with no significant change in glucose level. The EFE administration (250 mg/kg body wt/day) to alcohol-administered rats significantly modulated plasma lipids and lipoprotein patterns and also decreased nitrite/nitrate, total bilirubin and creatinine levels. EFE administration to alcohol receiving rats showed a significant \((P<0.05)\) increase in plasma total protein, A/G ratio and uric acid levels. Total cholesterol \((r = 0.466)\), triglycerides \((r = 0.574)\), VLDL-C \((r = 0.578)\), LDL-C \((r = 0.225)\) and total bilirubin \((r = 0.419)\) showed a stronger positive correlation with that of NOx in alcohol-treated rats. The concentration of nitric oxide (NOx) was negatively correlated with HDL-C \((r = -0.285)\) and uric acid \((r = 0.392)\) in alcohol-treated rats. The amelioration of alcohol-induced oxidative stress might be due to the combined effect of phytophenols, such as tannins and flavonoid compounds and vitamin C.

**Keywords:** Alcohol, *Emblica officinalis*, Lipid profile, Nitric oxide, Oxidative stress

Alcohol is known to cause disturbances in the metabolism of carbohydrates, lipids and proteins. Excessive alcohol consumption is associated with tissue and organ damage, leading to coronary heart disease, alcohol liver disease and several other manifestations including neurological disorders, for which therapeutic approaches are sought\(^1,2\). Enhanced oxidative stress and decreased antioxidant status induced by ethanol metabolism play a major role in the causation of alcohol toxicity and damage\(^3\). Research on alcoholism in the past has revealed much about the biochemical mechanisms, metabolic pathways, and effects on health and alcoholic damage as well as toxicity of body organs\(^4\). The etiology of many diseases is attributed to alcohol. Now, in view of increased prevalence of alcoholism, the interest of researchers has been shifted from toxicity to treatment\(^5\).

In biological systems, free radicals are generated in the form of reactive oxygen species (ROS), such as superoxide anion and hydroxyl radicals, hydrogen peroxide, singlet oxygen, nitric oxide, and peroxynitrite. These ROS cause destructive and irreversible damage to cellular components, such as lipids, proteins and DNA\(^6\). Recently, various phytochemicals and their related effects on health, especially suppression of active oxygen species by natural antioxidants from tea, spices and herbs have been intensively studied\(^7\). The unabated alcoholism has made researchers for the past two decades to develop remedies for alcoholism. However, developing low toxicity and high efficiency medicines has remained a challenging task\(^8\).

*Emblica officinalis* Gaertn (Phyllanthus Emblica. L.), commonly known as *amla* is a medium to large deciduous tree, belonging to Euphorbiaceae, widely growing in different parts of India, Sri lanka, Pakistan, Uzbekistan, South East Asia and China. *E. officinalis* is known for its antioxidant properties and for its therapeutic effects, and is a principle component in more than hundred herbal formulations that are widely used in India and other countries\(^9\). Studies have also demonstrated the potent antibacterial\(^10\), hepatoprotective\(^11\), anti-diabetic\(^12,13\), anti-mitochondrial dysfunction\(^14\) and antioxidant...
properties of *Embilca* fruit\textsuperscript{15,16}. The wide use of *E. officinalis* in India for various purposes has prompted us to select the same to treat alcohol toxicity which has not been done, so far. Thus, the present study has been designed to evaluate the preventive effect of *E. officinalis* fruit extract (EFE) on the plasma glucose, total protein, lipid peroxidation, protein sulfhydryl groups, carboxyls, lipid profile, nitrite/nitrate levels, uric acid in alcohol-treated rats.

**Materials and Methods**

**Chemicals**

The chemicals were procured from Sigma Chemical Co. (St. Louis, MO, USA) and SISCO Research Laboratories, Mumbai, India. Ethanol used for administration to rats was obtained by re-distillation. An aqueous *Embilca officinalis* fruit extract dry powder (90.8% water soluble extractives including 49.5% tannins) was obtained from Chemiloids Ltd., Vijayawada, India (Manufacturer and exporter of herbal extracts). All kits were purchased from Span Diagnosis Ltd, Surat, India.

