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# Dual protective effect of *Passiflora incarnata* in epilepsy and associated post-ictal depression

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ABSTRACT

*Ethnopharmacological relevance: Passiflora incarnata* L. (Passifloraceae) has been used for the treatment of epilepsy in several traditional systems of medicine.
*Aim of the study:* The aerial parts of *Passiflora incarnata* contain multiple bioactive metabolites such as, flavonoids (like, chrysin that show CNS depressant activity by agonizing GABA–benzodiazepine receptor), amino acids (like, GABA), harmala alkaloids (reversible monoamine oxidase-A inhibitor), etc. In view of this, the present study was designed to investigate dual protective effect of the hydroethanolic extract of *Passiflora incarnata* in pentylenetetrazol (PTZ)-induced seizure and associated post-ictal depression.
*Materials and methods:* Different groups of mice were administered with repeated subconvulsive doses of PTZ (50 mg/kg; *i.p.*) at an interval of 5 days for 15 days. From 5th to 15th day the animals in different groups were administered daily with varying doses of hydroethanolic extract of *Passiflora incarnata* (150, 300, and 600 mg/kg; *i.p.*), diazepam (2 mg/kg; *i.p.*) and vehicle. On every 5th day, after PTZ treatment, seizure severity (score) was noted. Following convulsive episodes the locomotor activity (using actophotometer) and immobility period (using forced swim test) were also determined. On 15th day after behavioral assessment, the brain serotonin and noradrenaline levels were determined using spectrofluorometric methods.
*Results:* Treatment with the extract significantly ($p < 0.05$) reduced the seizure severity and immobility period as compared to vehicle control, in a dose and time-dependent manner. Moreover, the extract treatment retained the serotonin and noradrenaline levels of the brain.
*Conclusions:* The results of present study concluded that the hydroethanolic extract of *Passiflora incarnata* suppress PTZ-induced seizures, and ameliorates its associated post-ictal depression, which has been found to be get worsened with the standard antiepileptic drug, diazepam.


## 1. Introduction

Epilepsy is a chronic neurological disorder characterized by episodic and unpredictable incidence of epileptic seizures. It is commonly associated with the brain dysfunctions leading to several behavioral comorbidities like, depressive disorders (DDs) (Hecimovic et al., 2003). There is a high prevalence of DDs among epileptic patients. In a population-based survey it has been found that 29% of epileptic patients suffer from depression, in contrast to 8.7% of healthy individuals (Seminario et al., 2009).

In spite of high prevalence, the treatment of depression in epilepsy still remained a question. Depression in epileptic patients often goes untreated due to inevitable fear of the clinicians that antidepressant drugs may lower seizures threshold, and thus may worsen the epileptic condition. Even if treatment is attempted, it is limited to antidepressant drugs, again on trial basis as there is no specific drug available for the treatment of depression in epilepsy (Mula and Schmitz, 2009). On one hand, most of the antidepressant agents have a tendency to lower the seizures threshold; on other hand majority of antiepileptic drugs themselves causes CNS depression (Kanner, 2003a). The possible reason is the common pathogenic mechanisms among these two disorders, epilepsy and depression (Montgomery, 2005). Elevation of one leads to treatment of other, for example, electroconvulsive therapy has efficacy in treatment of depression, and epileptic patients experience an increased occurrence of depressive episodes, when seizures frequency falls in response to antiepileptic therapy (Jobe, 2003).

All these issues indicate an unmet need for the discovery of remedies to treat depression in epilepsy, without affecting seizures

*Abbreviations:* CMC, Carboxymethyl cellulose; CNS, Central nervous system; DDs, Depressive disorders; DMSO, Dimethyl sulfoxide; FST, Forced swimming test; GABA, Gamma-aminobutyric acid; *i.p.*, Intraperitoneal; MAO, Monoamine oxidase; OTC, Over-the-counter; PI, *Passiflora incarnata* hydroethanolic extract; PTZ, Pentylenetetrazol; RP-HPLC–UV, Reversed phase-high performance liquid chromatography–ultraviolet detection; s, Seconds.

