RESEARCH ARTICLE

Neuroprotective Effect of Chlorogenic Acid against Pentylenetetrazol Induced Kindled Epilepsy in Mice

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ABSTRACT

Background: Epilepsy is a group of chronic neurological disorders characterized by seizures. Kindling, a chronic epileptic mouse model that was used to explore the epileptogenic mechanism and seeking new anti-epileptics. In kindling, sub-convulsive (chemical/ electrical) stimuli are delivered repeatedly and erratically, eventually causes massive convulsions. The aim of this study was to investigate the neuroprotective effects of chlorogenic acid, a phenolic acid derived from coffee, on seizure severity and kindling progression. Memory impairment inflammation due to oxidative stress by pentylenetetrazol (PTZ).

Objective: This study was used to investigate the neuroprotective effect of chlorogenic acid against pentylenetetrazol induced kindled epilepsy in mice.

Methods: Kindling was provoked by subsequent (one-day-gap) injections of PTZ (subconvulsive; 35 mg/kg; s.c.) for 29 days in mice. The experimental protocol included six groups (n=6) receiving proconvulsant doses of PTZ (35 mg/kg i.p.) every other day for 31 days. Alternating subcutaneous injections of PTZ induced priming with 15 injections of PTZ. Compared with the PTZ group, pre-treatment with chlorogenic acid (5 and 10 mg/kg) 1 h before PTZ administration reduced seizure score, reduced metastasis latency due to increased normal maze, and decreased metastasis latency extension at FST. PTZ-induced biochemical changes were enhanced in chlorogenic acid-treated animals, as indicated by decreased lipid peroxidation (MDA), nitric oxide and AChE levels, and increased SOD, GSH, catalase level. Following PTZ injection, convulsive behaviours were noted for 30 minutes. Open-field-test (locomotor activity), force swimming test (depressive behaviors), elevated plus-maze and passive avoidance tests were employed to evaluate cognition. Brain homogenate was used to estimate oxidative stress (glutathione,superoxide-dismutase, lipid-peroxidation), and acetylcholinesterase activity.

Results: This result suggest the neuroprotective potential of chlorogenic acid. This may be correlated with its ability to inhibit oxidative damage and reduce the occurrence of seizures and other related damage. It may be a promising candidate for mitigating the consequences of events.

Conclusion: Our findings suggest effect of chlorogenic acid against pentylenetetrazol-induced kindled epilepsy in mice which were established by behavioral and biochemical paradigms.

Keywords: Chemical kindling, Pentylenetetrazol, Chlorogenic acid, Immobility time.

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INTRODUCTION

Epilepsy, one of the most prevalent neurological illnesses, affects 1% of people worldwide.[1] Clinical studies have shown that more than half of people with epilepsy deteriorate from cognitive impairment.[2] Patients with epilepsy may experience cognitive impairment due to several factors, including psychosocial issues and the type of seizure they are experiencing.[3] The problem of effective epilepsy therapy is not adequately addressed even though seizure control is only one component of optimised treatment programs. Around 50 million individuals worldwide are diagnosed with epilepsy, contributing significantly to the severity of health globally. An estimated 4 to 10% per 1000 people in the wider community have active epilepsy at any given moment. Each year, epilepsy is thought to be diagnosed in 5 million persons worldwide. Epilepsy is thought to be diagnosed in 49 out of every 100,000 people annually in high-income countries. Memory problems affect a lot of epileptic patients. Goddard introduced the name “kindling” for these

