RESEARCH ARTICLE

Preventive Effect of *Phaseolus vulgaris* Seed Coats on Pentylenetetrazole (PTZ) induced Kindling and Behavioral Comorbidities

Sarika Chaudhary*, Sagarika Majhi, Madhu Verma

Abstract

Introduction: To investigate the preventive effect of *Phaseolus vulgaris* seed coats on pentylenetetrazole-induced kindling and behavioral comorbidities.

Material and Methods: Thirty Wistar albino rats were categorized into five groups. The first group received regular saline (0.9 % w/v NaCl) p.o.; the second group received PTZ (35 mg/kg b.w.) i.p.; the third group received valproic acid (200 mg/kg b.w.) p.o.; the fourth group received *P. vulgaris* extract (100 mg/kg b.w.) p.o.; the fifth group received *P. vulgaris* extract (200 mg/kg b.w.) p.o. on an alternate day for 21 days. PTZ improved lipid peroxidase levels, decreased Glutathione level, decreased superoxide dismutase activity, increased Nitric Oxide level.

Result: This study revealed that *P. vulgaris* (Hydroalcoholic extract) increased the anti-oxidant level of both 100 mg/kg and 200 mg/kg compared to the PTZ category. Histopathological findings revealed that the hippocampal section of the brain of rats receiving *P. vulgaris* extract had improved relative to the receiving PTZ group.

Conclusion: Based on the result, it is proposed that *Phaseolus vulgaris* has anti-oxidant properties. This is useful for the treatment of epilepsy.

Keywords: Epilepsy, Kindling, Oxidative stress, Pentylenetetrazole, *Phaseolus vulgaris*.

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Introduction

Epilepsy is a recurrent disease of the brain, mostly due to unexplained, unjustifiable cyclic seizures. The unrestrained, hypersynchronous movement of neurons to the brain triggers a nervous change in brain function that leads to seizures. The seizure is inflamed by frequent aggrieve vs. calenture, the insulin shock does not affect the trust of the epilepsy because it is a sudden subordinate condition, not a chronic disorder.[1]

Epilepsy is the most common neurological disease with a high degree of certainty of 50 new cases per 100,000 populations per year.[2] 1-2% of the population is diagnosed with epilepsy, but about one-third of the population has refractory epilepsy (including seizures that are not managed by two or more patients with prescribed antiepileptic aids or other forms of treatment). A 70–80 % of epilepsy develops in early childhood. Seizures are classified into a triad: generalized, focal (formerly referred to as especially susceptible) and epileptic spasms.

If a normal balance between excitation and brain inhibition is skewed, seizures that occur as a result of differences in several brain justification rates, from heredity and subcellular node cascades to extensive neuronal circuits.[3] Genetic and seized influences reorder the E/I equillibrium. Epilepsy can be exacerbated from the transistor level (e.g. abnormal nerve cell integration in cortical dysplasia) to the binding site level (e.g. aberrant γ-aminobutyric acid (GABA) binding site dimers in Angelman syndrome) to the anomalous ion channel activity caused by genetic disorders. (e.g. potassium channel genetic variations in the benign neonatal epilepsy family (BFNE). Acquired cerebral affronts can modify the role of the circuit (e.g., remolding anatomy of the hippocampal wiring after repeated febrile episodes or head trauma) accordingly. An advanced brain is especially vulnerable to seizures due to physiological case variability.[4]

Limited quantity, massive price, low efficiency and adverse effects, sedation, dizziness, coordination disturbances, mood disturbances, sexual dysfunction of antiepileptic drugs (AEDs) are prime concerns. Traditionally, herbal medicine has always been a portion of epilepsy therapy. Herbal medicines are usually well-tolerated and have fewer side effects.[5]

Most antiepileptic drugs do not prevent or reverse the pathological process that underlies epilepsy, hence the

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continued search for new treatments with fewer side effects and improved efficacy.[6]

Herbal medicine plays a crucial part in meeting the population's primary health care needs, with Africa and Asia being the continents with most users.[7] Several other medicinal plants have shown potential for new, safe possible treatments.[8] Herbal medicines generally have a broad spectrum because they are an assortment of bioactive compounds.[9]

P. vulgaris (red kidney beans) is an impressive source of proteins, energy, carbohydrates, minerals and vitamins. Red kidney beans have an excellent profile of amino acids, flavonoids such as kaempferol, quercetin, myricetin, naringin, and their derivatives and are used as anti-oxidants, anti-inflammatory, anti-diabetic, anti-proliferative activity and are effective in neurodegenerative diseases such as parkinsonism. The present study focuses on the preventive effect of kidney beans on epilepsy, which has been carried out to explore a wide range in the future.

