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ORIGINAL ARTICLE

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Effect of *Pergularia daemia* Forsk. aqueous extract in Pentylenetetrazole-induced wistar albino rats Antiseizure study

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Background: Epilepsy is a Neurological disease. It interferes with the brain's normal electrochemical functions. Similar to a seizure, Epilepsy is an indication of irregular brain activity.
Aim: The current study aims to determine if the Aqueous leaf extract of *Pergularia daemia* Forsk. (AEPD) has the same anti-seizure activity of Pentylenetetrazole (PTZ) induced rats.
Methods: Wistar albino rats were utilized in the studies, and the animals were randomly separated into groups. To determine the antiepileptic potential of rats given Pentylenetetrazole-induced epilepsy, doses of AEPD extract were administered at 100, 200, and 400 mg/Kg BW. Physical behaviors like immobility, swimming, and motor activities were assessed in the experimental animals. By analyzing the levels of neurotransmitters including GABA, Glutamate, Norepinephrine, serotonin, and Acetylcholinesterase in the animal's brain, the effect of PTZ was examined.
Results: The results showed that on the 30th day, acute PTZ administration at a dose of (75 mg/kg bw) produced seizure, which increased immobility and reduced swimming time. Although the plant treatment in PTZ-induced groups showed glutamate, dopamine, serotonin, acetylcholinesterase, and GABA levels were all restored. The PTZ increased oxidative stress followed by neural and non-neural cells (Glial cells) degeneration, which altered levels of neurotransmitters. The results indicate that ***Pergularia daemia*** **Forsk.** a traditional source can be utilized to treat seizures.

Key words: Epilepsy, GABA, Seizure, Pergularia daemia Forsk., Pentylenetetrazole

The rapid and unexpected modification in motor activity as well as behaviour, with or without abnormalities in awareness, is known as epilepsy, a chronic neurological disorder marked by recurring, unexplainable convulsions or seizures. The condition changes as a result of the group of epileptic neurons in the brain firing excessively and abnormally hypersynchronized (Fisher *et al.*, 2005). Around 50 million people are suffering from epilepsy worldwide and among them, 80% are in upward countries. Epilepsy affects all age ranges, particularly people in their first two decades of life, as well as the elderly. It is estimated that the mortality rate for those with epilepsy is 2-3 times more than the general population, and is significantly higher for young age people (Boer *et al.*, 2008). Epileptic foci were shown to have neurotransmitter abnormalities (Badawy *et al.*, 2009). Therefore, ictogenesis is exacerbated by a neurotransmitter imbalance characterized by decreased GABA activity through GABA A receptors as well as increased glutamate activity through ionotropic glutamate receptors (Werner, Coveñas, 2011). Moreover, ictogenesis is also heavily impacted by the actions of other neurotransmitters, namely (dopamine, serotonin hypoactivity, and noradrenaline hyperactivity) (Werner, Coveñas, 2011). In addition to adverse effects, teratogenic consequences, chronic toxicity, and dose-related around 30% of people with epilepsy still suffer seizures after being treated with new antiepileptic medicines (Smith, Bleck, 1991). Therefore, the discovery of natural anticonvulsants that are both efficacious and benign is of the utmost importance. Up to 80% of the population in certain underdeveloped nations relies on folk cures or traditional medicine (Akerele, 1988). Many people believe that medicinal plants might be a significant source of new therapeutic chemical compounds (Farnsworth, 1989). Several plants utilized in traditional medicine to treat epilepsy have yet to be scientifically examined although some have demonstrated action in recent bioassays for the identification of anticonvulsant activity (Raza, Choudary, 1999).

Herbaceous *Pergularia daemia* Forsk. belongs to the *Asclepiadaceae* family. This kind of plant is native to subtropical as well as tropical regions, mainly found in Tamil Nadu, India. Extremely widespread in the tropical regions of Africa, reaching as far east as Arabia. *Pergularia daemia* Forsk. has various medicinal properties for example antibacterial, cardioprotective, hepatoprotective, antidiabetic, wound healing, and antifertility (Golam *et al.*, 2001; Kumar *et al.*, 2006; Wahi *et al.*, 2002; Dhawan *et al.*, 1973; Senthilkumar *et al.*, 2003). The plant is effective in vatha, poisoning, asthma, and convulsion; while the root is beneficial in piles (Yoganarasimhan, 2000), leprosy, anemia, and mental disturbance. The Plant contains stomachic, diuretic, and laxative effects, beneficial in sore eyes, biliousness, and cough. Joints are treated with a mixture of leaf paste and castor oil is utilized for joints in spleen enlargement and liver problems; leaves have hypoglycemic action. This research was conducted to determine whether or not an Aqueous extract of *Pergularia daemia* Forsk has anticonvulsant effects using animal models.

