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## Effect of Repeated Administration of *Paeonia emodi* Wall Root Extract in Experimental Models of Epilepsy and Behavior

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### ABSTRACT

The root of *Paeonia emodi* wall has been used for thousands of years to treat epilepsy in Unani medicine. This study aimed to investigate the reported antiepileptic, neuroprotective and anxiolytic activities of *Paeonia emodi* root in Unani literature. *Paeonia emodi* root ethanolic extract (PEE) (300 and 600 mg kg<sup>-1</sup>) per orally was evaluated on Pentylenetetrazole (PTZ) kindling seizures in mice and was tested for its ability (1) to suppress the convulsive and lethal effects of PTZ in kindled mice (anti-epileptogenic effect) (2) to attenuate the PTZ-induced oxidative injury in the brain tissue (antioxidant effect). Besides, PEE was also evaluated to test its ability to prevent PTZ induced memory impairment using Morris water maze test, anxiolytic potential by examining the animal's performance on elevated plus maze test and to rule out any associated motor impairment of the test drug by using grip strength and Rotarod performance tests. ANOVA followed by Dunnett's test was used to evaluate the results along with Kruskal Wallis analysis by ranks was performed for evaluating kindling comparison. PEE significantly decreased oxidative injury in the mouse brain tissue in comparison with the PTZ-kindling group, however, PEE 600 mg kg<sup>-1</sup> was found to be the most effective in preventing PTZ-induced seizures relative to valproate. Besides showing antiepileptogenic potential, PEE also exhibited statistically significant anxiolytic, antioxidant activity without any evidence of causing any cognitive or motor impairment. The findings suggest the potential of PEE as adjuvant to antiepileptic drugs with an added advantage of preventing cognitive impairment.

**Key words:** Antiepileptic, *Paeonia emodi*, PTZ kindling

### INTRODUCTION

Epilepsy, which is characterized by recurrent seizures, is the second most common neurologic disorder after stroke. Besides causing impaired physical, psychological and social functioning (Ramezani *et al.*, 2008), in developing countries, it is also associated with "Epilepsy stigma" (Ahmad, 2011). Despite the rigorous efforts, 30% of epileptic patients continue to have seizures (Nikalje *et al.*, 2011). *Paeonia emodi* wall (Paeoniaceae) popularly known as "Ood-saleeb" is an erect glabrous, leafy perennial herb indigenous to North-west Himalayas from Kashmir to Kumaon (Chauhan, 1999; Ismail *et al.*, 2003), North Pakistan (Hamayun *et al.*, 2006), West Nepal and is

also known to grow in a single locality in China (De-Yuan, 1997). In traditional Unani system of medicine, the roots of this plant have been used to treat epilepsy over centuries. The recommended daily clinical dose is 6 g. The root of this plant was thought to prevent infantile convulsions when hung around their neck (Majusi, 1889). It also has antispasmodic, diuretic and neuroprotective properties (Sina, 1887). In the course of pharmacological studies, Various components of the plant root showed  $\beta$ -glucuronidase inhibitory activity (Nawaz *et al.*, 2000), lipoxygenase inhibiting activity, hydroxyl radical scavenging activity (Hall *et al.*, 1996), neuroprotection (Xiao *et al.*, 2005) and possible anticonvulsant activities (Tsai *et al.*, 2005; Tsuda *et al.*, 1997). Several Phytochemical studies have demonstrated the presence of important bioactive compounds in the plant's root: monoterpene glycosides (Muhammad *et al.*, 1999), monoterpene galactosides (Riaz *et al.*, 2003a), triterpene (Riaz *et al.*, 2003b), 1, 5-dihydroxy-3-methyl-anthraquinone, ethyl gallate and methyl grevillate (Nawaz *et al.*, 2000). The root also contains an essential oil with salicylaldehyde as the chief component (Asif *et al.*, 1983), a fixed oil, gallic acid, benzoic acid and sucrose (Kapoor *et al.*, 1968). Earlier Nizami and Jafri (2005) reported combination of *Paeonia emodi* root and *Delphinium denudatum*, ethanolic extracts possesses anticonvulsant, antianxiety and memory enhancing activities. Since, neither this plant was evaluated in single nor against chronic experimental models of epilepsy and behavior; hence, the aim of the present research work was to examine the traditional claim by repeated administration of ethanolic extract of *P. emodi* (PEE) in different models of experimental epilepsy and behavior.

## MATERIALS AND METHODS

The roots of *Paeonia emodi* were procured from a well known herbal supplier (Green Earth Pvt. Ltd. NewDelhi) which was harvested from the Pahalgam Valley of Kashmir during the early spring (February 2008). A voucher specimen no. NISCAIR/RHMD/CONSULT/-2007 08/921/105/PAEONIA EMODI /DATE 19NOV. 2007 of the plant was authenticated and deposited at NISCAIR herbarium, New Delhi. The plant roots were washed properly, dried at 45°C (48 h) and then powdered and stored in sterile containers at 4°C until used. The doses of extract were obtained that are used in Unani system of medicine by the standard method.

