Hydroalcoholic extract of *Emblica officinalis* Gaertn. affords protection against PTZ-induced seizures, oxidative stress and cognitive impairment in rats

Mahaveer Golechha, Jagriti Bhatia* & Dharmvira Singh Arya
Department of Pharmacology, All India Institute of Medical Sciences (AIIMS), Ansari Nagar, New Delhi 110 029, India

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The cognitive impairment seen in epileptics may be a consequence of either the underlying epileptogenic process alone or it could manifest on account of the use of antiepileptic drugs that cause cognitive impairment as an adverse effect or both. Thus, there is a need for drugs that can suppress epileptogenesis without contributing to or, if possible, by acting to prevent the development of cognitive impairment. *Emblica officinalis*, an Indian medicinal plant, has marked antioxidant property. The effect of seven days pretreatment of 300, 500 and 700 mg/kg doses of hydroalcoholic extract of *E. officinalis* (HAEEO) administered intraperitoneally to rats was evaluated on pentylenetetrazole (PTZ) induced seizures, cognitive deficit and oxidative stress markers viz malondialdehyde (MDA) and glutathione. The 500 and 700 mg/kg ip doses of HAEEO completely abolished the generalized tonic seizures and also improved the retention latency in passive avoidance task. Further, HAEEO dose-dependently ameliorated the oxidative stress induced by PTZ. These findings suggest the potential of HAEEO to be used as an adjuvant to treatment with antiepileptic drugs.

**Keywords:** Cognitive impairment, *Emblica officinalis*, Epilepsy, Oxidative stress, PTZ

Epilepsy is a common neurological disorder encountered in clinical practice. The incidence of epilepsy has been reported as 5-10 cases per 1000 persons and it affects approximately 1% of the world’s population. Epilepsy is characterized by abnormal episodic bursts of electrical activity in neurons which may spread in the entire brain. The excessive activation of excitatory amino acid receptors or the failure of inhibition due to altered functional properties of the GABA<sub>A</sub> receptor usually underlies the spread of epileptic activity. Such abnormal neuronal activities may have a significant impact on the normal cognitive processes and behaviour of the affected individual. The role of free radicals in epilepsy has also been recognized recently. Increased levels of free radicals have been demonstrated during seizures. The anticonvulsant effect of several agents having antioxidant property such as vineatrol, transresveratrol, melatonin and alpha lipoic acid has been demonstrated. The cognitive impairment observed in epilepsy is attributable both to the epileptogenic process as well as the antiepileptic medication. In addition to the decline in cognition, the present antiepileptic drugs (AEDs) are also associated with a host of adverse reactions, drug interactions and teratogenicity. Hence, in view of the above drawbacks of the presently available AEDs, there is need for development of newer drugs which are not only safe but also suppress epileptogenesis and prevent cognitive decline.

*Emblica officinalis* Gaertn., commonly known as amla (Hindi) and gooseberry (English), has been used in the traditional system of medicine to reduce fever, alleviate asthma, treat constipation and enhance digestion, strengthen the heart, benefit the eyes, enhance intellect and as a health tonic. The fruits of *E. officinalis* have potent antioxidant activity due to the presence of tannoids, tannins, vitamin C and flavonoids. The pharmacological studies on *E. officinalis* fruit have revealed that it has good antioxidant, cytoprotective and immunomodulatory, anti-diabetic, hypolipidemic, anti-tussive, cardioprotective, antiulcerogenic and hepatoprotective activity. Since, the primary underlying pathology of epilepsy is attributable to increased oxidative stress and in view of the fact that *E. officinalis* possesses very high antioxidant activity, it was interesting to investigate the effect of hydroalcoholic extract of *E. officinalis* (HAEEO) on pentylenetetrazole (PTZ)-induced seizures, oxidative stress and cognitive impairment in rats.