MATERIALS AND METHODS

Plant material collection and authentication

The plant source was found in the Trichy area, and the specimen was deposited in the RAPINAT Herbarium, St. Joseph's College, Trichy, Department of Botany, for authentication.

Preparation of Aqueous extract

The dried leaves were powdered using a commercial electrical blender. 200 g of dry powder was dissolved six times in water and mixed well. The content was boiled at 100^{o}C till the 1/3rd level of water. The final crude content was filtered utilizing muslin cloth and evaporated to dryness. The final obtained crude extract was used for experimental studies.

Antiepileptic Screening

Experimental Animals

Healthy adult Two to three-month-old Wistar strains of Albino rats measuring 150 g-200 g were purchased Biogen is located in Bangalore. The animals were permitted five days to become acclimated to laboratory conditions before the experiment. Polypropylene cages of standard size were used to house the animals. An animal consumed rat chow pellets from Sai Durga

Foods and Feeds in Bangalore, India, and water *ad libitum*. After receiving clearance from the commission (clearance No: 790/03/ac/CPCSEA), CPCSEA ethical guidelines were followed in all investigations.

Chemicals

The PTZ was purchased from Sigma Chemicals, USA. Diazepam was purchased from Ranbaxy Lab, Mumbai.

PTZ-induced seizures

In this experiment, six groups of rats were comprised of six rats each. Group I was considered as a control and the rats in Group II were given PTZ at the dosage of 75 mg/kg BW i.p. to cause clonic seizures in the rat. Groups III, IV, and V received dosages of 100, 200, and 400mg/kg of the AEPD extract administered orally into test groups, and Group VI was treated with oral administration of Diazepam (4 mg/BW). The PTZ was given after 60 minutes from the oral administration of the extract, and diazepam. The antiepileptic activity was accessed by its ability to delay the onset of myoclonic jerks and clonic convulsions. Animals were sacrificed via cervical decapitation after the experiment period. The serum was separated from the blood by centrifuging it for 10 minutes at 3000 rpm. The brain was removed and cleaned in ice-cold salt water. The brain was homogenized in pH 7.4 phosphate buffer (0.1M) and utilized in the various procedures.

The homogenate was used for the determination of the Neurotransmitter assay.

Statistical Analysis

Results were all based on mean ± SD. SPSS (or any statistical software) was used to do ANOVA (one–way analysis of variance) on P values less than 0.05 were considered significant for the data.

RESULTS and

Effect of *Pergularia daemia* Forsk on PTZ induced seizure.

Clonic and tonic seizures are caused by PTZ. The most common preliminary screening test for evaluating likely anticonvulsant medicines is the prevention of seizures in PTZ-induced animals. In the present study, when administered orally diazepam and aqueous leaf extract of *Pergluaria daemia* Forsk. at a dose of 4 mg/kg and (high dose (400 mg/kg bw), medium dose (200 mg/kg bw), and low dose(100 mg/kg bw) decreased the onset of seizures in comparison to control. The results of this study indicate that the leaf extract of *Pergularia daemia* Forsk possesses clinically appropriate antiseizure activity. It is useful in overcoming PTZ-induced convulsions, in animal models.

Figure 1. shows that the AEPD delays the onset and duration of PTZ-induced seizures.

Figure 2 showed that the PTZ exposure affected all the physical behaviors of the animals as it significantly increased immobility and decreased locomotor function of swimming in Group II rats in comparison to the normal group ($p<0.05$). In contrast, treatment using AEPD was effective in decreasing immobility ($p<0.05$) and in another way, in increasing locomotor function in swimming significantly ($p<0.05$), which helps the brain on a molecular and behavioral level by regulating neurotransmitters, which could be used to manage stress and mood reducing hormones.

Figure 3 showed that PTZ (75 mg/kg) administration in Group II significantly ($p<0.05$) decreased the nociceptive activity by affecting sensory neurons that respond to damaging or potentially damaging stimuli by the distribution of possible treatment signals to the spinal cord and the brain. PTZ indicated an important decrease in spontaneous motor activity. The aqueous extract of *Pergularia daemia* Forsk. at doses of (100, 200 and 400 mg/kg bw) was given to rats from Groups III, IV, and V rats. After the induction with PTZ, the rats from Groups III, IV, and V did not show any alteration in the nociceptive central nervous system function. Diazepam (4 mg/kg BW) treated rats also showed no modulation in the nociceptive activity of the central nervous system in group VI rats.

