Antioxidant activity of active tannoid principles of *Emblica officinalis* (amla)

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The antioxidant activity of tannoid active principles of *E. officinalis* consisting of emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%), was investigated on the basis of their effects on rat brain frontal cortical and striatal concentrations of the oxidative free radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), and lipid peroxidation, in terms of thiobarbituric acid-reactive products. The results were compared with effects induced by deprenyl, a selective monoamine oxidase (MAO) B inhibitor with well documented antioxidant activity. The active tannoids of *E. officinalis* (EOT), administered in the doses of 5 and 10 mg/kg, i.p., and deprenyl (2 mg/kg, i.p.), induced an increase in both frontal cortical and striatal SOD, CAT and GPX activity, with concomitant decrease in lipid peroxidation in these brain areas when administered once daily for 7 days. Acute single administration of EOT and deprenyl had insignificant effects. The results also indicate that the antioxidant activity of *E. officinalis* may reside in the tannoids of the fruits of the plant, which have vitamin C-like properties, rather than vitamin C itself.

*Emblica officinalis* Gaertn., known as *amla*, a member of a small genus *Emblica* (family Euphorbiaceae), is extensively found all over India, as well as Srilanka, Malaya, China, Pakistan and Bangladesh. The fruits of the plant have been used in Ayurveda as a potent *rasayana*. The *rasayanas* are used to promote health and longevity by increasing defence against disease, arresting the aging process and revitalizing the body in debilitated conditions. The clinical efficacy of the fruits of *E. officinalis* are held in high esteem in Ayurveda and *amla* is referred to as a *maharasyana*. The fruits form the major constituent of *Chyavanprash avelaha*, a polyherbal Ayurvedic *rasayana* preparation described in Charaka Samhita. This preparation is widely used in this country for its preventive, curative and health restorative properties. Experimental studies conducted with extracts of the fruits of *E. officinalis* indicate that they have significant cytoprotective effect against isoprenaline-induced myocardial injury, radiation induced chromosomal damage and heavy metal induced hepatotoxicity and nephrotoxicity. Clinical studies suggest that the fruits have anabolic activity. Experimental investigations on *Chyavanprash* have shown that it exhibits significant adaptogenic, immuno-potentiating and memory-facilitating effects.

During the second and third quarters of this century, several papers were published attributing the biological and therapeutic effects of *amla* fruits to their rich vitamin C (L-ascorbic acid) content, ranging from 0.1 to 0.7% in fresh pericarp. However, little was known about the chemistry and biological activity of its major constituents, the hydrolysable tannins (10 to 12% in pericarp), except that they contained gallic and ellagic acids, inhibited the degradation of vitamin C and had some pharmacological activity entirely unrelated to the clinical use of the fruits. A recent study, on fresh juice and solvent extractives of *E. officinalis* fruits, indicated the complete absence of vitamin C, dispelling the popular and long-existing belief that the clinical effects of *amla* and *Chyavanprash* were due to the rich vitamin C content of the fruits. The investigations indicated that the potent vitamin C-like activity of the fruits was due to low Mw (Mol. wt. <1000) hydrolysable tannins. Four such compounds, emblicanin A, emblicanin B, punigluconin and pedunculagin, were isolated from the fresh pericarp and their chemical structures were established by spectroscopic analyses and chemical transformations. Emblicanin A and B have been shown to exhibit

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significant antioxidant effect in vitro. The present study was designed to investigate the in vivo antioxidant activity of the active principles of the fruits of *E. officinalis* in terms of their effects on superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and lipid peroxidase activities in rat brain frontal cortical and striatal areas. Deprenyl, a standard antioxidant, was used for comparison.

**Materials and Methods**

The study was conducted on adult male CF strain rats (160-190 g), obtained from the Institute of Medical Sciences Central Animal House. They were housed in colony cages (3-4 rats per cage), at an ambient temperature of 25±2°C and 45-55% RH, with a 12 hr light/12 hr dark cycle. The rats had free access to standard pellet chow and drinking water. Experiments were conducted between 0900 and 1400 hrs.

The test compound, emblicanin A and B-enriched fraction was prepared from fresh juice of *E. officinalis* fruits, by deactivating the contained hydrolytic enzymes followed by column chromatography over Sephadex LH-20, using methanol and methanol-water as eluent.

The extract of *E. officinalis* hydrolysable tannins (EOT), was dissolved in 0.9% saline, and administered i.p. (volume 1 ml/kg) in doses of 5 and 10 mg/kg, once daily for 7 days. Deprenyl (Torrent Laboratories, 2 mg/kg) was similarly administered. Control animals received equivalent volume of the vehicle (0.9% saline) through the same route and for the same period. The rats were sacrificed by decapitation, either 1 hr after drug or vehicle administration on day 1, or 1 hr after the last drug administration on day 7. The brains were removed and the frontal cortex and striatum dissected. The tissues were weighed and homogenized in 2 ml of iced-cold triple distilled water and sonicated for 15 sec. The homogenates were then centrifuged (10,000×g, 2 min) and the supernatants used for biochemical estimations. However, for estimation of lipid peroxidation the homogenates were incubated at 37°C for 1 hr with arachidonic acid.