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liberal brain changes.[4] Chemical as well as electric techniques may cause kindling. Chemical kindling implies the systematic administering of many convulsions' chemical compounds. It triggers seizure development that leads to generalized tonic-clonic seizures and cognitive impairment. Chemical kindling is quicker and far less time-consuming than electrical kindling and will not include major surgery or implantation of electrodes. Various seizures like GABAergic pentylene tetrazole, picrotoxin, bicuculline N-methyl-D-aspartate and morphine can be used to cause chemical kindling.[5] It is an aromatic residue derived from the esters of cinnamic acids with hydroxyl groups like caffeoyl, tocopherol and caffeoylquinic acid. Hindering 11 βHSD1 an enzyme that is implicated in the formation of hormones that increases blood pressure by binding to the benzodiazepine sites to activate the GABAs receptor, leading to lower psychological distress.[6] Valproic acid (VPA) or valproate is an anti-epileptic therapeutic medication used as a medicinal product to cure epilepsy manic depression and avoid neuropathic pain. Negative effects of VPA include hepatic steatogenesis in mice and detrimental effects on the synthesis of hepatic protein and fats.[7] Currently, epileptic diseases are inadequately treated, and symptomatic drugs for treating epileptic diseases are inadequate and largely useless. In this regard, natural products (plant-based medicines) are ideal for developing safe and effective means to treat epilepsy. Neuroprotective methods begin to work effectively, implying that some safe and efficient prophylaxis treatment is hugely valuable. Attention is focused on investigating the effectiveness of herbal supplements used in traditional medicine since they are cheap and have few side effects. In animals of diabetics and Huntington's disease the most abundant catechin in green tea has been found to possess memory improvement and antioxidant activities. Consumption of chlorogenic acid to a broad myriad of health impacts like neuroprotective properties, cardio-protection, fat loss, chemo-preventive properties, anti-inflammatory properties greatly reduced cholesterol levels, lessened feed-induced insulin production, antianxiety properties and pro-hyperalgesia results. Due to the presence of chlorogenic acid in green coffee and wide pharmacological activities reported earlier this study has been planned to investigate the neuroprotective effect of chlorogenic acid in pentylene tetrazole-induced kindling in mice. Such type of study has not been studied earlier.

MATERIAL AND METHODS

Animal Care and Selection

Swiss albino mice (male; 22 to 30 g) were obtained from the Indian Veterinary Research institute, India, and sustained on standard laboratory environment with water *ad libitum*. The protocol was approved by Institutional Animals Ethics Committee (IAEC) (1024/PO/Re/S/08/CPCSEA). IAEC guidelines were obeyed to avoid any suffering to experimental animals during the entire procedure.

Drug and experimental protocol

Group 1: Control mice treated with Vehicle of the PTZ (0.9% saline) + Vehicle of the chlorogenic acid.
Group 2: Normal mice + chlorogenic acid (10 mg/kg) (Per se)
Group 3: PTZ treated group mice – PTZ (35 mg/kg, 0.9% saline) S.c. + Vehicle of chlorogenic acid.
Group 4: PTZ (35 mg/kg, S.c.) + Valproic acid (200 mg/kg) p.o.
Group 5: PTZ (35 mg/kg, S.c.) + chlorogenic acid (5 mg/kg) p.o.
Group 6: PTZ (35 mg/kg, S.c.) + chlorogenic acid (10 mg/kg) p.o.

PTZ Induced Seizure

Each group consists of 6 mice and study was conducted of 31 days. Subcutaneous injections of PTZ (15 injections) were given on alternate days up to 29 days, chlorogenic acid and valproic acid were given p.o. up to 29 days daily. Behavioral parameters were assessed on 29 days after PTZ injection, and 30 day by elevated plus maze test, passive avoidance test and 31 day forced swim test (FST) and open field test were performed. Biochemical estimations were done on 31 days after completion of behavioral parameters (Figure 1).

The conventional method specifies the exact steps to take to create a PTZ kindling model while following a low dosage PTZ administration schedule every other day. This protocol suggests that references/test compounds (treatment) that could influence the duration, intensity or occurrence of kindling will be examined by the researcher in parallel. Consequently, four categories of animal groups are required to that appropriate measure are in place to ensure that the effectiveness of the test/references compound is adequate determined. Pentylenetetrazole solution (35 mg/kg or 3.5 mg/mL, dose volume 10 mL/kg. Test/reference solution and vehicles 0.9% (w/v) saline.[8,9] Pentylenetetrazol (PTZ): PTZ can be used to build models of epilepsy for both acute and chronic animals example say an acute injecting of PTZ in mice at such a defined threshold (60–100 mg/kg, intraperitoneally or subcutaneously). Outcomes in myoclonic stroke, clonus, and tonic extension. Repetitive administering of PTZ at low dosage of the sub-threshold 20–40 mg/kg, intraperitoneally could produce kindling phenomenon. During each injecting of PTZ, the seizures rating is determined according to the following properties.[10]

- No changes in behaviour
- Blinking, ear and face twitching, isolated myoclonic
- Stare, nod
- Seizures, convulsions, and scratching limited to the forelimbs.
- Grand mal (generalized without tonic), double front leg lift; and
- Widespread muscle spasms, intermittent all-limb, and generalized tonic-clonic seizures

Behavioral Parameter

Elevated plus maze

The height of this maze was 50 cm above the ground, although it consists of two closed arms forming a cross with a square center. According to the described method, memory retention impairment was assessed using the elevated plus maze. Seizures were induced on the first day before the first test (initial transmission latency). However, mice were placed separately on the ends of the arms facing a central platform. was 60 seconds.