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threshold. In spite of all the marvelous advancements in modern medicine, traditional medicine has always been practiced (Shaikh and Hatcher, 2005). Plants and their extracts are better choice to treat such type of diseases (in which treatment of one worsens the other), because of presence of antagonistic substances in them, due to which they show lesser or no side effects (Farnsworth, 1966). While searching some herbal remedies, we found literature reports pertaining to the ethnomedical use of *Passiflora incarnata* L. (Passifloraceae) in treatment of epilepsy. Formerly its flower has been approved as an OTC sedative in USA (Khare, 2007). It is an official plant that has been included in official pharmacopeias of different countries. Phytochemical research carried out on *Passiflora incarnata* had lead to the isolation of several bioactive metabolites like, chrysin, apigenin, homoorientin, vitexin, luteolin, quercetin, kaempferol, isovitexin, orientin, isoorientin, schaftoside, isoschaftoside, harman, harmol, harmine, harmalol, and harmaline, etc. (Dhawan et al., 2004). Several CNS depressant effects of *Passiflora incarnata* have been experimentally explored and suggested principally due to the presence of flavonoids like chrysin that act by agonizing benzodiazepine receptor (Medina et al., 1990; Wolfman et al., 1994; Dhawan et al., 2004; Johnston and Beart, 2004; Nassiri-Asl et al., 2007). Recently the presence of GABA has been reported in *Passiflora incarnata* and suggested to elicit GABA currents in hippocampal neurons (Elsas et al., 2010). Apart from flavonoids and recently reported GABA, *Passiflora incarnata* also contains harmala alkaloids. These alkaloids are reversible monoamine oxidase (MAO)-A inhibitor in nature and show antidepressant activity (Callaway et al., 1999; Abourashad et al., 2003). Presence of these diverse bioactive metabolites in *Passiflora incarnata* led us to hypothesize that it might offer dual protection by ameliorating post-ictal depression as consequence of epilepsy, without affecting seizures threshold.

Based on this the present study was envisaged to investigate the effect of hydroethanolic extract of *Passiflora incarnata* on epilepsy and its associated post-ictal depression in mice. This is the first attempt made to explore the above mentioned dual effect of *Passiflora incarnata*.

## 2. Materials and methods

### 2.1. Drugs and chemicals

Pentylenetetrazol (dissolved in normal saline) was obtained from Sigma Chemical Company (USA), chrysin and serotonin from Acros Organics (Belgium), dimethyl sulfoxide (DMSO) from Qualigens Fine Chemicals (Mumbai, India), carboxymethyl cellulose (CMC) from s.d. Fine Chem Ltd. (Mumbai, India) and HPLC grade solvents from Spectrochem Pvt. Ltd. (India). A reference drug diazepam was obtained locally from Jackson Laboratories Ltd. (India). Noradrenaline was obtained as a gift sample from Troikaa Pharmaceuticals Ltd. (Dehradun, India). The standardized (previously standardized by manufacturers using isovitexin as a marker) hydroethanolic extract of *Passiflora incarnata* was supplied as a gift sample by Alchem International Ltd. (Ballabgargh, India).

### 2.2. Phytochemical analysis

*Passiflora incarnata* extract was subjected to preliminary phytochemical tests to determine the presence of alkaloid, carbohydrates, glycosides, saponins, steroids, triterpenoids, tannins, flavonoids, proteins and amino acids (Trease and Evans, 2002).

