

## Effect of *Emblica officinalis* tannoids on a rat model of tardive dyskinesia

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Effect of active tannoid principles of *E. officinalis*, comprising of emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%), was investigated on a rat model of tardive dyskinesia (TD) induced by once daily administration of haloperidol (1.5 mg/kg, ip) for 28 days. Involuntary orofacial movements (chewing movements, buccal tremors and tongue protrusion) were assessed as TD parameters. The tannoid principles of *E. officinalis* (EOT) were administered concomitantly with haloperidol in the doses of 10, 20 and 50 mg/kg, po, for 28 days. Sodium valproate (200 mg/kg, po), a Gaba-mimetic agent, and vitamin E (400 mg/kg, po), an antioxidant, were used as the standard drugs and administered for the same period. EOT induced a dose-related inhibition of all the three TD parameters assessed, as did vitamin E. The effect of sodium valproate remained statistically insignificant. The results suggest that EOT exerts a prophylactic effect against neuroleptic-induced TD which is likely to be due to its earlier reported antioxidant effects in rat brain areas, including striatum.

*Emblica officinalis* Gaertn., known in the subcontinent as *amla*, finds extensive use in Ayurveda. The juice and extracts of the fruits of the plant are used as *rasayanas* which are claimed to have preventive, curative and health restorative functions<sup>1</sup>. They are used to promote health and longevity by increasing defence of the body against disease and other noxious external factors, for arresting the aging process and for revitalizing the body in debilitating conditions<sup>1</sup>. Experimental studies conducted with the fruit extracts indicate that they have significant cytoprotective against radiation and heavy metal induced toxicities<sup>2</sup>. Recent investigations have shown that the tannoid principles of *E. officinalis*, comprising of emblicanin A and B, punigluconin and pedunculagin, have significant *per se* antioxidant effect in rat brain frontal cortex and striatum, evidenced by increases in superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities, concomitant with reduction in lipid peroxidation (LPO)<sup>3</sup>. This antioxidant effect was also noted against chronic stress-induced perturbations in SOD, CAT, and LPO activities<sup>4</sup>, and against iron-induced increase in hepatic LPO activity<sup>5</sup>.

Tardive dyskinesia (TD) is a delayed extrapyramidal neurological syndrome associated with continued neuroleptic therapy. The pathophysiological basis of TD remains obscure. It has been postulated that compensatory increases in the function of dopamine (DA)

in the basal ganglia, following neuroleptic blockade of postsynaptic DA receptors, is involved.<sup>6</sup> Animal models of TD have used the induction of abnormal involuntary movements following long term administration of neuroleptics, usually haloperidol, and such models have been utilized to investigate prophylactic and therapeutic effects of drugs for TD<sup>7</sup>. The other theories of TD implicate central cholinergic dysfunction following the development of DA receptor supersensitivity, which results in cholinergic-DA imbalance in striatum<sup>6</sup>, and the GABA deficiency<sup>8</sup> and oxidative stress<sup>9</sup> postulates. Ayurvedic *rasayanas*, including *E. officinalis* have been said to be effective in neurodegenerative conditions associated with aging<sup>1</sup>. Vitamin E has been reported to be effective in preventing experimental TD<sup>9</sup>. The present study was conducted to investigate the prophylactic effect of the tannoid principles isolated from *E. officinalis*, which have earlier been shown to induce significant antioxidant effects<sup>3-5</sup>, on an animal model of TD.

The investigations were conducted on adult male Wistar rats (160-180 g, housed in colony cages at an ambient temperature of 25<sup>0</sup> ± 2<sup>0</sup>C and 45-55% RH, with a 12 hr light/12 hr dark cycle. They had free access to standard pellet chow and drinking water. Experiments, including drug administrations, were conducted between 09.00 and 14.00 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 eluents. 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<sup>10</sup>. Details of extraction and structure elucidation of the emblicanins and other compounds from *E. officinalis* fruits, have been published earlier<sup>10</sup>.

The rats were administered an injectable preparation of haloperidol (Searle, India), diluted in normal saline, in the dose of 1.5 mg/kg, ip, once daily (including Sundays) for 28 days. The animals were shifted to the laboratory from the Department animal room two days after the final dose. After a two hour habituation period, the rats were placed individually in a transparent observation cage (25 x 15 x 10 cm). After a further 5 min of habituation, the rat was observed for involuntary orofacial movements (chewing movements, tongue protusions and buccal tremors) for the next 15 min by an unbiased 'blind' observer. Since these involuntary movements do not appear while the rat is walking, grooming, rearing or sleeping, the time period for these activities was deducted from the 15 min observation period and the remaining period was assessed as the final observation period. The total number of each abnormal orofacial movement was then divided by this observation period (min) to calculate the frequency of the abnormal involuntary orofacial movements<sup>7</sup>.