**Drugs and extract:** PTZ (Sigma, USA), Sodium Valproate (Wockhardt Ltd., India), Diazepam were prepared immediately before their use, however, ethanolic extracts were prepared one day before the experiments. For the preparation of ethanolic extract, coarsely ground powdered root was exhaustively extracted by Soxhlet apparatus (70-80°C) with 8 parts of 90% ethanol under boiling for 5 h and was filtered through a 400-mesh cloth to collect the extract. The extract was concentrated and finally spray dried to get a brown powder. The percentage w/w yield of extract was 31. The loss on drying at 105°C and ash content of the powder were 3 and 7% w/w, respectively. The extract was administered each time in by dissolving the calculated quantity in 0.2% w/v Tween 80.

**Phytochemical screening and LC-MS fingerprinting:** Preliminary phytochemical characterization of the PEE was done using the methods of Harborne (1984) and Ikhiri *et al.* (1992). The findings of the phytochemical screening of the plant's crude extract are shown in Table 1.

**Animals:** Swiss male albino mice weighing 20-35 g were procured from the central animal house, Jamia Hamdard, New Delhi. Animals were housed in groups of 6 per cage and maintained at

Table 1: Phytochemical screening of *Paeonia emodi* root ethanolic extract

Chemical compounds	Tests/reagents	Findings/results
Alkaloids	Dragendorff's reagent/Meyer's reagent	-
Flavonoids	Acid-alcohol/solid magnesium/amyl-alcohol	-
Saponins	Frothing test	-
Triterpene	Sulphuric acid reagent	++
Tannins	Ferric chloride reagent	++
Reducing sugars	Fehling's reagent	+
Anthraquinones	Borntrager's test, BPC	+
Glycosides	Lieberman's test/Keller-Killiani test	+++

-: Negative; +: Weakly positive; ++: Moderately positive; +++: Strongly positive

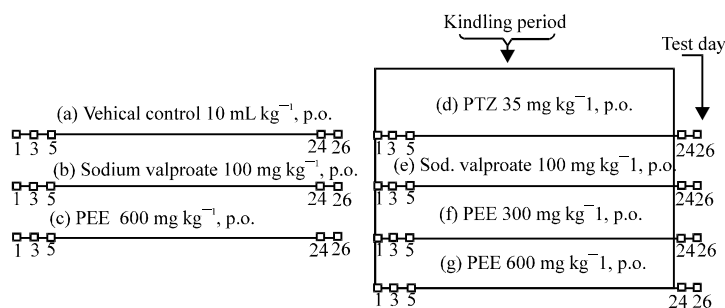


Fig. 1: Scheme illustrating the treatment protocols. “a,b,c” groups are non-kindling groups. In d, e, f and g groups (kindling groups); PTZ was administered at a dose of 35 mg kg<sup>-1</sup> (i.p.) on days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22 and 24 (kindling period) and at a dose of 75 mg kg<sup>-1</sup> on day 26 (test day). Group names were indicated from “a” to “g” and administrating solutions for each group are shown over the time line

20-30°C and 50-55% humidity in a natural light and dark cycle, with free access to food and water. The experiments were performed during the light cycle in awake, freely moving animals that were adjusted to laboratory conditions before proceeding with the experiments. The study was performed with prior approval of the Institutional Animal Ethics Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) with approval reg. no. JH/CPCSEA/31-01-2000/Project No. 418/2007-2010. Utmost care was taken to ensure that animals were treated in the most humane and ethically acceptable manner.

**Experimental procedures:** PTZ was dissolved in isotonic saline (0.9% NaCl) and administered intraperitoneally (i.p.). Sodium Valproate (SV) was diluted by addition of sterile isotonic saline solution. The volume of administration for both oral as well as i.p. route did not exceed 10 mL kg<sup>-1</sup>. SV and PEE were orally administered once daily by gavage.

Figure 1 shows the schematic treatment protocol of experiments as described below. The animals were randomly divided into 7 experimental groups. There were 10 mice in each group. In the last three groups, all PTZ injections were given 2 h after the oral administrations (Saline, SV, PEE 300 and PEE 600).