Immediately after assessing the first transmission latency, mice were removed in the maze arm and transferred to their home cages. A second hold transfer latency attempt was made after 24 hours in the same way inducing seizure (Figure 2).[11]

Open field test

was conducted to determine the chronic stress effects on mice. The OFT test designed to analyze PTZ- induced impact on the motivational activity of animals in the last day before the end of the trials. The unit consists of a rectangular box (80 × 80 × 50 cm) with floor separated into equivalent squares (25 × 16 × 16 cm). The mice were put in the open-field central area and given 3 minutes of free-roaming. During this test 2 criteria were quantified rate of locomotion (no. in which the mice has passed one of the four- paw grid lines). The animals were kept to the testing house for at least 2 hours prior to the start of the test. The OFT procedure was conducted in a sound proofing area there was no human intervention, even the room cleaning was performed with a 5% ethanol: water solution due to behavioral testing for bias elimination due to the odor left by retired rats. The study of the motivational activity of mice was carried out randomLy by two impartial observers the means outcome was statically evaluated according to the efficiency of the interobserver test (Figure 3).[12]

Forced swimming test

The device contains a cylinder (47 cm× 38 cm × 38 cm) of normal tap water temperature was maintained at 22 ± 1°C. The experiment subsisted 2 testing one is conditioning trial and another is test trial. In the conditioning trial, animals were smoothly kept in the cylinder for 15 minutes. Then the animals were dried and kept into a hot cage along with the paper napkins for 10–15 minutes. prior to return to their home cages. After 24 hours, another test trial was performed. Animals were put into the cylinder again for 5 minutes. Then after session of swim, animals were eliminated from the cylinder, swapped with a dry cloth, and put under the heating lamp for about 1/2 an hours prior to returning to their home cages. The immobilization time, defined as the loss of movement of the full body except that a little movement is important to keep the animal's head above the water, which was recorded. After test the cylinder was thoroughly cleaned. Animals were sacrificed under anesthesia and brain was removed. Brain tissue rinse with ice-cold normal saline prepare 10% homogenate with PBS centrifuge tissue at 10,000 for 20 minutes (Figure 4).[13]

Passive avoidance test

Mice underwent a passive avoidance test by placing the shuttle container in an 8W light tray. The light cabin was separated by a noose door from the dark room. The noose door was immediately entered and existed after a conditioning time 30 seconds after the mice enter the dark chamber. The dark room subjected experienced of a low intensity foot shocked (0.5 mA; 10 s) recorded the animals move from one chamber to the next which was reported in seconds as a transfer latency time (TLT). In a second trials (1st retention) given 24 hours after the 1st trials. The first trails were for acquisition and retention was evaluated. The tenure was 270 seconds. In retention trials the shock was not delivered to discourage reacquisition. In relation to the acquisition (1st) trials the criteria for learning was taken as arise in the TLT on retention (2nd or consequent) studies (Figure 5).[14]

Biochemical Parameters

Animals were sacrificed under anesthesia and brain was removed. Brain tissue rinse with ice-cold normal saline prepare 10% homogenate with PBS centrifuge tissue at 10,000 for 20 minutes.

Assessment of cerebral thiobarbituric acid reactive substances-TBARS

TBARS was estimated spectrophotometrically (at 532 nm, UV-1800 ENG 240V; Shimadzu Corporation Japan) using method depicted by Ohkawa in 1979. Supernatant-0.20 mL was pipetted out in a tube and then sodium dodecyl sulphate (0.20 mL of 8.1%), 1.50 mL-acetic acid (30% of pH 3.5), 1.50 mL-thiobarbituric-acid (0.8%) were mixed. Then, the volume was kept up to 4 mL by adding distilled water and incubated for 60 minutes (at 95° C) then permitted to cool. Subsequently cooling, distilled water (1-mL) and n-butanol-pyridine mixture (5 mL of 15:1 v/v) were mixed. These tubes were rotated at 4000 g for 10 minutes. The absorbance of emergent pinkish color was mentioned.[15,16]