Neurotransmitter assay

Table: 1 depicts that PTZ administration increased the Glutamate level significantly ($p<0.05$) in epileptic control animals forebrain. AEPD at doses of 100, 200, and 400 mg/kg bw, and standard drug diazepam-treated animals decreased significantly ($p<0.05$) in Glutamate levels in the forebrain of PTZ-induced rats. In the present study, GABA, Serotonin, Norepinephrine,

Dopamine, and Acetylcholine esterase levels were significantly ($p<0.05$) decreased in the forebrain of PTZ-induced animals were observed. AEPD at doses of 100, 200, and 400 mg/kg, standard drug diazepam-treated animals increased significantly ($p<0.05$) in the levels of GABA, Serotonin, Norepinephrine, Dopamine, and Acetylcholine esterase in the forebrain of PTZ-induced rats.

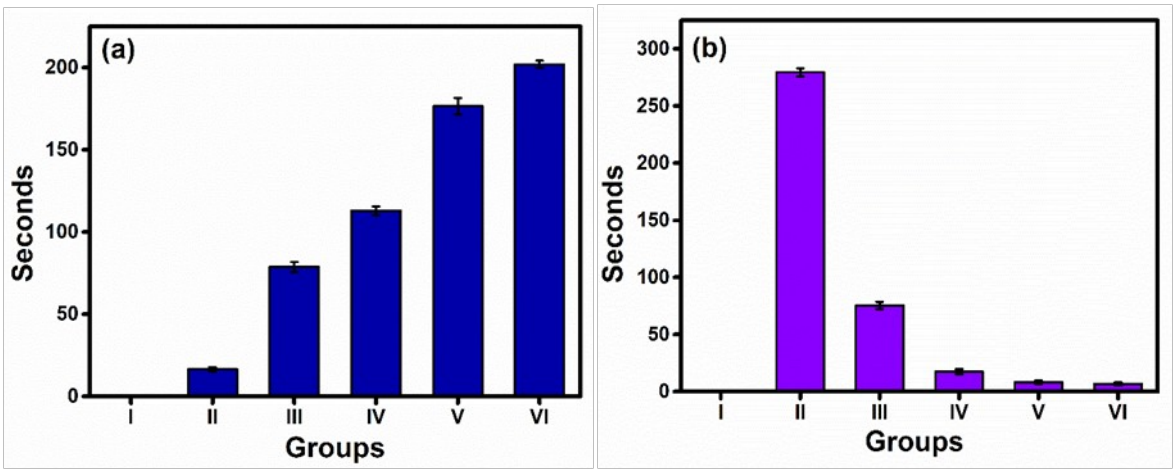

Figure 1. (a): Onset of seizure in experimental Rats, (b): Duration of seizure in experimental rats.

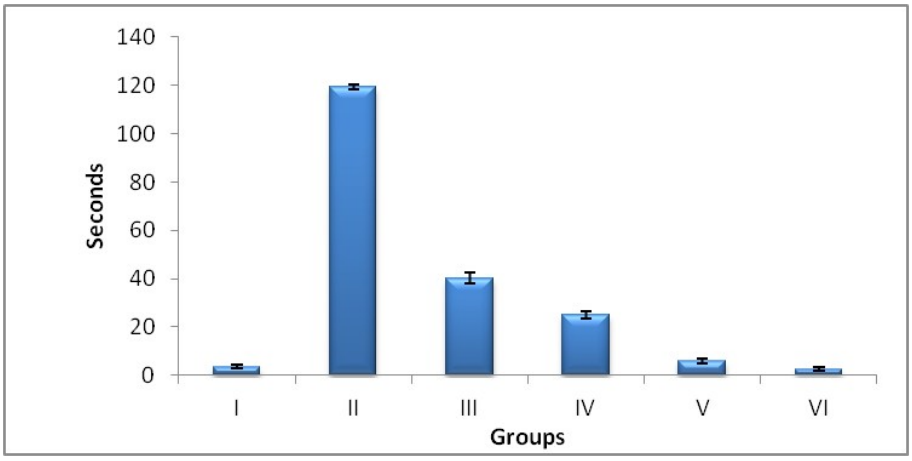

Figure 2. Effect of *Pergularia daemia* Forsk. on the immobility of rats during forced swimming test