Chrysin being one of the principal anticonvulsant flavonoid present in *Passiflora incarnata* was used as a biomarker for the standardization of the hydroethanolic extract. RP-HPLC–UV method was used for the determination of chrysin in the extract. Briefly, 500 mg of the extract was dispersed in water and defatted with petroleum ether. Aqueous portion was partitioned with ethyl acetate. The ethyl acetate fraction was dried and 100 μg/mL solution of the fraction made in methanol was used for chrysin determination. HPLC system consisted, 515 binary pump (Waters), 2487 dual wavelength UV detector (Waters) and Rheodyne manual injector. The chromatographic separation was achieved on reversed-phase analytical column (150 mm × 4.6 mm, 5 μm; Agilent, USA). Mobile phase comprised, methanol:water (85:15) which was filtered through a 0.45 μm membrane (Millipore, USA) and degassed on a sonicator (Transsonic T 570/H, Elma, Germany). The injection volume was kept 20 μL and the peaks were identified by comparison with the retention time of standard solution. Prior to HPLC analysis, the optimized UV spectra of standard chrysin solution was determined by wavelength scanning between 200 and 400 nm using a standard UV system (Beckman DU 640 B). Quantification was based on the standard curve of chrysin ($y = 295.6x + 6491$; $R^2 = 0.999$).

The determination test for the presence of harmala alkaloids was performed by the method described by Tauber (1949) with slight modifications. Briefly, the extract was dispersed in ethanol (1%) and the resultant solution was placed in a test tube (1 mL). To which 3 mL of perchloric acid (70–72%) and 0.1 mL of dichromate solution (0.01%) was added and contents of the tube were mixed. After observing the color changes, 0.1 mL of 1% ferric chloride solution was added and again observed for further color changes.

### 2.3. Animals

Male Swiss Albino mice, weighing 20–30 g obtained from Central Research Institute, Kausali, Himachal Pradesh, were employed in the present study in different groups ($n = 6$). The animals were housed in standard cages and were maintained at room temperature with natural day and night cycles. The animals were allowed free access to food (standard laboratory rodent's chow) and water during the study period. The experiments were conducted between 9.00 and 16.00 h. All procedures were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India. The experimental protocol was approved by the Institutional Animal Ethical Committee (107/99/CPCSEA-2008/01).

### 2.4. Preparation of test samples and dosing

The dose of extract was selected based on the literature reports (Soulimani et al., 1997) and administered at 150, 300 and 600 mg/kg doses. The extract was reconstituted by dissolving in DMSO and then dispersing the resultant solution in 0.5% CMC made in saline (DMSO 1: CMC 9) freshly before use and was injected intraperitoneally (*i.p.*). Vehicle control groups received equal volume of vehicle (DMSO 1: CMC 9) *i.p.* (injection volume 10 mL/kg).

### 2.5. Induction of post-ictal depression

The test procedure was based on the fact that, repeated convulsive attack leads to worsening of epileptic condition, which is associated with behavioral abnormalities (Hecimovic et al., 2003; Kanner, 2003b; Mazarati et al., 2008). The animals were divided into six different groups designated as, Group I: sham control (naïve), Group II: vehicle control, Group III: standard and Group IV–VI: extract treated. The animals in different groups were treated as shown in Fig. 1. Briefly, on 1st day, all the animals of vehicle control, standard and extract treated groups were injected with PTZ (50 mg/kg; *i.p.*). From 5th to 15th day, the extract treated groups were injected daily with varying doses of the extract (150, 300,

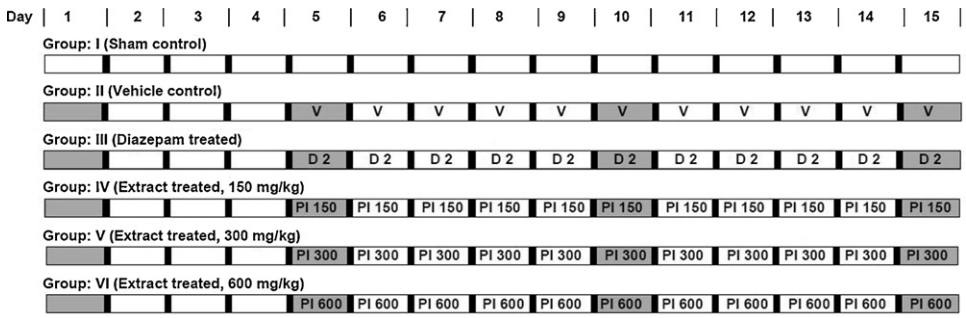


**Fig. 1.** Schematic representation of experimental protocol. Shaded boxes represent PTZ treatment. PTZ was administered 30 min after respective treatment on day 5, 10 and 15. V: vehicle (10 mL/kg); D2: diazepam (2 mg/kg); PI 150, 300 and 600: *Passiflora incarnata* hydroethanolic extract 150, 300 and 600 mg/kg, respectively.