**Animals and experimental design**

Two-months old male albino wistar rats, weighing about 120-140 g were procured from Sri Venkateswara Enterprises (Bangalore, India) and maintained under laboratory conditions of institutional animal house. They were fed with commercial balanced pellet diet (Hindustan Lever Ltd, Bombay, India) and tap water *ad libitum*. The animals were divided into four groups of eight rats in each group: Group I or control rats (C) received glucose instead of alcohol (i.e., caloric equivalent to alcohol); Group II alcohol-treated rats (A) received 20% (v/v) alcohol in water administered through stomach tube (5 g/kg body wt/day); Group III rats (A + EFE) received aqueous *Embilca* fruit extract (250 mg/kg body wt) and then 20% (v/v) alcohol in water (5 g/kg body wt/day) after 8 h; and Group IV rats received aqueous *Embilca* fruit extract (250 mg/kg body wt). The dose was fixed based on our previous study\textsuperscript{14}.

The aqueous *Embilca* fruit extract 250 mg dry powder/kg body wt was administered daily in water to each rat. All the groups received the treatment for 60 days. Food and water intake of all the animals was recorded daily and weight of rats was recorded on alternate days. The study was undertaken with prior approval by our Institution Ethics Committee. At the end of the experimental period, the rats in each group were fasted overnight and then sacrificed by cervical dislocation. Blood was collected by cardiac puncture into heparinized tubes and centrifuged (2000 g for 10 min) to separate plasma.

**Plasma biochemical analysis**

The plasma glucose\textsuperscript{17}, total protein, A:G ratio\textsuperscript{18}, total cholesterol\textsuperscript{19}, triglycerides\textsuperscript{20}, HDL-cholesterol\textsuperscript{21}, LDL-cholesterol, VLDL-cholesterol\textsuperscript{22}, atherogenic index\textsuperscript{23}, total bilirubin\textsuperscript{24}, creatinine\textsuperscript{25}, uric acid\textsuperscript{26} levels were determined using commercial kits. Lipid peroxides were measured by the formation of malondialdehyde (MDA) as described previously\textsuperscript{27}. The concentration of protein carbonyls was determined using 2,4-dinitrophenylhydrazine (DNPH) assay method\textsuperscript{28}. Total sulfhydryl groups were determined using Ellman’s reagent\textsuperscript{29}. Nitrite/nitrate levels were determined as described previously\textsuperscript{30}.

**Statistical analysis**

Mean and standard deviation (S.D) values of all the parameters were determined for each group. The data were subjected to ANOVA, followed by Duncan’s multiple range test\textsuperscript{31}. Level of significance was *P*<0.05. Correlations between variables were assessed with Pearson’s correlation coefficients (*r*).

**Results**

Table 1 depicts the levels of plasma nitric oxide (nitrite/nitrate), glucose, total proteins, uric acid, total bilirubin, creatinine and A/G ratio in control and different experimental groups. In alcohol-treated rats, the levels of total bilirubin, creatinine and nitric oxide were increased significantly (*P*<0.05) and those of plasma uric acid, total protein and A/G ratio were decreased significantly (*P*<0.05), when compared with control rats. Oral administration of EFE to alcohol administered rats significantly decreased (*P*<0.05) the levels of total bilirubin, creatinine and nitric oxide and increased the levels of plasma uric acid, total protein and A/G ratio when compared with alcohol-alone administered rats. No significant change in the glucose level was observed in alcohol-treated rats, when compared to controls.

The plasma lipid profile of control and different experimental rat groups is presented in Table 2. In alcohol-treated group, the plasma total cholesterol, triglycerides, LDL-C and VLDL-C as well atherogenic index were significantly (*P*<0.05) increased by 29, 28, 132, 28 and 182%, respectively.
Table 1—Effect of EFE on plasma biochemical profile in alcohol-treated rats  
[Values are mean ± SD of 8 rats in each group]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Alcohol-treated</th>
<th>+ EFE-treated</th>
<th>EFE-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOx (µmol/L)</td>
<td>27.29 ± 1.93c</td>
<td>43.44 ± 3.37a</td>
<td>31.38 ± 2.28b</td>
<td>27.03 ± 2.24c</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.92 ± 0.29a</td>
<td>5.77 ± 0.41c</td>
<td>6.38 ± 0.34b</td>
<td>6.98 ± 0.43a</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.48 ± 0.07a</td>
<td>1.13 ± 0.06c</td>
<td>1.37 ± 0.11b</td>
<td>1.51 ± 0.083a</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>80.40 ± 3.06a</td>
<td>84.05 ± 4.20a</td>
<td>79.37 ± 5.25a</td>
<td>78.53 ± 3.36a</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.61± 0.03a</td>
<td>1.02 ± 0.06b</td>
<td>0.71 ± 0.04a</td>
<td>0.64 ± 0.07a</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.48 ± 0.02a</td>
<td>0.81 ± 0.06b</td>
<td>0.56 ± 0.05a</td>
<td>0.46 ± 0.05a</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.46 ± 0.14a</td>
<td>1.72 ± 0.15c</td>
<td>2.43 ± 0.16b</td>
<td>2.51 ± 0.11a</td>
</tr>
</tbody>
</table>

Means in the same row not sharing a common superscript are significantly different (P<0.05) among groups.

