

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*^{1,2}. The *rasayanas* are used to promote health and longevity by increasing defence against disease, arresting the aging process and revitalizing the body in debilitated conditions². The clinical efficacy of the fruits of *E. officinalis* are held in high esteem in Ayurveda and *amla* is referred to as a *maharasayana*². The fruits form the major constituent of *Chyavanprash awaleha*, a polyherbal Ayurvedic *rasayana* preparation described in Charaka Samhita³. 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 nephrotoxicity⁴. Clinical studies suggest that the fruits have anabolic activity⁴. Experimental investigations on *Chyavanprash* have shown that it exhibits significant adaptogenic,

immuno-potentiating and memory-facilitating effects⁴.

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 pericarp⁵. 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 fruits⁵. A recent study⁵, 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 transformations⁵. Emblicanin A and B have been shown to exhibit

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significant antioxidant effect *in vitro*⁵. 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 antioxidant⁶, 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* by deactivating the contained hydrolytic enzymes followed by column chromatography over Sephadex LH-20, using methanol and methanol-water as eluent.

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 markers⁵. Details of extraction and structure elucidation of the emblicanins and other compounds from *E. officinalis* fruits, have been published elsewhere⁵.

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 dissected⁷. The tissues were weighed and homogenized in 2 ml of ice-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

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

Treatment (mg/kg, ip)	n	Frontal Cortex		Day 1	Striatum Day 7*
		Day 1	Day 7*		
[Values, expressed as U/mg protein, are mean±SE]					
SOD activity					
Vehicle	16	12.6 ± 0.9	14.4 ± 1.4	15.7 ± 1.9	17.4 ± 2.2
EOT (5)	6	15.9 ± 1.9	18.6 ± 0.9 ^a	18.4 ± 1.2	22.2 ± 1.6 ^a
EOT (10)	6	14.9 ± 1.4	24.9 ± 1.6 ^b	18.9 ± 1.8	28.2 ± 1.2 ^c
Deprenyl (2)	6	16.8 ± 1.5 ^a	24.2 ± 2.2 ^c	19.4 ± 1.9	30.4 ± 2.9 ^c
CAT activity					
Vehicle	16	15.0 ± 1.4	14.4 ± 1.4	15.7 ± 1.9	17.4 ± 2.2
EOT (5)	6	14.6 ± 1.2	19.0 ± 1.4 ^a	17.6 ± 1.9	24.8 ± 1.9 ^a
EOT (10)	6	18.2 ± 0.9	24.9 ± 0.8 ^b	19.0 ± 2.1	29.4 ± 1.6 ^c
Deprenyl (2)	6	19.8 ± 1.6	22.6 ± 2.0 ^b	29.6 ± 2.6 ^a	34.9 ± 3.3 ^c
GPX activity					
Vehicle	16	0.064 ± 0.01	0.052 ± 0.014	0.082 ± 0.008	0.073 ± 0.011
EOT (5)	5	0.071 ± 0.008	0.084 ± 0.008 ^a	0.093 ± 0.006	0.099 ± 0.006 ^a
EOT (10)	6	0.076 ± 0.008	0.099 ± 0.006 ^b	0.098 ± 0.009	0.16 ± 0.009 ^c
Deprenyl (2)	6	0.068 ± 0.004	0.084 ± 0.006 ^a	0.096 ± 0.01	0.099 ± 0.01 ^b

* The drugs were administered once daily for 7 days.

^{a,b,c} and ^c indicate $P < 0.05$, < 0.01 and < 0.001 , respectively, different from vehicle treated control group (Mann-Whitney U-test).

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

Treatment (mg/kg, ip)	n	[Lipid peroxidation (nmol/g) mean ± SE]			
		Frontal cortex		Striatum	
		Day 1	Day 7*	Day 1	Day 7*
Vehicle	16	186.4 ± 15.8	188.9 ± 23.2	198.2 ± 36.3	186.4 ± 28.2
EOT (5)	6	169.0 ± 12.8	132.9 ± 12.4 ^a	169.3 ± 22.4	129.0 ± 16.4 ^a
EOT (10)	6	164.3 ± 19.4	112.3 ± 13.9 ^b	156.4 ± 19.0	108.0 ± 11.6 ^c
Deprenyl (2)	6	156.6 ± 14.9	139.3 ± 12.6 ^b	161.6 ± 17.2	128.2 ± 10.3 ^c

* The drugs were administered once daily for 7 days

^{a,b} and ^c 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 Saggi *et al.*⁸. CAT activity: the assay was based on the method of Beers and Sizer⁹. 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 29.2% and 72.9%, 31.9% and 72.9%, 61.5% and 90.4%, respectively, whereas the increases in the striatal enzymes were 27.6% and 62.1%, 42.5% and 69.0%, and 35.6% and 119.8%, respectively (Table 1). Deprenyl elicited qualitatively a similar response and the increases induced by it in frontal cortical SOD, CAT and GPX activities were 68.0%, 56.9% and 61.5%, respectively, when treated for 7 days. Similar increases were recorded in concentrations of striatal SOD, CAT and GPX activities to the extent of 74.7%, 100.6% and 35.6%, respectively (Table 1). On the contrary, both doses of EOT, and deprenyl, induced a decrease in lipid peroxidation in the brain areas

investigated. The effects were statistically significant only after treatment for 7 days. The decreases recorded for EOT (5 and 10 mg/kg) and deprenyl in frontal cortex 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).

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 somnifera*¹⁴, *Ocimum sanctum*¹⁵, *Shilajit*¹⁶ and *Bacopa monniera*¹⁷, 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 disease¹⁸. 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 expected¹⁹. The choice of the brain area selected for this study was based on the evidence that they are highly vulnerable to oxidative stress induced injury²⁰. Free radical induced degeneration in corpus striatum and frontal cortex has been implicated with the aging process, Parkinsonism and Alzheimer's disease²⁰.

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 hippocampus¹⁴⁻¹⁷, and to reduce brain lipid peroxidation²¹. However, its effect on brain GPX activity remains controversial²². 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 animals²³. 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^{14-17,22}.

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 glutathione²⁴. Although vitamin C is thought to be an important antioxidant with protective effects against respiratory diseases, atherosclerosis and carcinogens²⁵, there is no clinical evidence in the form of controlled clinical trials which

confirm these attributes of vitamin C²⁵. *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 factor⁵. However, a recent comprehensive study⁵ has shown that vitamin C is not present in fresh juice extractives of *E. officinalis* fruits. It was suggested⁵ 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 well⁵. 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.

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