**Induction of Kindled seizures and seizure observation procedures:** Kindling was produced by a total of 11 treatments with 35 mg kg<sup>-1</sup> PTZ i.p. on every second day (Monday, Wednesday and Friday). Mice were observed for 30 min after the last drug administration. A clonic convulsion was

defined as lasting a minimum of 3 sec (Hansen *et al.*, 2004). After an additional 30 min, mice were observed for lethality before returning to the home cage. Seizure intensity was evaluated according to the following modified scale (Erakovic *et al.*, 2001):

- 0 : No response
- 1 : Ear and facial twitching
- 2 : Convulsive waves axially through the body
- 3 : Myoclonic body jerks
- 4 : Generalized clonic convulsions, turnover into side position
- 5 : Generalized convulsions with tonic extension episode and status epilepticus
- 6 : Mortality

The animals were said to be kindled after having received 11 PTZ injections and having reached atleast 3 consecutive stage 4 or 5 seizures.

In this study, 75 mg kg<sup>-1</sup> PTZ was selected as challenge dose in kindled mice on day 26 (test day). This dose of PTZ produced convulsions (tonic and clonic), status epilepticus and lethality. All groups were tested for PTZ challenge dose (75 mg kg<sup>-1</sup>) induced seizures and status in kindled mice.

**Dissection and homogenization:** Groups of non-kindling mice were sacrificed by decapitation 3 h after the last oral administration (Vehicle, Valproate and PEE 300 and 600 mg kg<sup>-1</sup>). Kindled mice were decapitated at the end of the observation period on the test day. The whole brain was quickly removed, rinsed with cold isotonic saline solution twice and weighed. A 10% (w/v) tissue homogenate was prepared with 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by centrifugation of the homogenate at 12,000x g for 20 min, at 4°C for enzyme assays.

**Measurement of lipid peroxidation:** The quantitative measurement of lipid peroxidation in the whole brain was done according to the method of Wills (1966). The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm using Perkin-Elmer lambda 20 spectrophotometer. The results were expressed as nmol of MDA/mg protein using the molar extinction coefficient of chromophore (1.56×10 M<sup>-1</sup> cm<sup>-1</sup>).

**Measurement of reduced glutathione:** Reduced glutathione in the whole brain was estimated according to the method of Ellman (1959). A 0.75 mL of homogenate was precipitated with 0.75 mL of 4% sulfosalicylic acid. The samples were centrifuged at 1200x g for 15 min at 48°C, the assay mixture containing 0.5 mL of supernatant and 4.5 mL of 0.01 M DTNB. The yellow color developed was read immediately at 412 nm using Perkin-Elmer lambda 20 spectrophotometer. The results were expressed as nmol GSH per mg protein.

### **Behavioral tests**

**Morris water maze:** Morris water maze learning task adapted for mice was conducted to assess spatial learning ability (Morris, 1984) which is used to evaluate the hippocampus dependent learning and memory in animal models (De-Qiang *et al.*, 2011). It consisted of a circular pool (130 cm in diameter, 70 cm deep) filled with water (25±1°C) to a depth of 20 cm having a platform (6 cm in diameter) placed 1 cm below the water surface in a constant position in the middle of the southwest (SW) quadrant. The water was made opaque by mixing a non-toxic white opacifier.

Curtains were drawn around the pool and contained distinctive visual marks. The maze was operationally sectioned into four equal quadrants: NW, NE, SW and SE. Twenty four hours after the last administration of PTZ, this experiment was started. Entry points were at the quadrant corners (i.e., N, S, E and W) and were pseudo-randomly assigned so that each trial began at a different entry point than the preceding trial. In each trial, the mouse was released gently into the pool facing the wall of one of four starting positions and was allowed to swim until it reached onto the platform. When a mouse could not reach the platform after 60 sec, it was placed on the platform by the experimenter. In either case, the mouse was left on the platform for 30 sec and then removed from the pool, dried with a towel and returned to the cage. On day 0, a 60 sec free-swim trial without the platform was carried out for habituation. Training consisted of four trials per day for 5 consecutive days. The time interval between each training sessions was 30 min (Kim *et al.*, 2003). The latency to find the hidden platform, distance traveled and path of travel were recorded and analyzed using the Ethovision Behavioral Analysis System version 2.3 (Nodulus, Wageningen, The Netherlands).

**Elevated plus maze:** This apparatus comprised two open arms (16×5 cm) and two closed arms (16×5×10 cm) that extended from a common central platform (5×5 cm). The entire maze was elevated to a height 50 cm above floor level. The negative control group received distilled water, the positive control group received diazepam (3 mg kg<sup>-1</sup>) and the three test groups received three different doses of PEE for 21 days. After treatment of different groups, the mice were individually placed on the Elevated Plus Maze (EPM) center platform facing an open arm observed for 5 min (Bum *et al.*, 2009). The number of entries by each animal into open or closed arms and the time spent by each animal in either open or closed arms (conventional parameters) were recorded with stopwatches by two trained experimenters.

**Rotarod test:** Effects on motor function were assessed in a Rotarod test (Techno) using a rod with a diameter of 3 cm rotating at a constant speed of 6 rpm. The mice were placed on the rotating rod for 2 min and the fall off time was noted.