Table 2—Effect of EFE administration on plasma lipid profile in alcohol treated rats  
[Values are mean ± SD of 8 rats in each group]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Alcohol-treated</th>
<th>+ EFE-treated</th>
<th>EFE-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>76.80 ± 5.03b</td>
<td>99.42 ± 5.27a</td>
<td>80.90 ± 5.86b</td>
<td>74.03 ± 5.61b</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>75.61 ± 5.12b</td>
<td>97.10 ± 3.11a</td>
<td>77.10 ± 8.14b</td>
<td>70.83 ± 7.90b</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>38.60 ± 3.48a</td>
<td>26.41 ± 3.71c</td>
<td>34.15 ± 3.12b</td>
<td>32.90 ± 1.62b</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>21.14 ± 1.97c</td>
<td>51.20 ± 1.43a</td>
<td>31.11 ± 1.67b</td>
<td>23.97 ± 1.42c</td>
</tr>
<tr>
<td>VLDL-Cholesterol</td>
<td>15.12 ± 1.22b</td>
<td>19.42 ± 1.62a</td>
<td>15.42 ± 1.42b</td>
<td>14.16 ± 1.60b</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>0.98 ± 0.02c</td>
<td>2.76 ± 0.05b</td>
<td>1.36 ± 0.01b</td>
<td>1.15 ± 0.01c</td>
</tr>
</tbody>
</table>

All the values are expressed as mg/dl. Means in the same row not sharing a common superscript are significantly different (P < 0.05) among groups.

Fig. 1—Effect of EFE administration on plasma lipid peroxidation (A), protein carbonyls (B), protein sulfhydryl (C) in control and experimental groups. A comparison of the changes in levels of plasma lipids and lipoproteins with NOx in controls and experimental groups (D) [Values are mean ± SD of eight rats in each group. The means sharing a common letter are not significantly different (P<0.05) among groups]
with decreased HDL-C (31%) compared to controls. Treatment with EFE to alcohol-treated rats showed significant ($P<0.05$) decrease in plasma cholesterol, triglycerides, LDL-C, VLDL-C, atherogenic index (21, 26, 20, 8.2 and 49%) and a significant ($P<0.05$) rise in HDL-C (42%), compared to alcohol-treated rats.

Fig. 1A shows the extent of lipid peroxidation in plasma of control and experimental animals. The concentration of thiobarbituric acid reactive substances (TBARS) was significantly ($P<0.05$) higher in alcohol-administered rats than the control rats. Treatment with EFE together with alcohol reduced the level of TBARS significantly ($P<0.05$), as compared with those of the alcohol-alone administered rats. Increased carbonyls with decreased sulfhydryl group content were observed in plasma of alcoholic rats than in experimental control rats (Figs 1B and C). Administration of EFE along with alcohol lowered the concentration of carbonyls with significantly increased sulfhydryl content ($P<0.05$), as compared with alcohol-alone administered rats.

Fig. 1D shows the relationship between levels of plasma nitric oxide (nitrite/nitrate) and cardiovascular risk factors measured in the present study. Table 3 reveals correlation analysis between nitric oxide and biochemical parameters. Total cholesterol ($r = 0.466; P<0.001$), triglycerides ($r = 0.574; P<0.001$), LDL-cholesterol ($r = 0.225; P<0.014$), VLDL-cholesterol ($r = 0.578; P<0.001$) and total bilirubin ($r = 0.419; P<0.001$) showed positive correlation with that of NOx in alcohol-treated rats. The concentration of NOx was negatively correlated with HDL-cholesterol ($r = -0.285; P<0.018$) and uric acid ($r = -0.392; P<0.001$) in alcohol-treated rats.