and 600 mg/kg; *i.p.*), vehicle treated group with vehicle (10 mL/kg; *i.p.*) and standard group with diazepam (2 mg/kg; *i.p.*), whereas no treatment was given to the sham control group. On every 5th day, after 30 min of the treatment the animals of vehicle control, standard and extract treated groups were injected with 50 mg/kg; *i.p.* of PTZ. Induced seizures as a consequence of PTZ were given a numeric score as: unresponsiveness = 0, mild contractions = 1, clonic seizures = 2, tonic seizures = 3 (forelimb and then hindlimb rigidly extended to rear) and death = 4 (Balter-Seri et al., 1999). After cessation of seizures, locomotor activity of the animals was accessed using an actophotometer (INCO, India). Animals were individually placed in the actophotometer and total activity count was registered for 5 min. The locomotor activity was expressed in terms of total photo beam interruption counts/5 min (Singh and Goel, 2009).

Thereafter the animals were subjected to forced swimming test (FST) to assess the depressive behavior. Briefly, the animals were placed individually in a glass cylinder ($25 \times 12 \times 25\ cm^3$), containing water at 25 °C (±3 °C) up to a level 15 cm for 5 min and total immobility period (seconds) was recorded. The animals were judged to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water (Porsolt et al., 1977).

### 2.6. Estimation of noradrenaline and serotonin levels of the brain

Mice were sacrificed by decapitation on 15th day, 10 min after behavioral assessment. The whole brain was removed and homogenized in phosphate buffer. The brain tissue samples were taken for the determination of noradrenaline and serotonin levels by fluorometric methods described by Weil-Malherbe (1971) and Snyder et al. (1965) respectively, using a spectrofluorometer (ELICO, SL-174). Noradrenaline and serotonin levels of the brain were expressed as percentage of control.

### 2.7. Statistical analysis

Results were expressed as mean ± SEM. The intraday significance of difference in the responses between sham control, vehicle control, standard and extract treated groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's test. The results were regarded as significant at $p < 0.05$.

## 3. Results

### 3.1. Phytochemical analysis

Preliminary phytochemical screening of hydroethanolic extract of *Passiflora incarnata* showed the presence of alkaloid, carbohydrates, glycosides, flavonoids, proteins and amino acids.

Solvent–solvent partitioning of the hydroethanolic extract of *Passiflora incarnata* yielded 0.95% (w/w) of ethyl acetate fraction. The optimal UV spectrum for chrysin detection was calibrated at 269 nm. The HPLC chromatogram of standard chrysin solution showed an absorption peak with a retention time of 4.3 min (Fig. 2). A similar peak was observed in the HPLC chromatogram of the ethyl acetate fraction at similar retention time (Fig. 3), indicating the presence of chrysin. Based on the calibration curve, the quantity of chrysin was found to be 32.5 ± 0.60 ($n = 3$) μg/g of the hydroethanolic extract.

In specific alkaloid determination test, addition of perchloric acid and dichromate solution a light yellow to almost colorless mixture was formed. On subsequent addition of ferric chloride gave