**Grip strength:** The effects of PEE (600 mg kg<sup>-1</sup>) on muscular strength (tone) in mice were assessed by the grip-strength test. The grip-strength apparatus comprised a wire grid (8×8 cm) connected to an isometric force transducer (dynamometer). The mice were lifted by the tails so that their forepaws could grasp the grid. The mice were then gently pulled backward by the tail until the grid was released. The maximal force exerted by the mouse before losing grip was recorded. The mean of 3 measurements for each animal was calculated and subsequently, the mean maximal force of 8 animals per group was determined. The neuromuscular strength in mice was expressed in N (Newtons) as Means±S.D. of at least 24 determinations (3 measurements for each of 8 animals per group) (Ali *et al.*, 2004; Luszczki and Czuczwar, 2007).

**Statistical analysis:** Seizure severity scores were compared using Kruskal-Wallis one-way analysis of variance on ranks followed by multiple comparison tests. For incidence %, Fisher's exact probability test was used. The p-values <0.05 were considered significant.

## RESULTS

**Phytochemical characterization and LC-MS fingerprinting:** Chemical characterization showed that PEE mainly contained: glycosides, terpenoids, anthraquinones, tannins, carbohydrates and traces of alkaloids but not saponins and flavonoids (Fig. 2).

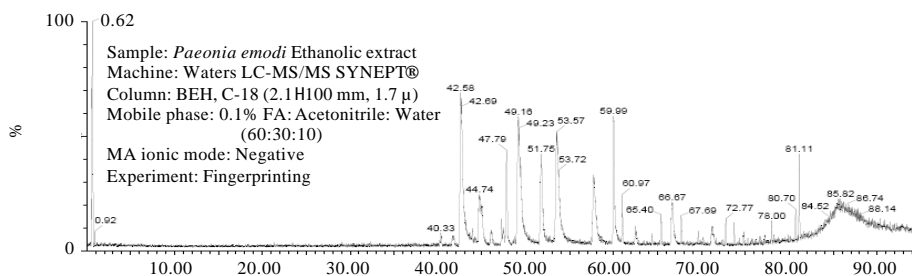


Fig. 2: LC-MS fingerprinting mass spectrum of *Paeonia emodi* ethanolic extract

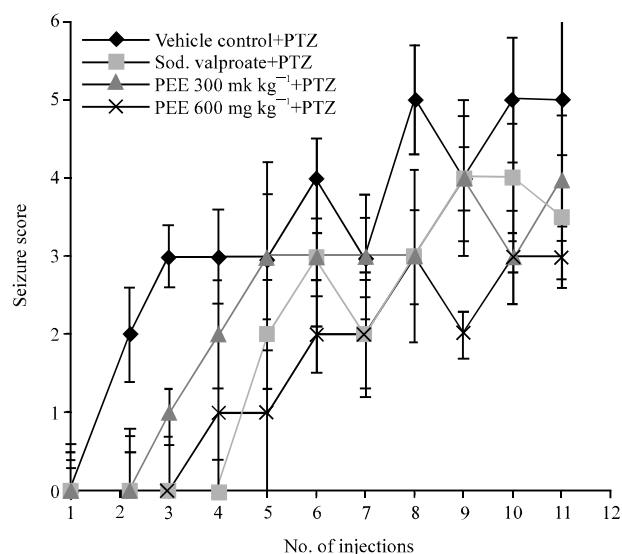


Fig. 3: Effect of PEE on seizure severity during induction of kindling by PTZ in mice. Vehicle, Sodium Valproate and PEE were administered daily, while PTZ ( $35 \text{ mg kg}^{-1}$ , i.p.) was given once every 2 days for 26 days. Data are presented as median seizure score with upper and lower quartiles. The number of animals per group was 20

**Effect of sodium valproate and PEE on induction of PTZ-kindling:** All mice in each group survived without any complications at the end of the kindling period. In the PTZk group, repeated administration of a subconvulsant dose of PTZ ( $35 \text{ mg kg}^{-1}$ ) on every second day (for 24 days, 11 injections) resulted in increasing convulsive activity leading to generalized clonic-tonic seizure score of 5. Administration of Sodium valproate ( $100 \text{ mg kg}^{-1}$ ) did not alter the course of kindling induced by PTZ. However, treatment with PEE at the dose of  $600 \text{ mg kg}^{-1}$  suppressed the kindled seizure significantly, as none of the animals could achieve a score of 5 with 11 injections of PTZ ( $35 \text{ mg kg}^{-1}$ ). PEE  $600 \text{ mg kg}^{-1}$  treated mice revealed significant decrease in seizure scores on day 26th relative to control group ( $p < 0.05$ ). Administration of PEE ( $600 \text{ mg kg}^{-1}$ ) significantly decreased the seizure score on day 26th when compared with Sodium valproate+PTZk mice ( $p < 0.01$ ) (Fig. 3).