Materials and Methods

The seeds of *P. vulgaris* were identified and procured from the local market of Modinagar, Ghaziabad. The material was authenticated by Dr. Sunita Garg, Emeritus Scientist, CSIR-National Institute of Science Communication and Information Resources (NISCAIR), Pusa Campus, New Delhi. A voucher specimen was deposited at RHMD, New Delhi. Authentication number- NISCAIR/RHMD/consult/2019/3511-12.

Pentylenetetrazole (PTZ) (CAS No- 54-95-5) was purchased from Sisco Research Laboratory Pvt. Ltd, New Delhi. Valproic acid was obtained as Epilex 200mg from New Delhi. All other chemicals and reagents used in the experiments were of analytical grade.

Animals

Adult male wistar albino rats (150-250) were obtained from Animal House, ITS College of Pharmacy, Muradnagar, Ghaziabad. They were maintained at a controlled temperature (23±2 and relative humidity (50-70%) under 12-12 hour light-dark cycle with free access to food and water ad libitum. All the experimental procedures were carried out in accordance with the guidance of the Institutional Animal Ethics Committee (IAEC).

Experimental Design

Animals are randomly divided into five groups and each containing six animals.

Group 1: Normal control received 0.9 % w/v NaCl
Group 2: PTZ Control received vehicle + PTZ 35mg/kg i.p.
Group 3: Standard control received valproic acid 200 mg/kg p.o + PTZ 35 mg/kg i.p
Group 4: Treated group I received *P. vulgaris* extract 100 mg/kg p.o + PTZ 35 mg/kg i.p.
Group 5: Treated group II received *P. vulgaris* extract 200 mg/kg p.o. + PTZ 35 mg/kg i.p.

PTZ was dissolved in sterile saline (0.9 % w/v NaCl).

Induction of Kindling

PTZ has been used to induce kindling in rats. With the exception of control groups, the dose of 35 mg/kg of PTZ intra-peritoneal was administered into all rats on an alternate day for 21 days. Standard and extract were given 30 minutes before each injection. Following PTZ injection, the seizure score was determined as stage 0 (No response); stage 1(Myoclonic jerk); 2 (Straub tail); 3 (clonic jerk without loss of righting reflex); 4 (Clonic seizures with loss of righting reflex); 5 (clonic-tonic seizures). The animals were considered to have been kindled after attaining a seizure score of 4 on three consecutive days. In the present study, 11 injections of PTZ were required to acquire kindling. After that, behavioral parameters, such as the rotarod test and the forced swim test, were evaluated. At the end of the study, animals were sacrificed, and their brains were isolated for further biochemical assessment and histopathology.

Assessment of Behavioral Parameters

Rotarod tests were performed to determine fine motor control. The task consists of one training session and one test session, conducted 24 hours apart. The trial begins with the rat being put in the appliance and ends when the rat falls off the rod or two consecutive times after hitting the termination time of 60 seconds. The overall number of tryouts was 10 during the training session. A 60 seconds rest time was allowed between each trial. Rats were observed for 40 min in the test session, and the latency was reported up to the first fall. Extract or its vehicle was provided 60 minutes prior to the start of the test session.[10]

Forced swim test was conducted based on the method previously described with some modifications [11]. Rats were individually placed in open cylindrical container (40 cm in diameter × 80 cm in height) containing water at 45 cm height. The total time of immobility was recorded for 5 minutes after 1-minute of habituation. Immobility was defined as the animal floating in the water without struggling and making only very minimal movements necessary to keep its head above the water. An increase in the duration of immobility is indicative of depressive-like behavior.