Assessment of cerebral reduced glutathione (GSH) contents

Reduced GSH was assessed according to the method published by Beutler in 1963. Absorbance was noted through a spectrophotometer (at 412 nm). Trichloroacetic-acid (10% w/v) was added in the supernatant (1:1 proportion). Disodium-hydrogen-phosphate (2.0 mL of 0.30 M) was added in the half mL of supernatant and [5, 5`-dithiobis (2- nitro-benzoic-acid- DTNB) in 1% w/v sodium-citrate] (0.25 mL of 0.001 M). A standard plot was prepared using reduced GSH (10–100 µM) (Figure 6).[15,17]

Assessment of cerebral superoxide dismutase (SOD)

Brain SOD was assayed spectrophotometrically (560 nm) as methods depicted by Beauchamp and Fridovich (1971). In 0.5 mL of tissue-homogenate was mixed with 0.10 mM ethylene diamine-tetra-acetic-acid (EDTA-0.20 mL) 50 mM sodium carbonate (1.0 mL) and 24 µM nitro-blue tetrazolium-chloride (NBT-0.40 mL). The reaction started after adding of 1.0 mM hydroxylamine-hydrochloride (0.40 mL). The emerging blue tint was estimated at 560 nm.[18]

Estimation of cerebral acetylcholinesterase (AChE) activity

AChE was assessed spectrophotometrically (at 420 nm) by Ellman methods.[19] The interaction of dithiobisnitro-benzoate and thiocholine develops yellow tint. The supernatant (0.5 mL) was taken in a 25 mL flask. DTNB solution was added for preparing dilutions. Two parts of 4.0 mL were taken in to distinct test tubes. Eserine solution was mixed in one tube. In 1.0 mL of substrate solution was mixed in both tubes. The tube containing eserine drops was taken as blank.[15,19]

Measurement of Protein

Protein was determined in all brain samples by the Lowry method using bovine serum albumin (BSA) (1 mg/mL) as definitive.[20]

Statistical Analysis

The outcomes were represented as mean and standard error of mean-SEM. All the outcomes were analysed employing one-way analysis of variance-ANOVA preceded by Tukey's multiple comparison tests. A value of $p < 0.05$ was regarded as significant statistically.

RESULT AND DISCUSSION

Repeated injections of PTZ (35 mg/kg) every other day for 29 days (up to 15 times) resulted in firing, indicated by a continuous increase in the source (mean score 4-4 for up to 2 consecutive days). Like an animal set on fire. However, administration of 5 and 10 mg/kg chlorogenic acid significantly reduced kindling seizure scores compared to the PTZ-treated group ($p < 0.05$) (Figure 1). Valproate also significantly ($p < 0.05$) reduced seizures compared to PTZ. A dose-dependent effect in reducing seizure scores was observed, with admirable protection compared to lower doses.

Although there was no significant difference between different groups in (acquisition study), repeated administration of PTZ significantly ($p < 0.05$) prolonged transmission latency in comparison with group (retention study). However, administration of chlorogenic acid (5 and 10 mg/kg) 30 minutes prior to PTZ administration significantly reduced metastasis latency compared to the PTZ group. Administration of valproic acid also significantly ($p < 0.05$) reduced the transduction latency binding compared to the PTZ group.

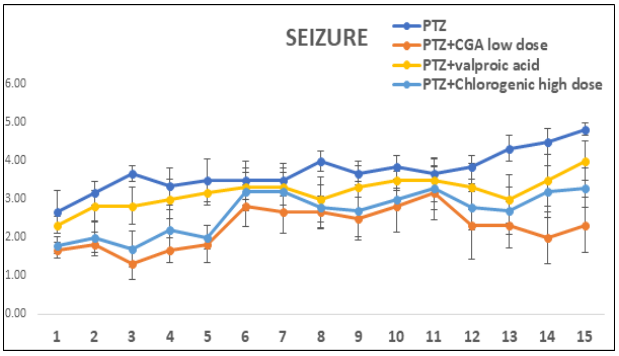
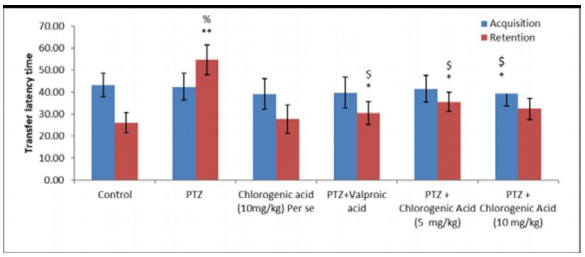