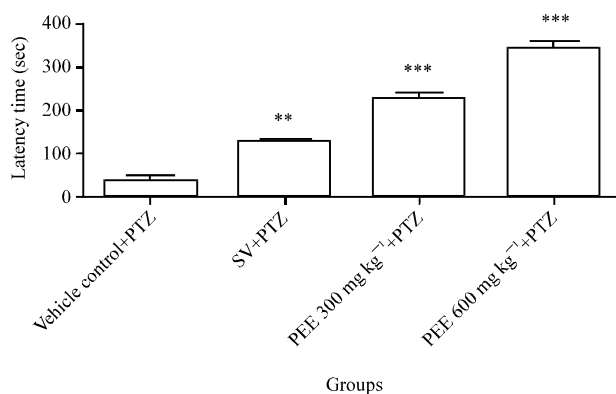


Fig. 4: Comparison of the latency of the seizures to PTZ challenge dose ( $75 \text{ mg kg}^{-1}$ , i.p.) on 26th day in kindled mice. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  as compared to vehicle control+saline group (Group 1)

**PTZ test dose:** On the test day (PTZ  $75 \text{ mg kg}^{-1}$ , i.p.), PTZ-kindled mice developed a classical pattern of limbic type motor seizures with a median score of 5. The convulsive response consisted of first twitch, short-lasting episodes of clonic seizures and then continuous clonic seizures with wild running ending with falling of the animal and tonic seizures. Pretreatment with SV did not prevent the development of seizure. SV pretreated PTZ kindled animals produced a seizure intensity which was the same as the saline treated PTZ kindled mice. However, in this group, a higher latent period was observed when compared with the PTZk group ( $p < 0.01$ ). PEE ( $600 \text{ mg kg}^{-1}$ ) treated mice displayed a significant attenuated response to PTZ on the test day (26) compared with saline treated and Sodium Valproate treated animals ( $p < 0.001$ ) (Fig. 4). Pretreatment with PEE ( $600 \text{ mg kg}^{-1}$ ) significantly protected against the convulsive behaviors (seizure latency, seizure score) and mortality induced by PTZ. All mice survived without any complications in the PEE ( $600 \text{ mg kg}^{-1}$ ) group, while seven mice in the PTZk group and five mice in the Sodium Valproate+PTZk group died, because of severity of seizure.

**Effect of PEE (300 and  $600 \text{ mg kg}^{-1}$  p.o.) on PTZ-kindling induced brain biochemical changes:** Repeated treatment with PTZ induced oxidative stress as indicated by a significant rise in the whole brain MDA levels, an indicator of lipid peroxidation. PEE ( $600 \text{ mg kg}^{-1}$ , p.o.) (Group 4) treatment attenuated the increased malondialdehyde levels ( $p < 0.001$ ) as compared to PTZ treated group (Fig. 5).

In addition, PTZ treatment also decreased whole brain glutathione (GSH) levels, as compared to vehicle treated group. Pretreatment with PEE ( $300$  and  $600 \text{ mg kg}^{-1}$  p.o.) significantly improved ( $p < 0.05$  and  $p < 0.001$ ) the depleted GSH levels respectively (Fig. 6).

**Effect of PEE on morris water maze:** Water maze performance in control and kindled groups with and without PEE administration tested 24 h after last injection.

**Escape latency:** PTZ induced kindling significantly impaired the acquisition of spatial learning compared with saline-treated control. The animals of all groups showed improved Morris water maze acquisition performance, i.e., decreased latency to find the platform from the first to last day of training and it was highly improved in control and PEE groups. However, PTZ kindled (PTZk) group animals presented a higher latency to find the platform than control but PTZk+PEE group exhibited significant improvement over PTZk group (Fig. 7).



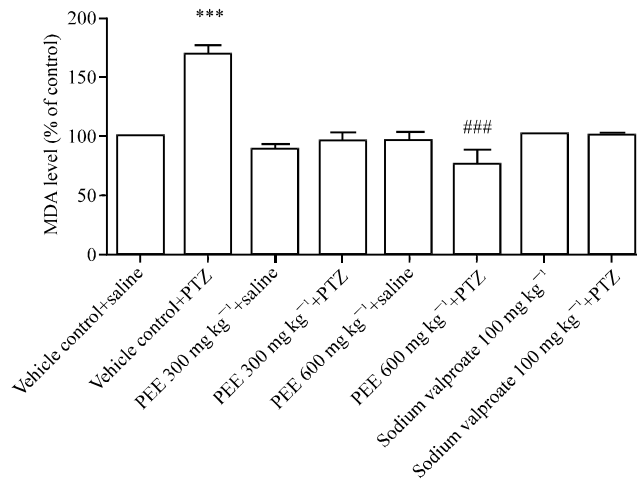


Fig. 5: Effect of PEE pretreatment (300 and 600 mg kg<sup>-1</sup> p.o.) on PTZ-induced lipid peroxidation in mice. \*\*\*p<0.001 as compared to vehicle control +saline group (Group 1). ###p<0.001 as compared to control ± PTZ treated group (Group 2)