The tannoid principles of *E. officinalis* (EOT) (10, 20 and 50 mg/kg), sodium valproate (Sun Pharma, India, 200 mg/kg) and vitamin E (Torrent Laboratories, India, 400 mg/kg) were administered orally (po), suspended in 0.3% carboxymethylcellulose in distilled water, once daily for 28 days, one hour prior to

haloperidol administration. Control animals received equivalent volume of the vehicle (2.5 ml/kg, po) for the same period and at the same time before haloperidol.

Statistical analysis was done first by one-way analysis of variance (ANOVA), followed by post-hoc use of the Tukey test. A probability value of < 0.05 was accepted as being statistically significant.

The results of the study confirm the utility of the haloperidol test as an animal model of TD, as indicated by the marked induction of involuntary abnormal orofacial movements. The results indicate that EOT has a dose-related preventive effect on neuroleptic-induced TD in rats. Vitamin E proved effective whereas sodium valproate remained ineffective in ameliorating the induced signs of TD, confirming the results of earlier studies<sup>7,11</sup>. Clonazepam, another GABA-mimetic agent, also proved ineffective like sodium valproate<sup>9</sup>. It was reported that the bioactive glycowithanolides of *Withania somnifera*, which have both GABA-mimetic and antioxidant effects, appear to prevent haloperidol-induced TD in rats by the latter action<sup>11</sup>. EOT is known to exert significant antioxidant effect in the striatum<sup>3,4</sup> of rats in the doses used in this study. It was earlier reported that *E. officinalis* fruits were a rich source of vitamin C which was held responsible for its major biological actions, including antioxidant effect<sup>12</sup>. However, more recent investigations have indicated that vitamin C is not present in fresh juice of *E. officinalis* fruits<sup>10</sup>. It was shown that the vitamin C like activity was due to the presence of low molecular weight tannoids, mainly emblicanin A and B, which had significant reducing activity and exerted antioxidant effect *in vitro*<sup>10</sup>. Ayurvedic *rasayanas*, including *W. somnifera*, appear to have significant antioxidant effects which may be responsible for several of their

Table 1—Effect of bioactive tannoid principles of *Emblica officinalis* (EOT), sodium valproate and vitamin E on haloperidol (HP, 1.5 mg/kg, ip x 28 days) induced rat model of tardive dyskinesia

Treatments (mg/kg, po)	n	[Values are mean ± SE]		
		Chewing <sup>a</sup> movements	Tongue <sup>a</sup> protusions	Buccal <sup>a</sup> tremors
Vehicle	12	5.8 ± 0.9	1.9 ± 0.6	0.7 ± 0.2
Haloperidol (HP)	10	14.4 ± 1.6 <sup>a</sup>	4.8 ± 0.8 <sup>a</sup>	2.1 ± 0.4 <sup>a</sup>
EOT (10) + HP	8	10.4 ± 1.9	2.9 ± 0.9	1.8 ± 0.5
EOT (20) + HP	8	8.4 ± 0.9 <sup>b</sup>	2.0 ± 0.6 <sup>b</sup>	1.0 ± 0.4 <sup>b</sup>
EOT (50) + HP	8	7.0 ± 0.6 <sup>b</sup>	1.6 ± 0.4 <sup>b</sup>	0.8 ± 0.3 <sup>b</sup>
Sodium valproate + HP (200)	8	10.9 ± 1.3	3.2 ± 0.9	1.9 ± 0.8
Vitamin E (400) + HP	6	6.6 ± 0.4 <sup>b</sup>	1.9 ± 0.4 <sup>b</sup>	0.9 ± 0.5 <sup>b</sup>

<sup>a</sup>Data represents frequency of the test parameters

<sup>a</sup>P < 0.05 different from vehicle - treated group; <sup>b</sup>P < 0.05 different from HP group

reported clinical and biological effects, including neurodegenerative disorders<sup>13</sup>. Since TD is an inevitable consequence of neuroleptic therapy in psychotic disorders<sup>6</sup>, it may be worthwhile trying to prevent TD by co-administering *E. officinalis* fruit extracts during neuroleptic therapy.

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