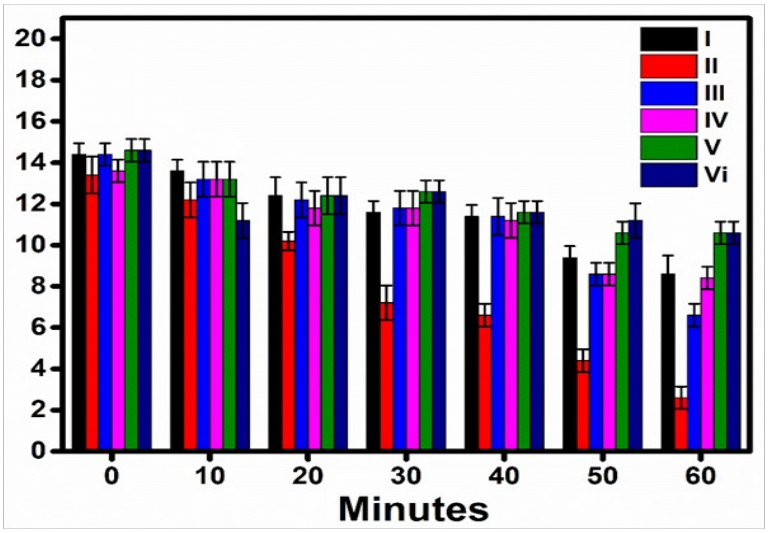

Figure 3. Effect of *Pergularia daemia* Forsk. on Hole cross test of PTZ induced epileptic rats

Table: 1 Effect of *Pergularia daemia* Forsk. on Glutamate, GABA, Serotonin, Norepinephrine, Dopamine, and Acetylcholine esterase activity in PTZ-induced epileptic rats

Group	Glutamate (µ mole/g tissue)	GABA (µg/ g tissue)	Serotonin (ng/ g tissue)	Nor Epinephrine (Pg/ g tissue)	Dopamine (Pg/ g tissue)	Acetylcholine esterase (µg of acetylcholine hydrolyzed/ g tissue)
I	41.29*±0.94	2.725*±0.009	50.76*±0.43	2.05*±0.129	1.37*±0.045	7.978*±0.305
II	62.17*±0.54	0.375*±0.170	31.16*±0.53	0.845*± 0.06	0.832* ± 0.052	1.556*±0.094
III	58.69±0.70	0.682±0.013	44.73±0.51	1.26 ± 0.069	0.813 ± 0.042	1.574±0.067
IV	54.9±0.53	1.136±0.010	46.48±0.16	1.557 ± 0.129	1.077 ± 0.040	2.166±0.056
V	51.42**±0.70	1.246**±0.017	47.33**±0.17	1.932** ± 0.068	1.164** ± 0.039	4.182**±0.244
VI	40.66**±0.53	1.423**±0.024	48.93**±0.29	2.01** ± 0.077	1.27** ± 0.039	6.956**±0.028

*p<0.05 statistically significant compared between Normal and Disease Control Group, **p<0.05 statistically significant compared between Disease Control and Drug treated Group

Discussion

The current study's findings show that AEPD had Antiseizure activity. The onset and duration of convulsion results showed that no death was found in the rats besides PTZ-induced seizure. After the treatment with an AEPD dose level of 400 mg/kg substantially late the onset of convulsions, significantly reduced the interval of convulsion. Diazepam (4 mg/Kg) is a common anti-epileptic medication that completely antagonizes the seizure induced by PTZ.

A forced swimming test is a commonly used animal model for assessing depression resulting from molecules of medication (Herrera *et al.*, 2011). Depression and psychiatric conditions are caused by antiepileptic drugs. The preparation of herbal compounds or herbal medicine is one of the most interesting aspects of medication development is antiepileptic action without producing depression. This herbal medicine reduces depression (Jianming *et al.*, 2011). In the current study, locomotor activity was used to assess for possible sedative effects of the antioxidant activity of AEPD. In this test, the locomotor activity of the AEPD was inhibited. As was found in the diazepam control, the effective dosages of AEPD 400 mg/kg were found to induce a significant reduction in locomotor activity and a reduction in motor coordination. Diazepam, which enhances GABAergic activity, is also a highly effective and extensively used treatment for absence seizures (Macdonald, Kelly, 1995). Diazepam and other Benzodiazepines, on the other hand, reduce locomotor activity (Turski *et al.*, 2006).