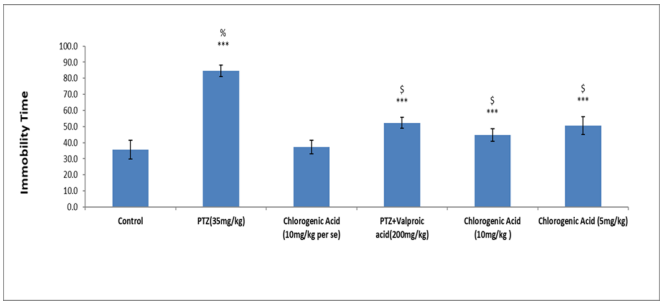
Figure 1: Effect of chlorogenic acid on seizure score



All values are expressed as mean ± SEM %**p < 0.05 compared with the control group, $**p < 0.05 compared with the PTZ-treated group.

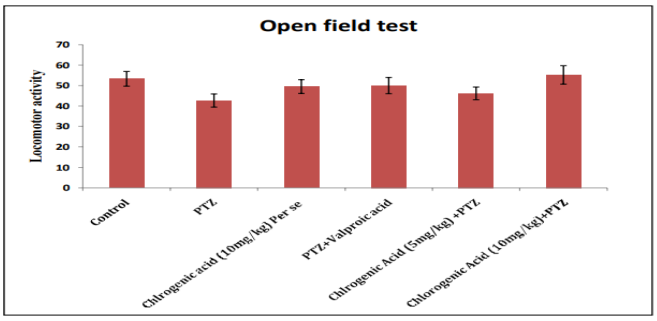
Significant increase in Transfer latency time (***p < 0.001) vs. acquisition trial.

Figure 2: Effect of chlorogenic acid on elevated plus maze



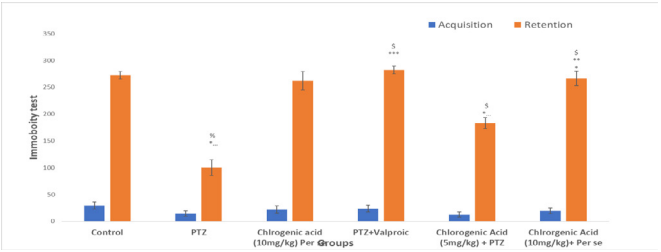
All values expressed in Mean ± SEM. %***($p < 0.001$), when compared with control group, $*** ($p < 0.001$), when compared with treated group.

Figure 3: Effect of chlorogenic acid on FST



There is no significant difference in locomotor activity between different treatment groups ($p > 0.05$).

Figure 4: Effect of chlorogenic acid on open field test

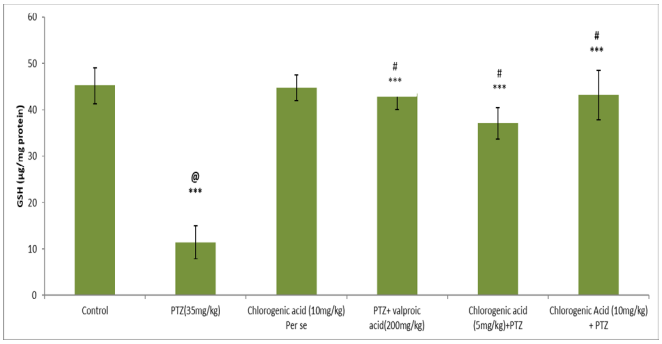


All values are expressed as mean ± SEM %**p < 0.001 compared with control group, $**p < 0.001 compared with PTZ-treated group.

Figure 5: Effect of chlorogenic acid on passive avoidance test

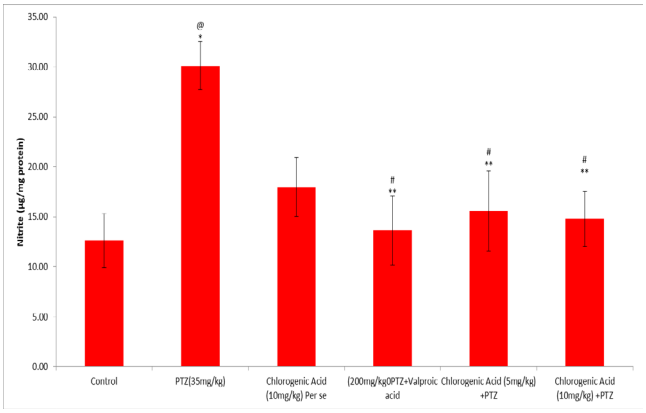
FST was performed on day 30 to evaluate the immobility time after repeated administration of PTZ ($p < 0.001$), which increased the immobility time compared to the control treated group. However, administration chlorogenic acid (5 and 10 mg/kg) significantly ($p < 0.001$), decreased the immobility time when the PTZ kindled groups. Administration of valproic acid decreased the immobility tie as compared to PTZ treated groups.