Sample Preparations and Assessment of Biochemical Parameters

Groups of the non-kindling rat were sacrificed by decapitation 3h after the last oral administration. Kindled rats were sacrificed by decapitation at the end of the observation period on test day and they were anesthetized before decollated. The brain had been taken away and wipe two times with freezing saline solution, placed in glass bottles, labeled, and stored in a deep freezer (-30 °C) until processing. One volume of hippocampal tissue was homogenized in four volume of an ice-cold tris-HCL buffer by using a homogenizer after cutting the brains into small pieces with scissors [12]. The following parameters were observed:

MDA test: One ml of the medium suspension was taken from the 10% homogeneous tissue and added 1 mL of 30 percent

TCA and 1 mL of 0.8 percent TBA reagent. The tubes were lined with aluminum foil, and held at 80 degrees centigrade in a shaking water bath for 30 minutes. Tubes were taken out after 30 minutes, and placed in ice-cold water for 30 minutes. Those were centrifuged for 15 minutes at 3000 rpm. The absorbance of the supernatant was read at 535 nm at room temperature against the appropriate blank. Blank consists of 1mL distilled water, 1mL of 30 % TCA and 1mL of 0.8 %TBA.

GSH test: 2 mL of 10% homogeneous, which had been concocted in KCl mixture, then added 2.5 mL of 0.02 M EDTA. 2 mL of the above mixture had been taken and added 4mL of icy filtered water and 1mL of fifty percent TCA, then quiver it for 10 minutes. The material had been moved to the centrifugal tube 10 minutes later, centrifuged for 15 minutes at 300 RPM. Upon centrifugation, 2 mL of the supernatant was combined with 4 mL 0.4 M tris buffer (PH 8.9). The whole solution was well combined and 0.1 mL 0.01 M DTNB was added, the absorbance was read in 5 minutes, applied 412 nm DTNB to blank reagent without homogeneous. For blank readings, instead of 2mL of homogenate, 2 mL of distilled water was added.

SOD test: In 5 mL of phosphate-buffered saline containing 100 mM of nitro blue tetrazolium (NBT) at 37 for 1.5 hours a weighed volume of nerve tissue was taken and used 5 mL of 0.5 M hydrochloric acid (HCl) to avoid the NBT reduction. The mixture was centrifuged for 20 min and the resultant pellet was suspended in 1.5 mL of pyridine and kept at 80°C for 1.5 hr to extract formazan, an adduct formed after reaction of NBT with superoxide anions. The mixture was again centrifuged at 10,000 g for 10 minutes and absorbance of formazan was determined spectrophotometrically at 540 nm. The tissue was minced and homogenized in water containing 40 mg/L diethylene triamine penta acetic acid in a mixture of 0.1 M sodium hydroxide (NaOH) and 0.1% sodium dodecyl sulfate (SDS).

Nitric oxide test: 400 µL carbonate buffer (pH 9.0) was applied to 100 µl serum sample in test tube, followed by the addition of approximately 0.15 g of copper cadmium alloy fillings. The test tube was incubated with an occasional shaking at room temperature for 1 hours. The reaction was prevented by introducing 0.35 M sodium hydroxide to 100 µL and 120 mM zinc sulfate solution to 400 µL (in distilled water) under a vortex mixing. The solution was allowed to stand for 10 minutes and centrifuged for 10 minutes at 4000 rpm. 500µL of the clear supernatant was moved to another test tube and 250 µL of 1% sulphanilamide (in 3N HCL) and 250 µL of 0.1% N-naphthyl ethylenediamine (in distilled water) was added. After 10 minutes the absorption was spectrophotometrically noted at 545 nm against suitably prepared blank solution (100 µL of distilled water was used instead of serum).

Histopathological examination

For histopathological examination, brain was dissected and hippocampal tissue was removed from the brain. It was fixed with 10% formalin after the hippocampus dissection and exposed to hemotoxyl and eosin. Degenerative changes in the neurons, such as cytoplasmic vacuolation, clumping of nuclear chromatin, hypereosinophilia and condensed cytoplasm, and cell fragmentation, were used to assess the relative percentage of neuronal damage.[13]

Statistical Analysis

Seizure stage scores and other examinations were analyzed by unpaired t-test, ANOVA and Tukey post-test.