*Correspondent author
Telephone: 91-11-2659 3282, 2654 6476, 2659 4266
Fax: 91-11-2658 8641, 2658 8663
E-mail: jagriti2012@rediffmail.com
Materials and Methods

Animals—Male wistar rats weighing 150-200 g body weight, obtained from the central animal house facility of All India Institute of Medical Sciences, New Delhi, were group housed in polyacrylic cages (38 × 23 × 10 cm) with each cage containing not more than four animals. The animals were maintained under standard laboratory conditions with natural dark and light cycles (12:12 h) and ambient room temperature. They were allowed free access to standard dry diet (Golden Feeds, India) and tap water ad libitum. All the behavioral procedures were carried out between 0900 and 1300 hrs. All procedures used in the study were reviewed and approved by the Institutional Committee for Ethical Use of Animals (application approval no. 384/IAEC/07). The care of animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Plant extract—The standardized hydroalcoholic extract of E. officinalis (HAEEO) was procured from Sanat Products Pvt. Ltd, New Delhi, India.

Drugs and chemicals—Pentylenetetrazole (PTZ) was purchased from Sigma Chemicals, USA. All other chemicals and solvents were obtained from standard commercial suppliers and were of highest purity and analytical grade.

Pentylenetetrazole (PTZ) induced seizures—PTZ was dissolved in saline. PTZ at the dose of 60 mg/kg consistently produces seizures with least mortality as standardized previously21. This is the minimal dose of chemoconvulsant PTZ at which 99.0% of the animals showed a generalized tonic seizure (GTS). The GTS is characterized by symmetric forelimb and hind limb tonus, and then hind limb clonus and flipping activity. The rats were divided into 5 groups of 6 animals each for evaluation of antiepileptic activity of HAEEO as detailed under:

Group I (Control group): The rats were injected with saline, ip, for 7 days (this group was used for comparison of results in the biochemical and behavioral studies)

Group II (Vehicle + PTZ group): Vehicle (saline) was administered, ip, for 7 successive days before PTZ (60 mg/kg, ip) administration.

Group III, IV and V (HAEEO + PTZ group): HAEEO in the dose of 300, 500 and 700 mg/kg/day, ip, was administered for 7 days respectively to rats in different groups.

On the seventh day, 30 min after the drug treatment, 60 mg/kg, ip, PTZ was administered and the animals were observed for 30 min for latencies to myoclonic jerks and GTS as well as duration of GTS.

One-trial passive avoidance test—Memory retention deficit was evaluated by step through passive avoidance apparatus22. This apparatus consists of equal sized light and dark compartments (30 × 20 × 30 cm). A 40 W lamp is fixed 30 cm above its floor in the center of the light compartment. The floor consists of metal grid connected to a shock chamber. The two compartments are separated by a trap door that can be raised upto 10 cm. Two hours after completing the assessment of behavioural changes due to PTZ (on the seventh day in all the groups), the rats were placed in the light compartment and the time lapse before each animal entered the dark compartment and had all four paws inside it was measured in seconds and termed as ‘initial latency’ (IL). Immediately after the rat entered the dark chamber with all the four paws inside the dark chamber, the trap door was closed and an electric foot shock (50 V AC) was delivered for 3 sec. After 5 sec, the rat was removed from the dark chamber and returned to its home cage. After 24 h of IL determination, the latency time was again measured in the same way as above in the acquisition trial and was termed as the retention latency (RL). However, during the retention trial, no foot shock was delivered, and the latency time was recorded to a maximum of 600 sec. To improve the reliability and validity of the foot shock avoidance test, the grid as well as the rat paw was moistened with water before foot shock as this is known to reduce the wide inter-animal variability in paw skin resistance of the rats.

Tissue preparation—Following assessment of behavioural parameters, the rats were sacrificed with overdose of pentobarbitone, ip, the brain removed and stored in liquid nitrogen. Thereafter, at the time of undertaking biochemical analysis, the brain tissue samples were thawed and homogenized with 10 times (w/v) ice-cold 0.1M phosphate buffer (pH 7.4) Aliquots of homogenates from rat brain were used to determine brain malondialdehyde (MDA)23 and glutathione (GSH)24 levels.