The concentrations of emblicanin A (37%), emblicanin B (33%), punigluconin (12%), pedunculagin (14%), rutin (3%) and gallic acid (1%) in the extract were established by HPTLC, using authentic markers. Details of extraction and structure elucidation of the emblicanins and other compounds from *E. officinalis* fruits, have been published elsewhere.

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### Table 1 — Effects of *Emblica officinalis* tannins (EOT) and deprenyl on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activity in rat brain frontal cortex and striatum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug Dose</th>
<th>Frontal Cortex</th>
<th>Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
<td>Day 7*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SOD activity</td>
<td>CAT activity</td>
</tr>
<tr>
<td>Vehicle</td>
<td>16</td>
<td>12.6 ± 0.9</td>
<td>15.7 ± 1.9</td>
</tr>
<tr>
<td>EOT (5)</td>
<td>6</td>
<td>15.9 ± 1.9</td>
<td>18.4 ± 1.2</td>
</tr>
<tr>
<td>EOT (10)</td>
<td>6</td>
<td>14.9 ± 1.4</td>
<td>18.9 ± 1.8</td>
</tr>
<tr>
<td>Deprenyl</td>
<td>6</td>
<td>16.8 ± 1.5*</td>
<td>19.4 ± 1.9</td>
</tr>
<tr>
<td>Vehicle</td>
<td>16</td>
<td>15.0 ± 1.4</td>
<td>15.7 ± 1.9</td>
</tr>
<tr>
<td>EOT (5)</td>
<td>6</td>
<td>14.6 ± 1.2</td>
<td>17.6 ± 1.9</td>
</tr>
<tr>
<td>EOT (10)</td>
<td>6</td>
<td>18.2 ± 0.9</td>
<td>19.0 ± 1.4*</td>
</tr>
<tr>
<td>Deprenyl</td>
<td>6</td>
<td>19.8 ± 1.6</td>
<td>22.6 ± 2.0b</td>
</tr>
<tr>
<td>Vehicle</td>
<td>16</td>
<td>0.064 ± 0.01</td>
<td>0.082 ± 0.008</td>
</tr>
<tr>
<td>EOT (5)</td>
<td>5</td>
<td>0.071 ± 0.008</td>
<td>0.093 ± 0.006</td>
</tr>
<tr>
<td>EOT (10)</td>
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<td>0.076 ± 0.008</td>
<td>0.078 ± 0.009</td>
</tr>
<tr>
<td>Deprenyl</td>
<td>6</td>
<td>0.068 ± 0.004</td>
<td>0.096 ± 0.01</td>
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</tbody>
</table>

* The drugs were administered once daily for 7 days.

** and *** indicate P < 0.05, < 0.01 and < 0.001, respectively, different from vehicle treated control group (Mann-Whitney U-test).
peroxidation, the tissues were homogenized in cold potassium chloride (1.15%) solution. The following methods were used: SOD activity was measured following the method of SAGGU et al.5. CAT activity: the assay was based on the method of Beers and SIZER. GPX activity was measured by the method of Paglia and Valentine10. Lipid peroxidation was assayed by the method of Ohkawa et al.11 Protein estimation was done by the method of Lowry et al.12

The data were analysed by the Mann-Witney U-test. P values less than 0.05 were considered statistically significant when compared to the vehicle-treated control group.

Results

E. officinalis tannins (EOT) (5 and 10 mg/kg, i.p.) induced a dose-related increase in SOD, CAT and GPX activities in frontal cortex and striatum of rats. However, the effects induced by a single acute administration of EOT were statistically insignificant. After 7 days of drug administration the increases induced by these two doses of EOT in frontal cortical SOD, CAT and GPX activities were 29.7%, 40.6% and 26.3%, respectively, whereas the decreases induced after 7 days treatment in lipid peroxidation in striatum were 30.8, 42.1 and 31.2% respectively (Table 2).