Results

Effects of *P. vulgaris* on Pentylenetetrazole Induced Kindling

The continual administration of Pentylenetetrazole (PTZ) (35 mg/kg b.w.) each alternative day (for 21 days, 11 injections) tend to result in kindling, ajust like implied via progression increased in the seizure score. *P. vulgaris* at the dose 100 mg/kg and 200 mg/kg and valproic acid at dose 200 mg/kg appreciably ($p<0.001$) counteracted the intensification of kindling. The protection afforded at 200 mg/kg was commendable as compared to 100 mg/kg of *P. vulgaris*. (Figure 1).

Effects of Behavioral Parameters

For examine, if effects on actions or motor skills followed the anticonvulsant effect of P. vulgaris. The individual rat groups were tested using the rotarod test and forced swim test. Statistical analysis showed that there were substantial variations in the time spent on the rod between the groups followed by unpaired t-test ($p < 0.0001$) and ANOVA + tukey t testing. In dose 100 mg/kg ($p < 0.05$), 200 mg/kg ($p < 0.001$) *P. vulgaris* and valproic acid ($p < 0.001$), the animals receiving *P. vulgaris* spent substantially more time on the rod relative to the PTZ control group. The significant result at the dose of 200 mg/kg is more commendable as compared to 100mg/kg (Figure 2).

Effects of MDA, GSH, SOD, NO activity on pentylenetetrazole induced kindled rats

PTZ-prompted kindling tends to result in increased oxidative stress leading to a reduction of SOD, GSH activities as seen

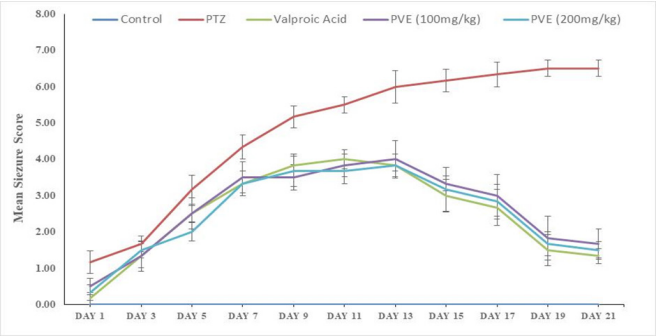

Figure 1: Effect of Drugs on Seizure Score
All values are expressed as mean+S.E.M, n= number of rats, PTZ= Pentylenetetrazole, PVE= *Phaseolus vulgaris* extract, significant different was observed for groups viz. valproic acid 200mg/kg, PVE 100 mg/kg and PVE 200 mg/kg when compared with PTZ group.

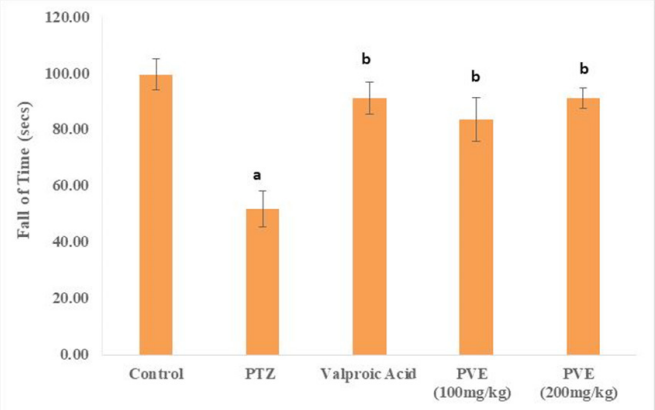

Figure 2: Effect of Rotarod test in Epilepsy
PTZ= Pentylenetetrazole, PVE= *Phaseolus vulgaris* extract, Values are expressed in Mean ± SEM (n = 6 in every group); [a]P < 0.0001; when PTZ group compared with normal control (Unpaired t-test); [b]P < 0.01; when other groups compared with PTZ group (One Way ANOVA, followed by Tukey's test).