Statistical analysis—Data were expressed as mean±SE. Statistical differences between the treatment and the control groups were calculated by One-way ANOVA followed by Tukey-Kramer post test. P<0.05 was considered to be significant.
Results

Effect of HAEEO on latency of myoclonic jerks—All the rats treated for 7 days with HAEEO at 300, 500 and 700 mg/kg, ip, doses exhibited myoclonic jerks following PTZ administration. However, there was a significant increase in the latency of myoclonic jerks in a dose dependent manner as compared to the Group II (Table 1; \(P<0.001\)).

Effect of HAEEO on GTS and duration of GTS—The GTS was not observed in groups pretreated with 500 and 700 mg/kg, ip, doses of HAEEO for 7 days. The latency of GTS as well as the duration of GTS was increased at 300 mg/kg, ip dose significantly (\(P<0.001\)) as compared to Group II (Table 1).

Effect of HAEEO on cognitive impairment induced by PTZ induced seizures in rats—The mean initial latency recorded 30 min after the administration of PTZ did not differ significantly between any of the groups (Table 2). Retention latency 24 h after the administration of PTZ in the Group II was significantly less (\(P < 0.001\)), compared with the Group I rats. This indicates significant cognitive impairment following PTZ administration. The Groups III, IV and V showed significant reversal of PTZ induced cognitive deficit as evidenced by dose-dependent increase in the retention latencies in these groups (\(P<0.001\)).

Effect of HAEEO pretreatment on the brain levels of MDA and GSH following PTZ induced seizures in rats—The brain levels of MDA were significantly raised after PTZ administration in Group II as compared to Group I rats (Table 3; \(P<0.001\)). However, pretreatment with HAEEO (Groups III, IV and V) for 7 consecutive days prior to PTZ treatment, produced significant decrease in the MDA levels as compared to Group II. Similarly, the brain level of glutathione was significantly decreased in Group II as compared to Group I (\(P<0.001\)). The HAEEO pretreatment with 500 and 700 mg/kg, ip, dose (Groups IV and V) led to a significant decrease in the oxidative stress level (\(P<0.05\) and \(P<0.001\) respectively) as indicated by the significant and dose-dependent increase in brain GSH levels as compared to Group II.

Discussion

The results of the present study show that pretreatment with HAEEO dose-dependently increased the latencies of myoclonic jerks and at doses 500 and 700 mg/kg ip completely abolished generalized tonic seizures (GTS) produced by PTZ. The PTZ induced seizure is most frequently employed in the preliminary screening of potential anticonvulsant drugs\(^{25}\). The mechanism by which PTZ is believed to exert its action is by acting as an antagonist at the GABAA receptor complex\(^{26}\). GABA is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will

<table>
<thead>
<tr>
<th>Group</th>
<th>Latency of myoclonic jerks (sec)</th>
<th>Latency of GTS (sec)</th>
<th>Duration of GTS (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + PTZ</td>
<td>34.66±3.05</td>
<td>43.5±3.09</td>
<td>18.5±1.25</td>
</tr>
<tr>
<td>HAEEO (300mg/kg) + PTZ</td>
<td>110.16±5.94*</td>
<td>151.83±6.50*</td>
<td>9.5±7.6*</td>
</tr>
<tr>
<td>HAEEO (500mg/kg) + PTZ</td>
<td>207.16±9.01*</td>
<td>No GTS*</td>
<td>No GTS*</td>
</tr>
<tr>
<td>HAEEO (700mg/kg) + PTZ</td>
<td>290.16±10.42*</td>
<td>No GTS*</td>
<td>No GTS*</td>
</tr>
</tbody>
</table>

\(*P<0.001\) compared to vehicle + PTZ group. (ANOVA followed by Tukey-kramer post test).

HAEEO: Hydroalcoholic extract of *E. officinalis*; PTZ: Pentylenetetrazole (60 mg/kg, ip).