Repeated administration of PTZ significantly decreased P and L, although there was no significant difference between the different groups in (acquisition study). 0.001, transmission delay compared to controls in retention trials. However, $p < 1$ was significantly prolonged when chlorogenic acid (5 and 10 mg/kg) was administered 30 minutes before PTZ



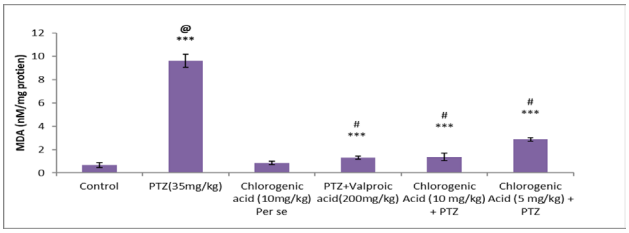
All values are expressed as mean ± SEM@***p <. 0.001 compared with the control group, #*** p < 0.001 compared with the PTZ-treated group.

Figure 6: Effect of chlorogenic acid on GSH



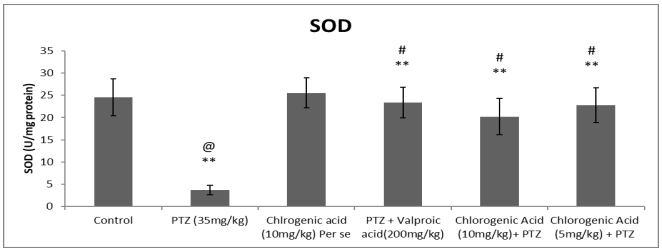
All values expressed in Mean ± SEM@*** < 0.05, when compared with control group, #*** <0.05.

Figure 7: Effect of chlorogenic acid on nitrite oxide



All values expressed in Mean ± SEM@*** p <0.001, when compared with control group, #*** p <0.001, When compared with PTZ treated groups.

Figure 8: Effect of chlorogenic acid on MDA (lipid peroxidation)



All values expressed in Mean ± SEM@** p <0.05, when compared with control group, #** p <0.05, when compared with PTZ treated group.

Figure 9: Effect of chlorogenic acid on SOD

administration. 0.001 transmission delay compared to PTZ group. Administration of valproic acid also significantly decreased p < 0.001, and reduced transmission late binding compared to PTZ group.

PTZ-induced flare-ups showed an increase in oxidative stress, resulting in a significant decrease (< 0.001) compared to the PTZ-treated group. Valproic acid showed an increase in GSH levels compared to the PTZ group (< 0.001). Treatments at 5 and 10 mg/kg showed dose-dependent effects.

Repeated administration of PTZ resulted in increased free radical and caused oxidative stress as soon by significant elevated level of nitrite oxide in PTZ treated groups as compared to control group (Figure 7). However, administration of chlorogenic acid at (5 and 10 mg/kg) significantly (p <0.05) reduced the nitrite oxide level as compared to PTZ treated group.

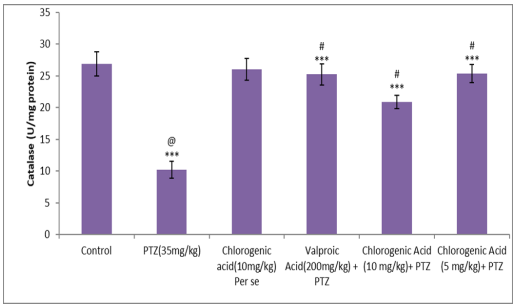
Valproic acid also significantly reduced the level of (p <0.05), in comparison to PTZ group.

Repeated administration of PTZ resulted in increased free radical and caused oxidative stress as soon as elevated level of MDA (Lipid peroxidation) in PTZ treated groups compared to the control group. However, administration of chlorogenic acid at (5 and 10 mg/kg) significantly (p <0.005), reduced the MDA level as compared to PTZ treated group. Valproic acid also significantly reduced the level of (p <0.005), in comparison to PTZ group (Figure 8).