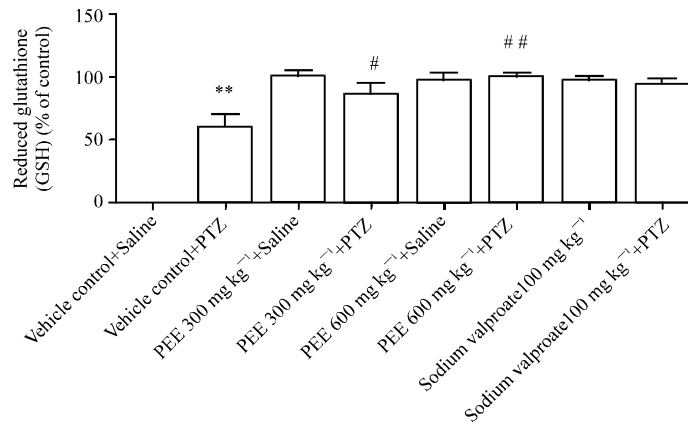


Fig. 6: Effect of PEE pretreatment (300 and 600 mg kg<sup>-1</sup> p.o.) on PTZ-induced glutathione (GSH) depletion in mice. Values expressed as percent response of vehicle treated control group. \*p<0.01 as compared to vehicle control+saline group (Group 1), #p<0.05, ##p<0.01 as compared to vehicle control+PTZ treated group (Group 2)

**Path length:** The animals of all groups performed improved Morris water-maze acquisition i.e., decreased path length to find the platform from the first to last day of training and it was highly improved in saline and PEE groups. However, the animals of PTZk group travelled farther in finding the platform than control and it was significantly improved in PTZk+PEE group as compared with PTZk group (Fig. 8).

**Effect of PEE on elevated plus maze:** Compared with distilled water, PEE 600 mg kg<sup>-1</sup> when given orally resulted in a significant increase in the percentage number of entries into open arms (p<0.001) and the percentage of time spent in open arms (p<0.01) of the EPM. As expected for a

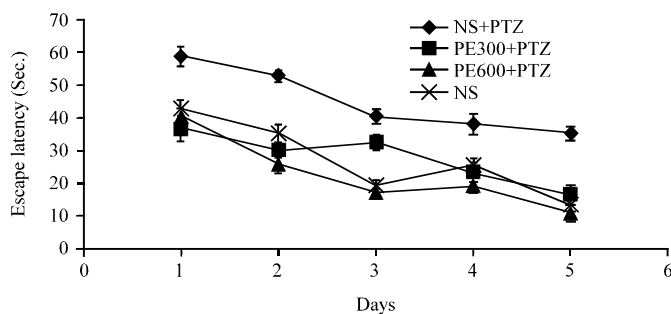


Fig. 7: Comparison of Escape Latency by mice to acquire platform on the Morris water maze task among the four groups of mice. Mean values of the four trials per day for 6 days for each of the three groups are shown. Repeated measures of ANOVA of swimming time among the groups followed by Dennett's test. The  $p < 0.05$ , as compared with the corresponding data of the Vehicle group

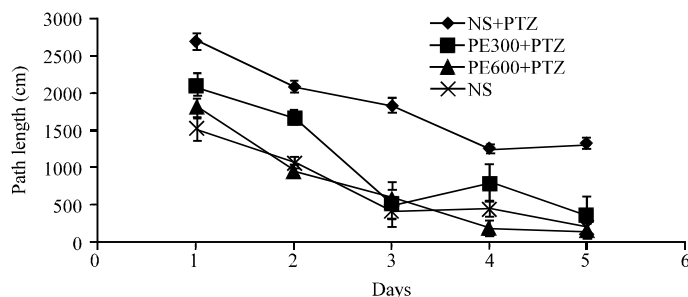


Fig. 8: Comparison of path length travelled by mice to acquire platform on the Morris water maze task among the four groups of mice. Mean values of the four trials per day for 6 days for each of the three groups are shown. Repeated measures of ANOVA of swimming time among the groups followed by Dennett's test. The  $p < 0.05$ , as compared with the corresponding data of the Vehicle group