### Discussion

Ethanol promotes oxidative stress, both by increased formation of ROS and by depletion of antioxidant status\(^\text{32}\). In the present study, plasma carbonyl groups and protein sulfhydryl groups levels were used as markers of oxidative modification of proteins in alcohol-treated rats. Observed increase in plasma lipid peroxidation, carbonyl groups and decrease in sulfhydryl groups showed the increased susceptibility of lipids and proteins to oxidation in alcohol-treated rats, which could be due to enhanced oxidative stress\(^\text{33}\). Evidences indicate that remedies for alcoholism, involving phytochemicals and the use of natural extracts from plant foods are very promising in reducing the risk of oxidative stress\(^\text{8,34}\). In the present study, administration of EFE to alcohol-treated rats significantly decreased plasma lipid peroxidation, protein carbonyls and increased sulfhydryl groups. This might be due to the free radical and hydroxyl radical scavenging activity of tannoid compounds present in EFE\(^\text{9,35}\).

In the present study, higher NOx level in plasma of alcoholic rats might be due to the up-regulation of NOS and/or its iso-forms contributing to the increased production of NOx, and was in agreement with earlier studies\(^\text{36,37}\). The characteristic changes observed in the concentrations of plasma lipids (cholesterol and triglycerides) and lipoproteins (LDL, VLDL and HDL) in alcohol-treated rats compared to other experimental groups suggested cardiac risk. A modest increase in plasma NOx of alcohol-treated rats receiving EFE was observed in the study. It is well-known that modest increase in NOx production exerts beneficial effect and overproduction causes detrimental effects by modulating signal transduction in animals and humans\(^\text{38}\). NOx behaves as antioxidant and prooxidants, depending on oxidative stress\(^\text{39}\). Increased NOx was positively correlated with plasma total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol in the present study. Many studies have confirmed nitrosation of proteins and lipids affecting cellular signaling events as well metabolic functions\(^\text{40,41}\).

Observed moderate levels of plasma nitrite and nitrate in alcohol-treated rats receiving EFE might be mainly due to inhibition of the hepatic cytosolic iNOS enzyme activity by EFE extract\(^\text{42}\). NOx-mediated regulation of increasing triglyceride lipases and production or secretion of apolipoprotein particles in liver, and decreasing the removal of circulating HDL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alcohol</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>$r = 0.466$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>$r = 0.574$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>HDL-C</td>
<td>$r = -0.285$</td>
<td>$&lt;0.018$</td>
</tr>
<tr>
<td>LDL-C</td>
<td>$r = 0.225$</td>
<td>$&lt;0.014$</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>$r = 0.578$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Glucose</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>$r = 0.419$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Creatinine</td>
<td>NS</td>
<td>—</td>
</tr>
<tr>
<td>Uric acid</td>
<td>$r = -0.392$</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>
might have played a role in the observed effect\textsuperscript{43,44}. Amelioration of the cardiac risk by EFE is due to the involvement of various therapeutic compounds present in EFE, which has NOx scavenging effects\textsuperscript{45}. Besides, EFE has hypcholesteremic activity by regulating/inhibiting the activity of the cholesterol synthesis rate-limiting enzyme HMG-CoA reductase as reported by earlier workers\textsuperscript{46,47}. It is also relevant to note that principles of Emblica prevent LDL-oxidation, thereby contributing to anti-atherogenic effect\textsuperscript{48}.

Disturbance in glucose homeostasis may lead to abnormal functioning of cells and their maintenance. Blood glucose level is maintained stable by interplay of various hormones, enzymes, tissues and other factors. Results of the present study showed no change in glucose homeostasis and confirmed earlier observations\textsuperscript{49,50}. A decrease in the levels of plasma total proteins, A/G ratio with significant increase in total bilirubin observed in alcohol-treated rats could be due to increased free radical production by alcohol. The NOX was positively correlated with total bilirubin and negatively correlated with uric acid. Treatment with EFE significantly increased the levels of plasma total proteins, A/G ratio, and uric acid with decreased total bilirubin and creatinine content. The decrease in plasma uric acid in alcohol-treated rats might be due to its continuous utilization by the system during free radical quenching reaction. It has been reported that uric acid serves as antioxidant in vivo, scavenging singlet oxygen, peroxyl and hydroxyl radicals\textsuperscript{51}. However, it is degraded on continuous exposure to peroxyl and hydroxyl radicals. In addition, increased plasma creatinine and decreased uric acid levels were observed in alcohol-treated rats, which showed the kidney dysfunction.

In conclusion, the alterations observed in the above parameters in alcohol-treated rats were normalized to a greater extent in EFE-treated animals, thus demonstrating the beneficial effects of EFE on hepatic, as well as renal function. The amelioration of alcohol-induced oxidative stress by EFE might be due to the combined effect of polyphenols such as flavonoids, tannins and other compounds such as vitamin C.

References

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