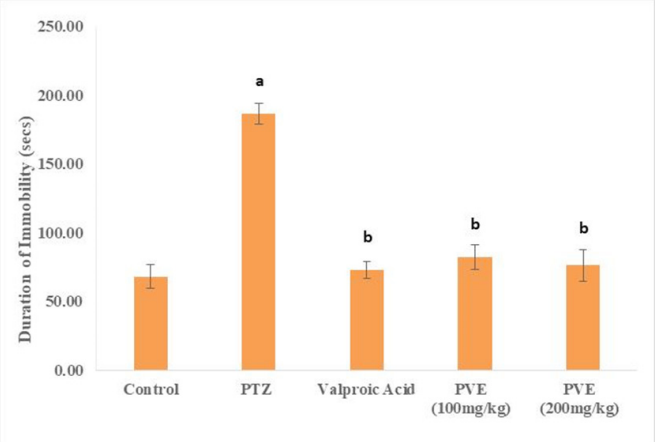

Figure 3: Effect of Forced swim test in epilepsy
PTZ= Pentylenetetrazole, PVE= *Phaseolus vulgaris* extract, Values are expressed in Mean ± SEM (n = 6 in every group); [a]P < 0.0001; when PTZ group compared with normal control (Unpaired t-test); [b]P < 0.0001; when other groups compared with PTZ group (One Way ANOVA, followed by Tukey's test).

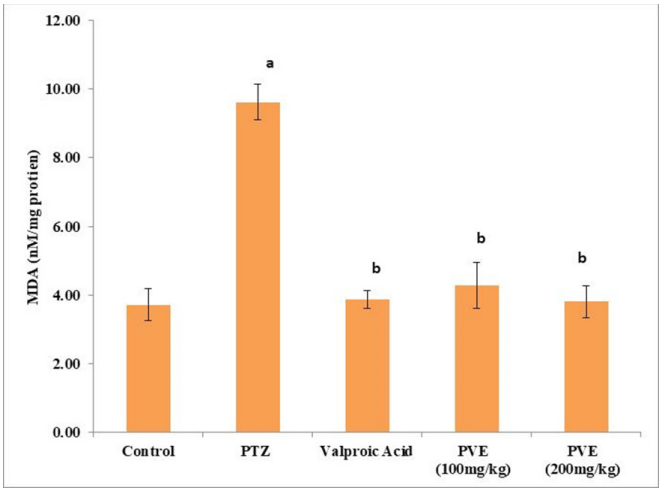

Figure 4: Effect of MDA levels in rats
PTZ= Pentylenetetrazole, PVE= *Phaseolus vulgaris* extract, Values are expressed in Mean ± SEM (n = 6 in every group); [a]P < 0.0001; when PTZ group compared with normal control (Unpaired t-test); [b]P < 0.0001; when other groups compared with PTZ group (One Way ANOVA, followed by Tukey's test).

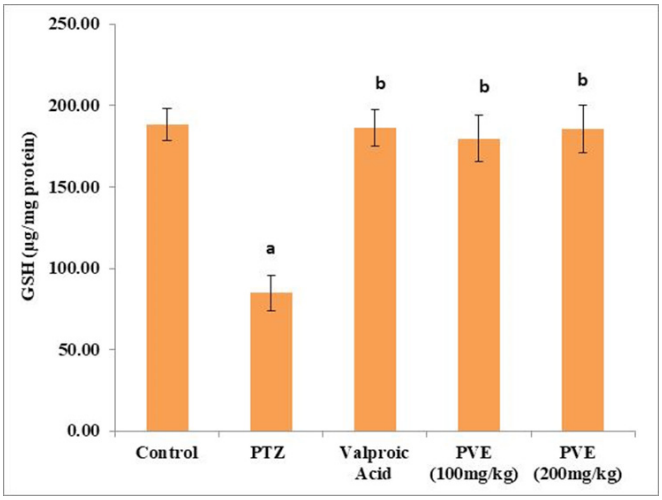

Figure 5: Effect of GSH levels in rats
PTZ= Pentylenetetrazole, PVE= *Phaseolus vulgaris* extract, Values are expressed in Mean ± SEM (n = 6 in every group); [a]P < 0.0001; when PTZ group compared with normal control (Unpaired t-test); [b]P < 0.0001; when other groups compared with PTZ group (One Way ANOVA, followed by Tukey's test).