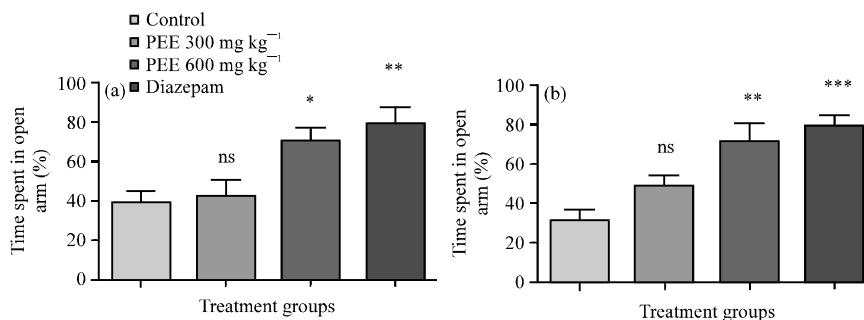


Fig. 9(a-b): Effects of PEE on percent time spent and number of entries in the open arm of the elevated plus maze. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns: Not significant

positive control group, diazepam 3 mg kg<sup>-1</sup> i.p., also caused an increase in the percentage of entries into and time spent in the open arms of the EPM (Fig. 9).

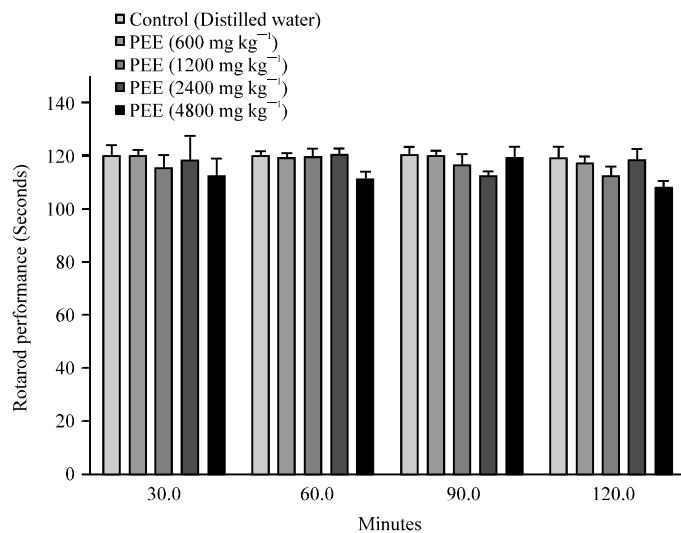


Fig. 10: Rotarod performance of the mice at various doses of PEE on different time intervals

Table 2: Effect of PEE (600 mg kg<sup>-1</sup>) on Grip strength

Treatment	Grip strength (N)
Vehicle control	84.36±3.77
PEE 300 mg kg <sup>-1</sup>	81.25±3.29
PEE 600 mg kg <sup>-1</sup>	86.75±2.59

### Effect of PEE on motor impairment

**Rotarod test:** PEE (600, 1200, 2400 and 4800 mg kg<sup>-1</sup>) on repeated administration did not exhibit any significant difference in the fall-off time when compared with control group (Fig. 10).

**Grip strength:** PEE (600 mg kg<sup>-1</sup>) did not elicit any significant impact on muscular strength of animals as assessed by the grip-strength test (Table 2).

## DISCUSSION

Kindling is a model of epilepsy and epileptogenesis and stands as a model to investigate effects of potential antiepileptogenic compounds on the reorganization of neuronal circuits which have similarities to those that occur after status epilepticus and lead to development of spontaneous seizures (Temkin *et al.*, 2001). Kindling also induces behavioral alterations and causes long term deficits in cognitive function (Sutula *et al.*, 1995; Gilbert *et al.*, 2000; Hannesson *et al.*, 2001). Various previous studies have shown that the kindling model allows the distinction between antiepileptogenic and anticonvulsant effects of a compound. The term “antiepileptogenic” refers to inhibition of processes underlying the development of the epileptic condition, whereas “anticonvulsant” refers to inhibition of seizures in an epileptic condition. Drugs that inhibit the development of kindling may have antiepileptogenic properties in humans, whereas drugs that inhibit seizures evoked in kindled animals may have anticonvulsant properties in humans (Sato *et al.*, 1990). In the present study, it was observed that the repeated administration of subconvulsant dose of PTZ (35 mg kg<sup>-1</sup>, i.p.), a blocker of GABA<sub>A</sub> receptor mediated Cl<sup>-</sup> channel, for 11 times resulted in stage 5 seizures, which was associated with cognitive impairment as

evidenced by altered performance on morris water maze. Our study demonstrated that PEE produces potent antiepileptogenic effects in the kindling model during kindling development and protects PTZ induced memory and other neurobehavioral impairments. However, additional strong evidence using objective measures such as EEG is also needed.