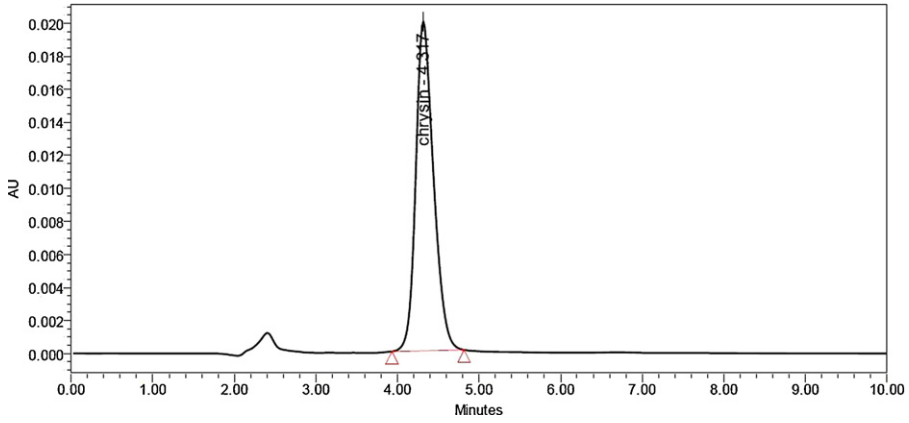


**Fig. 2.** HPLC chromatogram of standard chrysin.

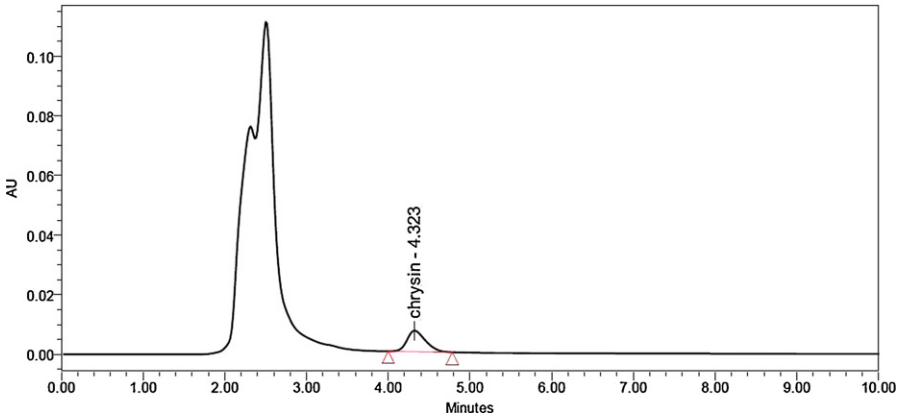


**Fig. 3.** HPLC chromatogram of ethyl acetate fraction of *Passiflora incarnata* hydroethanolic extract.

greenish blue color, thus confirmed the presence of indole component (harmala alkaloids) in the extract.

### 3.2. Effect of the extract on post-ictal depression

The seizure severity score in vehicle control group increases with subsequent doses of PTZ from 1st to 15th day. Treatment with the extract significantly ($p < 0.05$) decreased the seizure severity score at 300 and 600 mg/kg dose on 5th day, and at all doses i.e., 150, 300 and 600 mg/kg dose on 10th and 15th day as compared to vehicle control group. Moreover, the extract treatment at a dose of 300 and 600 mg/kg showed no seizures on 15th day, and 10th and 15th days, respectively (Fig. 4). The extract at 600 mg/kg dose showed similar anticonvulsant effects as that of diazepam. Since the sham control group was not injected with PTZ, hence not shown in Fig. 4. Treatment with the extract (150, 300 and 600 mg/kg) and diazepam significantly ($p < 0.05$) reduced the locomotor activity as compared to sham control group (Fig. 5). In case of antidepressant studies, vehicle control and diazepam treated groups showed significant ($p < 0.05$) increase in immobility period as compared to sham control group on 10th and 15th day, exhibiting the induction of depression. Whereas, the extract treated groups (150, 300 and 600 mg/kg) showed no change in immobility period as compared to sham control group. Moreover treatment with the extract at 600 mg/kg dose showed significant ($p < 0.05$) decrease in the immobility period on 15th day as compared to vehicle control group (Fig. 6).