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH(µg/g tissue)</th>
<th>MDA(nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.33±3.11</td>
<td>181.44±6.32</td>
</tr>
<tr>
<td>Vehicle + PTZ group</td>
<td>54.58±3.71*</td>
<td>282.67±7.93*</td>
</tr>
<tr>
<td>HAEEO (300mg/kg) + PTZ</td>
<td>65.62±3.27</td>
<td>236.24±13.74*</td>
</tr>
<tr>
<td>HAEEO (500mg/kg) + PTZ</td>
<td>72.50±4.48*</td>
<td>210.13±9.73*</td>
</tr>
<tr>
<td>HAEEO (700mg/kg) + PTZ</td>
<td>89.79±3.75*</td>
<td>190.00±13.40*</td>
</tr>
</tbody>
</table>

HAEEO (300, 500 and 700 mg/kg, ip) was administered for 7 days. *P values: *<0.001 compared to control; **<0.001 compared to vehicle + PTZ group. (ANOVA followed by Tukey-Kramer post test). Other details are same as in Table 1.
attenuate and enhance seizures respectively. Phenobarbitone and diazepam antagonize PTZ-induced convulsions by enhancing GABA neurotransmission. Since, in the present study HAEEO also showed protective effect against seizures induced by PTZ, it is probable that HAEEO may be acting via a GABAergic mechanism(s) to exert its anticonvulsant effect. The anticonvulsant drugs like benzodiazepines and valproic acid cause an increase in GABA levels in the brain. Hence, it is quite possible that the HAEEO may additionally exert its anticonvulsant effect by increasing the level of GABA in the brain.

In the present study, the administration of PTZ (60 mg/kg, ip) resulted in seizures that were associated with cognitive impairment as evidenced by reduction of retention latency in passive avoidance behavior. These results are in conformity with the findings of other workers who also demonstrated cognitive impairment after administration of chemo-convulsants. HAEEO prevented cognitive deficit associated with PTZ-induced seizures as evidenced by increased retention latency in passive avoidance behaviour. Interestingly, even though the lower dose of HAEEO (300 mg/kg, ip) failed to abolish the seizures induced by PTZ, it ameliorated the associated cognitive deficits.

Free radicals have been suggested to be the most likely candidate responsible for producing the neuronal changes mediating the behavioural deficits in neurodegenerative disorders. Antioxidants are effective in rodent models of epilepsy, stroke and Alzheimer’s disease. Therefore, the effect of HAEEO on oxidative stress in PTZ-induced seizures was also evaluated. The increase in the levels of MDA, a marker of lipid peroxidation, in the present study, indicates increased free radical generation in the Group II rats. The significantly lower level of MDA in the brains of the HAEEO + PTZ rats as compared with the Group II rats indicates attenuation of lipid peroxidation. There was a simultaneous significant decrease in the reduced glutathione levels in Group II rats. Glutathione is an endogenous antioxidant present mainly in the reduced form within the cells. It reacts with the free radicals and prevents the generation of hydroxyl radicals, the most toxic form of free radicals. The decreased level of reduced glutathione in Group II rats seen in the present study indicates that there was an increased generation of free radicals and that the reduced glutathione was depleted during the process of combating oxidative stress. The decrease in MDA levels and increase in the glutathione levels in HAEEO + PTZ rats indicates that HAEEO exerted good antioxidant effect. The potent antioxidant property of the fruit of E. officinalis has been reported in other studies also. Its fruit contains various phytochemical constituents like emblicanins A and B, gallic acid and ellagic acid which are powerful free radical scavengers. Moreover, other phytochemicals with NO scavenging properties like geraniin, corilagin and furosin have also been reported to be present in the E. officinalis fruit extract. In another study, it has been reported that the superoxide scavenging property of E. officinalis extract approximates that of L-ascorbic acid, a well established antioxidant. Thus, the observed anticonvulsant effect and amelioration of the cognitive deficit in rats in this study by HAEEO is very likely a result of the good antioxidant property of E. officinalis.

In conclusion, the results obtained in the present study suggest that HAEEO has anticonvulsant activity which may be mediated by multiple mechanisms. In addition, the present study also demonstrates that HAEEO can significantly prevent cognitive impairment and may act as a useful adjuvant to the conventional antiepileptic therapy.

References
aged 35-55 years, Indian gooseberry (amla) on serum cholesterol levels in men