in PTZ control rats compared to normal rats. Protein was estimated for further investigations. In PTZ–induced group at dose 35 mg/kg (158 %) increased the MDA level as compared to the control group ($p < 0.001$) followed by unpaired t-test. Administration of Valproic acid at dose 200 mg/kg (59.74 %) decreased the MDA level, *P. vulgaris* extract at dose 100 mg/kg (55%) decreased MDA level, and *P. vulgaris* extract at dose 200 mg/kg significantly (60.43%) decreased MDA level ($p < 0.001$) as compared to PTZ group using ANOVA followed by Tukey test (Figure 4). Administration of PTZ 35mg/kg in PTZ group (54.93%) decreased GSH level ($p < 0.0001$) as compared to control group followed by unpaired t-test. Treatment with Valproic acid at dose 200 mg/kg (116%) increased GSH level, *P. vulgaris* extract at dose 100 mg/kg (108%) increased and at 200 mg/kg significantly (115%) increased GSH level as compared to PTZ group followed by both ANOVA and Tukey test ($p < 0.0001$) (Figure 5). PTZ-induced group at dose 35 mg/kg (73%) decreased SOD level as compared to control group ($p < 0.0001$) followed by unpaired- t-test. Treatment with valproic acid at 200 mg/kg (265%) increased SOD level, *P. vulgaris* extract at dose 100 mg/kg (236%) increased, and at 200 mg/kg (253%) increased significantly ($p < 0.05$) as compared to PTZ group followed by ANOVA test. Nitric oxide test evaluated followed by unpaired t-test ($p < 0.001$) and both ANOVA and Tukey test ($p < 0.001$) (Figure 6). Administration of PTZ at 35 mg/kg in PTZ group (119 %) increased NO level as compared to control group ($p < 0.0001$) followed by unpaired t-test. Treatment with Valproic acid at 200 mg/kg (48.53 %) decreased NO level, *P. vulgaris* extract at dose 100 mg/kg (37.58 %) decreased, and at 200 mg/kg (48.26 %) decreased

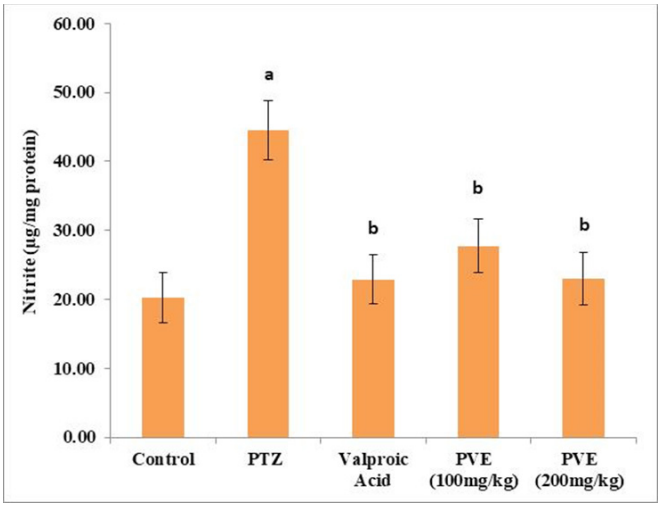

Figure 7: Effect of NO levels in rats
PTZ= Pentylenetetrazole, PVE= *Phaseolus vulgaris* extract, Values are expressed in Mean ± SEM (n = 6 in every group); [a]P < 0.0001; when PTZ group compared with normal control (Unpaired t-test); [b]P < 0.05; when other groups compared with PTZ group (One Way ANOVA, followed by Tukey's test).