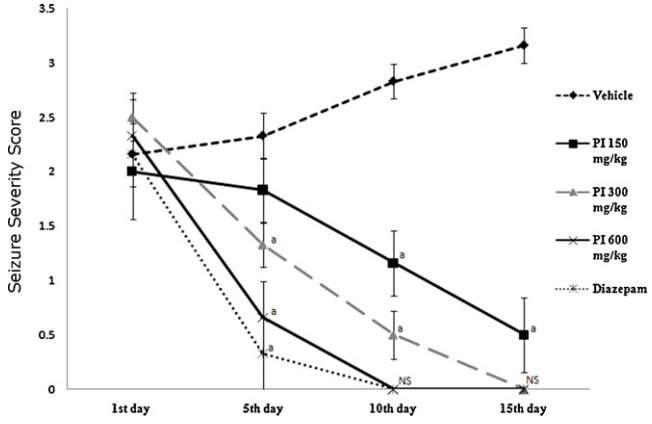


**Fig. 4.** Effect of *Passiflora incarnata* hydroethanolic extract on PTZ-induced seizure severity score. Sham control group is not shown as was not injected with PTZ. [a] $p < 0.05$ as compared to vehicle control (group wise comparison). PI 150, 300 and 600 mg/kg: *Passiflora incarnata* hydroethanolic extract 150, 300 and 600 mg/kg, respectively; NS: no seizure; diazepam 2 mg/kg.

### 3.3. Effect on the brain serotonin and noradrenaline levels

The brain noradrenaline and serotonin levels were significantly ($p < 0.05$) decreased in vehicle control group as compared to sham control group. Treatment with the extract attenuated the levels of serotonin and noradrenaline in a dose-dependent manner. The extract treated groups showed significant ($p < 0.05$) increase in the levels of noradrenaline at 300 and 600 mg/kg doses, and serotonin at 150, 300 and 600 mg/kg doses as compared to vehicle control group. Treatment with diazepam significantly ($p < 0.05$) increased the level of serotonin as compared to vehicle control group (Fig. 7).

## 4. Discussion

It has been well established that repeated induction of seizures lead to induction of depression (Hecimovic et al., 2003; Kanner, 2003b; Mazarati et al., 2008). Therefore in the present study repeated subconvulsive doses of PTZ were injected to induce depression in mice. As expected subsequent administration of PTZ in mice of vehicle control group resulted in increased immobility period in forced swim test, hence considered as depressed. The immobility period in vehicle control group increases with subsequent doses of PTZ. This is in accordance with literature that depression in epilepsy depends upon the severity and frequency of epileptic seizures (Barry et al., 2000).

Treatment with the varying doses of hydroethanolic extract of *Passiflora incarnata* resulted in a significant dose-dependent decrease in seizure severity score and immobility period, indicating its anticonvulsant and ameliorative effect against post-ictal depression. The anticonvulsant effect shown by the extract is in line with previous studies and might be due to the presence of flavonoids, which act by agonizing GABA–benzodiazepine receptor and/or by GABA itself (Medina et al., 1990; Wolfman et al., 1994; Dhawan et al., 2004; Nassiri-Asl et al., 2007). As the depression was induced by repetitive seizures, therefore it can be correlated that the amelioration of depression can be due to suppression of seizures by the extract. But several clinical and preclinical studies carried out in past indicated that the antiepileptic drugs themselves worsen the depressive condition due to their depressant nature (Srivastava, 2000; Tamarelle et al., 2009; Cramer et al., 2010). Moreover if the antidepressant effect observed in extract treated groups was due to seizures suppression, then it must be observed in case of diazepam treated group. But the diazepam treatment worsened the depressive condition, indicated by increased immobility period. Therefore, protection against the post-ictal depression indicated the involvement of other bioactive metabolites present in the extract for the activity.