The products of oxidation are produced during seizures and are involved in the tissue injury due to epileptic attacks. Oxidative injury has been implicated in the development of seizures (Sejima *et al.*, 1997; Sperk, 1994). Therefore, one of the main trends of the current investigations is the search of novel antiepileptic drugs with neuroprotective properties. Some antioxidants have been shown to be effective in reducing the oxidative stress in the models of epilepsy and other neurotoxicity induced by other agents (Willimore and Rubin, 1981; Kabuto *et al.*, 1998; Elsaid *et al.*, 2011). This study clearly demonstrated a potent anticonvulsant property of PEE against the course of kindling consequences in PTZ-kindled mice. PEE 600 mg kg<sup>-1</sup> was more potent as an anticonvulsant agent than Valproate against the effects of the test dose PTZ (75 mg kg<sup>-1</sup>) in PTZ-kindled mice. Moreover, our results showed that PEE was a very effective neuroprotective agent against PTZ-kindled seizures via its potent antioxidant activity.

The increase in the levels of MDA, a marker of lipid peroxidation in our study, indicates increased free radical generation in the vehicle-treated PTZ mice. The significantly lower levels of MDA in brain of the 300 and 600 mg kg<sup>-1</sup> PEE treated PTZ mice as compared with the vehicle treated PTZ mice indicate attenuation of lipid peroxidation. There was a simultaneous significant decrease in the reduced glutathione levels in vehicle-treated PTZ mice. 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. During this defensive process, reduced glutathione gets converted to its oxidized form with the help of the enzyme glutathione peroxidase. The decreased level of reduced glutathione in vehicle-treated PTZ seen in our study indicates that there was an increased generation of free radicals and the reduced glutathione was depleted during the process of combating oxidative stress (Schulz *et al.*, 2000). The decrease in MDA levels and increase in PEE-treated PTZ groups may be due to its antioxidant property.

As evidenced by the morris water maze test performance, the spatial learning and memory ability of the PTZ-kindled mice was impaired, which further proved that PTZ kindling could impair cognitive ability. However, long term treatment of PEE effectively improved the impaired learning and memory performance in PTZ-kindled mice. This result indicates that PEE ameliorates cognitive decline induced by PTZ kindling.

The anxiolytic potential was confirmed by the Elevated Plus Maze (EPM) test, where PEE increased the number of entries and time spent in open arms and reduced the time spent in closed arms. Results for 600 mg kg<sup>-1</sup> dose of PEE are up to some extent comparable to diazepam. These anxiolytic properties could be mediated by some components in the extract interacting with benzodiazepine/ GABA<sub>A</sub> receptors as agonists, with the NMDA receptors as antagonists, or with any other mechanisms (Finn, 2001; Tunnickliff, 1991; Somani *et al.*, 2010).

Besides showing antiepileptic and other behavioral modulation, PEE did not elicit any adverse effect on motor impairment or muscular strength at active anticonvulsant dose level (600 mg kg<sup>-1</sup>) which was evidenced by their performance on Rotarod and grip strength test, respectively. Though we used the maximum dose for Rotarod test (4800 mg kg<sup>-1</sup>) which was six times higher the active anticonvulsant dose and did not find any motor impairment till this dose level but, the limitation of our study was that we could not calculate the exact ED<sub>50</sub> and TD<sub>50</sub> values and hence, were unable to determine the proper protective index of the drug.

The mechanism by which PEE exerts this antiepileptogenic action is uncertain. Interestingly, similar to valproate and phenobarbital, there is some evidence that PEE may act, at least in part, through potentiation of GABAergic inhibition (Loscher *et al.*, 1996), by stimulating the activity of GABA synthetic enzyme Glutamate decarboxylase and inhibit GABA degradive enzymes (Abdel-Wahab and Metwally, 2011). Impairment of GABAergic inhibition is thought to play a crucial role in the processes underlying epileptogenesis in the kindling model (Sato *et al.*, 1990). Therefore, the GABA-mimetic effects of valproate, phenobarbital and levetiracetam could explain, at least in part, their antiepileptogenic activity in this model. As we are aware that the pharmacological activity of the medicinal plants are due to the synergistic action of the various components which exert their effects in a holistic way, hence, we are not able to pin point any individual component for the observed therapeutic activity. Nonetheless, paeoniflorin, has been reported to possess anticonvulsant and other neurobehavioral modulatory potential. Though we performed UPLC-MS fingerprinting of the crude PEE but non-availability of the marker compound restricted us to qualitative analysis only. Therefore, analytical profile of the extract using marker compounds and their pharmacokinetic data is also an important need to have insight to the exact mechanism of action.

## CONCLUSION

The present study demonstrates that PEE significantly attenuated PTZ-kindling and prevented associated memory impairment, oxidative damage, anxiety without showing any sign of motor impairment. Therefore, it could offer an alternate support to the antiepileptic therapy in preventing the development of cognitive impairment reported with several AEDs. However, we emphasize the preliminary nature of this study.

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