GABA, the cerebral cortex's key inhibitory neurotransmitter, maintains the inhibitory tone that balances neuronal activity. Seizures may develop if this balance is disturbed. GABA is generated within GABAergic axon terminals and released into the synapse, where it acts on one of two receptors that are: GABA-A, which regulates chloride entry into the cell, and GABA-B, which raises potassium conductance, reduces calcium entry, and inhibits presynaptic release of other transmitters (Sulthana and Naz, 2013). PTZ may cause convulsions by preventing GABA from acting on GABA-A receptors (Madhan *et al.*, 2010). Decreasing and enhancing convulsion occurs by increasing and decreasing GABA neurotransmission (Balamurugan *et al.*, 2009). In the present study treatment with effective doses of AEPD 400 mg/Kg has been shown to cause enhances the GABA levels. Diazepam and other common antiepileptic medications are thought to be effective by improving GABA-mediated inhibition and acting as an anticonvulsant against PTZ seizures.

In the vertebrate nervous system, the most prevalent excitatory neurotransmitter is glutamate. At chemical synapses, glutamate is held in vesicles. Glutamate is released from the presynaptic cell in response to nerve impulses. The glutamate receptors, such as the NMDA receptor, bind glutamate and activate opposing post-

synaptic cells. They can function in reverse in brain injury or disease, and excess glutamate can be stored outside of cells. This procedure, known as excitotoxicity, allows calcium ions to enter cells via NMDA receptor channels, causing neuronal damage and eventually cell death (Premanand and Ganesh, 2010). In the present study, the established antiepileptic drugs such as diazepam restored the glutamate levels in the brain. Similarly, AEPD at the dose level of 400mg/kg decreased significantly ($p<0.05$ & $p<0.01$) glutamate levels in the forebrain of rats.

Inhibiting monoamine oxidase (MAO) increases dopamine, noradrenaline, and serotonin (Monoamine) levels in the brain. MAO is a type of enzyme that degrades biogenic amines and raises the convulsion threshold (Srinivas *et al.*, 1997). Serotonin (5-Hydroxytryptamine) is a neurotransmitter that regulates mood, sleep, anxiety, alertness, and aggression. It has been suggested that serotonin agonists, precursors, and inhibitors of neuronal absorption promote narcolepsy (Bhattacharyya *et al.*, 1983). In many animal test systems, Serotonergic transmission increases, lowering the threshold for pentylenetetrazole (PTZ)-induced seizures and protecting against PTZ-induced convulsions (Srinivas *et al.*, 1997). It has been demonstrated that dopamine activation is essential for good internal encoding of motor abilities.

Dopamine is also a neurotransmitter that is determined to function as a signal transmission to the brain regions involved in learning new behaviours. Octopamine, a chemical related to dopamine, has been shown to have a comparable effect on insects (Barron *et al.*, 2007). Dopaminergic receptors, found in the substantia nigra and other areas of the brain, mediate these actions. Noradrenaline, which is primarily involved with blood pressure control, also has a function to play a role in seizure control, though less significantly than other biogenic amines. The cerebellum may have a biphasic impact of glutamate, which would block glutamate release at low concentrations (Dolphin, 1982). In the present study, AEPD at the dose level of 400 mg/Kg elevates monoamines such as serotonin, dopamine, and noradrenaline, both of which reduce seizure activity.

The central cholinergic system is essential for the development of memories (Blake *et al.*, 2014). In the elderly, acetylcholine-containing neurons become dysfunctional, leading to memory loss (Bachurin *et al.*, 2017). According to the results of this study, PTZ injections to epileptic rats considerably reduced their levels of acetylcholine. The rats' memory is altered along with this decrease in memory. Furthermore, acetylcholinesterase activity biochemical study shows that AEPD extract caused a suppression of the enzyme's activities. During the study, there was an improvement in memory in the physicochemical test, and this was followed by a rise in the acetylcholine levels in the hippocampus of treated rats when given various extract doses. While the cure with AEPD at the dose level of 400 mg/kg showed that AChE activity was significantly decreased and acetylcholine activity increased.

CONCLUSION

The current work, which studies PTZ-induced convulsion models, shows that neurotransmitters and neuropeptides in epileptic foci of the hippocampus are altered. Ictogenesis is facilitated by a neurotransmitter imbalance characterized by hypoactivity in presynaptic GABAergic neurons and excitotoxicity in glutaminergic neurons. Because dopamine hyperactivity through D2 receptors and serotonin hypoactivity through 5-HT receptors both have a proconvulsant effect, postsynaptic excitatory neurotransmitters also play a role in neural networks. The animals treated with AEPD significantly restore all neurotransmitter levels in the brain. We conclude that our AEPD acquires antiepileptic activity.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflict of interest.

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