### Table 2 — Effects of Emblica officinalis tannins (EOT) and deprenyl on lipid peroxidation in rat brain frontal cortex and striatum

<table>
<thead>
<tr>
<th>Treatment (mg/kg, ip)</th>
<th>n</th>
<th>Frontal cortex</th>
<th>Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
<td>Day 7*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>16</td>
<td>186.4 ± 15.8</td>
<td>198.2 ± 36.3</td>
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<tr>
<td>EOT (5)</td>
<td>6</td>
<td>169.0 ± 12.2</td>
<td>132.9 ± 12.4</td>
</tr>
<tr>
<td>EOT (10)</td>
<td>6</td>
<td>164.0 ± 19.4</td>
<td>156.4 ± 19.0</td>
</tr>
<tr>
<td>Deprenyl (2)</td>
<td>6</td>
<td>155.6 ± 14.9</td>
<td>161.6 ± 17.2</td>
</tr>
</tbody>
</table>

* The drugs were administered once daily for 7 days.

For significance levels, * denotes P < 0.05, ** denotes P < 0.01, and *** denotes P < 0.001, respectively, different from vehicle treated control group (Mann-Whitney U-test).

Discussion

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defences. Potential antioxidant therapy should, therefore, include either natural free radical scavenging antioxidant enzymes or agents which are capable of augmenting the activity of these enzymes, which include SOD, CAT and GPX13. If human disease is considered to result from an imbalance between oxidative stress and antioxidant defence, then it is conceivable that it may be possible to limit oxidative tissue damage and, hence, prevent or ameliorate disease progression, by supplementing antioxidant defence13. By virtue of their properties and clinical use in Ayurveda, the rasayanas may provide potential therapeutic intervention against oxidative threats, both in health and disease14. Earlier studies from this laboratory have indicated that Withania somnifera14, Ocimum sanctum15, Shilajit16 and Bacopa monniera17, categorized as Ayurvedic rasayanas augment anti-oxidant activity in experimental animals.

The E. officinalis extract, rich in emblicanin A and B, was found to significantly increase the cortical and striatal concentrations of the anti-oxidant enzymes, SOD, CAT and GPX, and to reduce lipid peroxidation in these rat brain areas. The most abundant oxidative
free radicals generated in living cells are superoxide anions and derivatives, particularly the highly reactive and damaging hydroxyl radical, which induces peroxidation of cell membrane lipids. The end products of lipid peroxidation are known to induce cellular damage and have been shown to be responsible for oxidative free radical induced human disease. Superoxide is inactivated by SOD, the only enzyme known to use a free radical as a substrate. However, the free radical scavenging activity of SOD is effective only when it is followed up by increases in the activity of CAT and/or GPX, since SOD generates hydrogen peroxide as a metabolite, which is more tissue-toxic than oxygen radicals and has to be scavenged by CAT or GPX. Thus, a concomitant increase in CAT and/or GPX activity is essential if a beneficial effect from increase in SOD activity is to be expected. The choice of the brain area selected for this study was based on the evidence that they are highly vulnerable to oxidative stress induced injury.

Free radical induced degeneration in corpus striatum and frontal cortex has been implicated with the aging process, Parkinsonism and Alzheimer's disease.

Deprenyl, the standard antioxidant agent used for comparison, has been earlier shown to increase SOD, CAT and GPX activities of several brain areas, including the frontal cortex, striatum and hippocampus, and to reduce brain lipid peroxidation. However, its effect on brain GPX activity remains controversial. Deprenyl has been reported to arrest the progression of Parkinson's disease and to retard the process of aging, leading to dramatic increase in life-span, in experimental animals. The antioxidant activity of deprenyl, like that of EOT, was minimal on acute single administration but became evident after subchronic administration, as has been reported earlier as well.

Vitamin C is regarded as the first line natural antioxidant defence in plasma and a powerful inhibitor of lipid peroxidation. It also regenerates the major antioxidant tocopherol (vitamin E) in lipoproteins and cell membranes. Intracellular mechanisms exist which can regenerate vitamin C (ascorbate) from its inactive metabolite dehydroascorbate by reduced glutathione. Although vitamin C is thought to be an important antioxidant with protective effects against respiratory diseases, atherosclerosis and carcinogens, there is no clinical evidence in the form of controlled clinical trials which confirm these attributes of vitamin C. E. officinalis fruits have long been postulated to be a rich source of vitamin C and the prophylactic, curative and restorative effects of the fruits and that of Chyavanprash, which has a very high content of the fruit pulp, were thought to be mainly due to this factor. However, a recent comprehensive study has shown that vitamin C is not present in fresh juice extracts of E. officinalis fruits. It was suggested that the vitamin C-like activity of the fruits was due to the presence of low molecular weight tannoids, mainly emblicanin A and B. The in vitro antioxidant activity of tannoids was demonstrated as well. The present study confirms the anti-oxidant activity of these active principles of E. officinalis fruits in terms of augmentation of brain SOD, CAT and GPX activity, concomitant with reduction in lipid peroxidation. Apart from potential clinical use of these tannoids, emblicanin A and B can be used to standardize Chyavanprash preparations, which appears to be problematic in assessing the clinical efficacy of different marketed preparations.

References