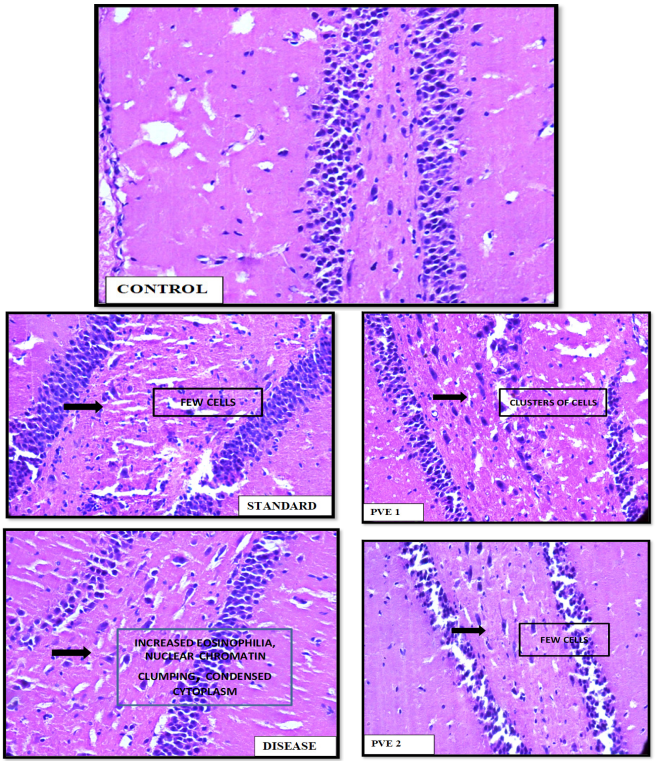

Figure 8: Histopathological examination of rat's brain in control, disease, standard, PVE 1 and PVE 2 groups

significantly nitric oxide level ($p < 0.05$) as compared to PTZ group using ANOVA followed by Tukey test. (Figure 7).

Result of Histopathological Examination

According to Fujikawa scaling system Hematoxylin and eosin staining were applied. Group II (PTZ group) showed dead neurons with pyknotic nuclei which were clearly showed as increased eosinophilia, chromatin clumping and condensed cytoplasm as compared to control group. Group III (Standard group), Group IV (PVE 1) and Group V (PVE 2) have been shown improved neuronal damage compared with PTZ group. (Figure 8)

Discussion

The current study showed *P. vulgaris*' preventive effect on PTZ-induced kindling and behavioral comorbidities. Medicinal plants are very advantageous and have been used by humanity to prevent and treat multiple diseases for more than hundred years.[14] A subconvulsive dose of PTZ is applied repeatedly and intermittently for many days, leading to seizures eventually developing. After PTZ injection the seizure score is determined.[15] Based on the seizure score in this study, *P. vulgaris* is advantageous compared with the PTZ group. Experiments also validated the findings of a study of behavioral seizures, showing that *P. vulgaris* can enhance behavioral comorbidities. In the present study, we found that the P. vulgaris increased the forced swim test's immobility time Previous research was recorded in the rotarod test, epileptic rats presented with motor dysfunction.[17] Rats treated with *P. vulgaris* stayed on the advancing rotarod slightly longer than the control group PTZ.

The increased level of MDA, which is a lipid peroxidation marker, indicated high production of free radicals in PTZ kindled rats.[13] The rise in MDA levels was greatly impeded by treatment with *P. vulgaris*. GSH is a natural anti-oxidant used to detoxify reactive oxygen species in the cells.[18] In model epilepsy the amount of GSH is reduced.[19] PTZ treatment diminished the level of GSH that was reinstated through treatment with *P. vulgaris*. There was a subsequent drop in the activity of SOD in rats that had been kindled by PTZ. SOD is the main enzyme of which deleterious reactive species such as superoxide are removed.[20] The treatment with *P. vulgaris* increased the amount of SOD while exhibiting a defensive effect against rats that have been kindled with PTZ. In the kindled rats PTZ increased NO levels in the hippocampus at a dose of 35 mg/kg.[21] Treatment with *P. vulgaris* at dose 100 mg/kg and 200 mg/kg lowered the NO level greatly.

Conclusion

Epilepsy is a chronic, uncommunicable condition of the brain that affects people of all ages. About half a million people have epilepsy, making it one of the world's most common neurological diseases. Nearly 80% of people with epilepsy live in low-and middle-income countries. Approximately 70% of people with epilepsy are reported to have seizure-free experience if they are correctly diagnosed and treated. In epileptic seizures, oxidative stress functions as an etiological component. PTZ initiates an oxidative stress mechanism that leads to epileptic seizures. *P. vulgaris* is a major source of protein, minerals, vitamins and dietary fibres. *P. vulgaris* is previously proposed to comprise flavonoids such as quercetin, kaempferol, naringin, naringenin, ferulic acid, and myrcetin as anti-diabetic, anti-inflammatory , anti-depressant, anti-

parkinsonic, analgesic, anti-proliferative, cardio-protective and hepato-protective. PTZ altered behavioral parameters such as Rotarod, Forced swim test, and biochemical parameters such as MDA, GSH, SOD, NO. The results of this study showed that PVE improved these parameters. PVE has an important impact in the treatment of epilepsy.

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