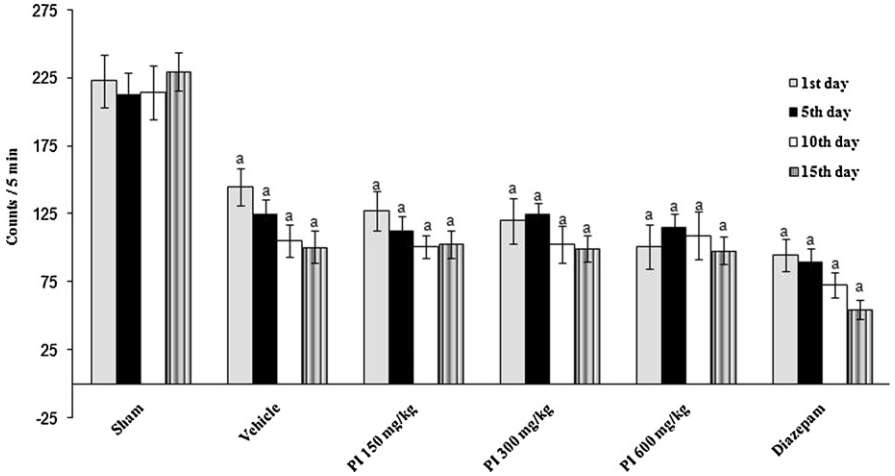


**Fig. 5.** Effect of *Passiflora incarnata* hydroethanolic extract on locomotor activity. [a]$p < 0.05$ as compared to sham control (group wise comparison). PI 150, 300 and 600 mg/kg: *Passiflora incarnata* hydroethanolic extract 150, 300 and 600 mg/kg, respectively; diazepam 2 mg/kg.

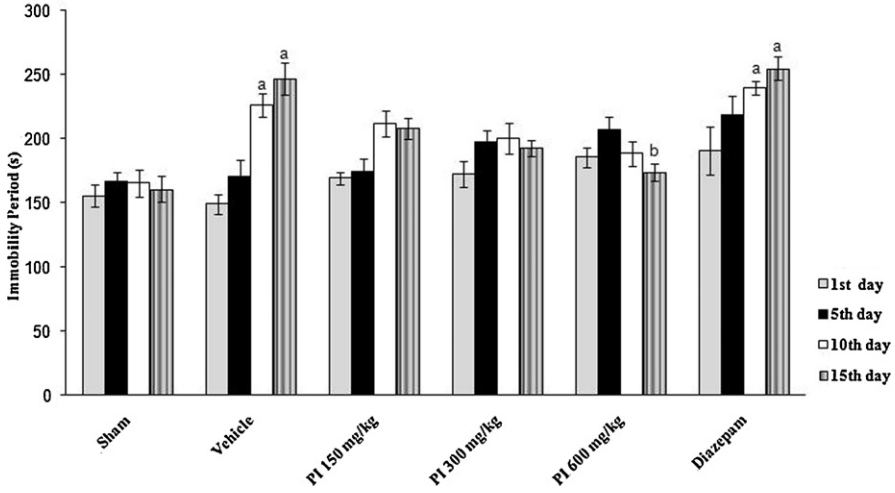


**Fig. 6.** Effect of *Passiflora incarnata* hydroethanolic extract on immobility period. [a]$p < 0.05$ as compared to sham control; [b]$p < 0.05$ as compared to vehicle control (group wise comparison). PI 150, 300 and 600 mg/kg: *Passiflora incarnata* hydroethanolic extract 150, 300 and 600 mg/kg, respectively; s: seconds; diazepam 2 mg/kg.

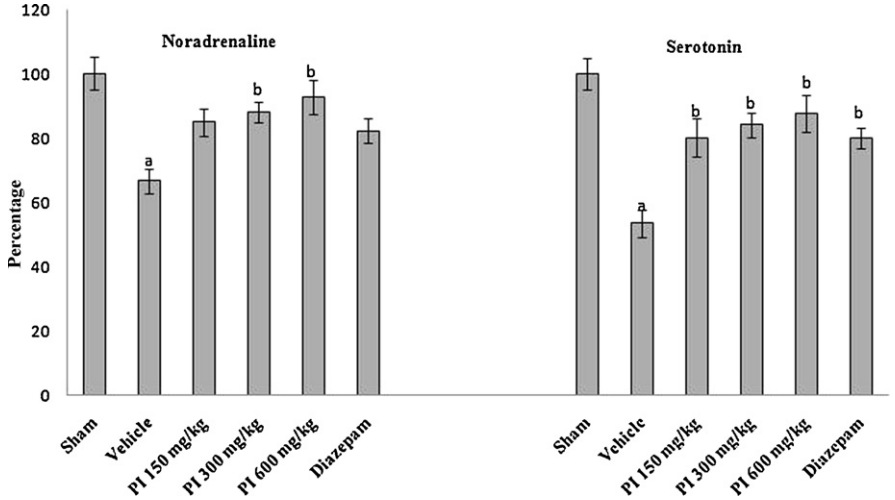


**Fig. 7.** Effect of *Passiflora incarnata* hydroethanolic extract on the brain noradrenaline and serotonin level. [a]$p < 0.05$ as compared to sham control; [b]$p < 0.05$ as compared to vehicle control. PI 150, 300 and 600 mg/kg: *Passiflora incarnata* hydroethanolic extract 150, 300 and 600 mg/kg, respectively; diazepam 2 mg/kg.

A number of possible reasons have been proposed that are responsible for the induction of depression in epilepsy like, abnormal activity of neurotransmitters (serotonin, noradrenalin, etc.), structural changes in temporal and frontal lobe structures, abnormalities in specific receptor binding and abnormal function of hypothalamic–pituitary–adrenal axis (Mula and Schmitz, 2009). It has been reported that experimentally induced seizures in animal can lead to decrease in levels of noradrenaline and serotonin in the brain (Hamdi et al., 1992; Madhyastha et al., 2005). The decreased noradrenaline and serotonin levels observed in the epileptic animals of vehicle control group in our study is in line with the literature findings, and might be a possible cause of induced depression. Substances that increase catecholamine levels of the brain have been reported to ameliorate the post-ictal depression (Montgomery, 2005). Hence, retention of noradrenaline and serotonin levels of the brain after the extract treatment might be a possible cause of post-ictal depression protection. As the initial phytochemical screening showed the presence of harmala alkaloids in the extract that causes inhibition of MAO-A, thereby prevents the degradation of noradrenaline and serotonin (Callaway et al., 1999). Therefore the increased levels of noradrenaline and serotonin in the brains of extract treated animals might be due to MAO-A inhibitory action of harmala alkaloids present in it.

As described, all of the antidepressant drugs have a risk of decreased seizures threshold. The same has been reported for harmala alkaloids in some previous studies in which their *per se* treatment induced convulsions in experimental animals. The convulsive effects of harmala alkaloids were suppressed by administration of GABAergic drugs like, phenobarbitone and diazepam (Pranzatelli and Snodgrass, 1987; Lutes et al., 1988). Thus, indicated the inhibition of convulsive effects of harmala alkaloids by GABAergic substances. This might be a possible reason that why *Passiflora incarnata* extract used in our study containing harmala alkaloids showed no convulsive effect, as the same extract contained GABAergic components like, flavonoids and GABA itself.

The animals receiving PTZ in all treated groups showed decreased locomotor activity in our study. It is in line with the previous reports suggesting, repeated epileptic attack decreases locomotor activity in post-ictal state. The decreased locomotor activity in this state has been suggested due to modulation of mesolimbic dopaminergic neuronal system (Ehlers et al., 1982; Ehlers and Koob, 1985). The decreased locomoter activity was not normalized by either the extract (at all doses) or by diazepam. As the extract due to presence of benzodiazepine agonistic components decreases locomotor activity, moreover harmala alkaloids having selectivity for MAO-A produces no effect on the metabolism of dopamine, therefore do not alter the locomoter activity in post-ictal state.

It has been reported that majority of clinically used anticonvulsant drugs increases the level of endogenous extracellular serotonin, as part of their antiepileptic action (Singh and Goel, 2010). In line to this statement the diazepam treatment showed the retention of serotonin level, but however was devoid of ameliorative effect against post-ictal depression.

## 5. Conclusions

From the results of present study it can be concluded that the hydroethanolic extract of *Passiflora incarnata* has protective effect against epilepsy associated post-ictal depression, without effecting seizures threshold. Further support to this hypothesis may be borrowed from critical clinical observations in future.

## Conflict of interest

The authors report no conflict of interests. The authors alone are responsible for the content and writing of this paper.

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