PTZ induced kindling exhibited increased oxidative stress leading significantly (p <0.05), decreased SOD compared to the normal control groups. However, the administration of chlorogenic acid (5 and 10 mg/kg) significantly increased SOD (p <0.05), as compared to PTZ treated group. Valproic acid exhibited increased SOD (p <0.05) compared to the PTZ group. Treatment with (5 and 10 mg/kg) showed the dose-dependent effect (Figure 9).

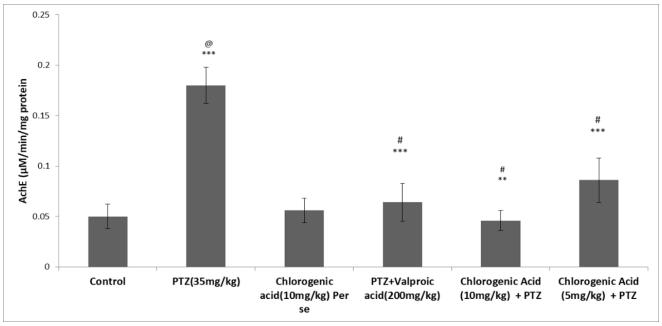
PTZ induced kindling exhibited increased oxidative stress leading significantly (p <0.001), decreased catalase compared to the normal control groups. However, the administration of chlorogenic acid (5 and 10 mg/kg) significantly increased catalase (p <0.001), as compared to PTZ treated group (Figure 10). Valproic acid exhibited increased catalase (p <0.001) compared to the PTZ group. Treatment with (5 and 10 mg/kg) showed the dose-dependent effect.

PTZ induced kindling exhibited increased oxidative stress



All values expressed in Mean ± SEM@*** p < 0.001, when compared with control group, #*** p <0.001, When compared with PTZ treated group.

Figure 10: Effect of chlorogenic acid on catalase



All values expressed in Mean ± SEM@*** $p < 0.001$, when compared with control group, #*** $p < 0.001$, When compared with PTZ treated group.

Figure 11: Effect of chlorogenic acid on AChE

leading significantly ($p < 0.001$), decreased AChE compared to the normal control groups. However, the administration of chlorogenic acid (5 and 10 mg/kg) significantly increased AChE ($p < 0.001$) as compared to PTZ treated group. Valproic acid exhibited increased AChE ($p < 0.001$) compared to the PTZ group. Treatment with (5 and 10 mg/kg) showed the dose-dependent effect (Figure 11).

DISCUSSION

Flavonoids, which are natural polyphenolic compounds, have gained increasing importance in recent years in alleviating various ailments, as they are safe and effective natural substances. Several studies on flavonoids have reported potentiating effects on various epileptogenic agents. Flavonoids such as resveratrol,[21] quercetin,[22] epigallocatechin-3-gallate,[23] naringin,[24] and rutin[25] are associated with electrically and chemically induced epilepsy and inflammation. It shows promising anti-epileptic activity against the model. They are effective in PTZ-induced kindling and PTZ-induced tonic-clonic seizures, which resemble generalized tonic-clonic seizures in humans.[26] In this study, long-term use of valproic acid protects against PTZ-induced firing. This may be due to the ability of PTZ to suppress oxidative stress. Furthermore, pretreatment with chlorogenic acid attenuated seizure scoring and kindling by PTZ. General stimulatory or inhibitory effects of central nervous system active drugs can influence animal responses to behavioral paradigms. Therefore, we also investigated the effects of chlorogenic acid on transition latency in the elevated plus maze, transition latency in the passive avoidance test, immobility time in the forced swim test, and locomotor activity. n the begun study, pretreatment with 10 mg/kg chlorogenic acid provided better protection against PTZ-induced neurobehavioral changes. This dose level of chlorogenic acid significantly decreased transition latency to exit the first arm, significantly increased transition latency to entering the dark chamber, significantly decreased immobility time on the FST, It was observed that it did not result in significant changes in locomotor activity. All his PTZ pretreatment groups (except the valproic acid treatment group) and activity compared to the PTZ group. The above results are consistent with previous reports that dietary flavonoids have shown improvements in cognition and memory in animal models. In addition, there is considerable evidence that flavonoids (particularly flavonols, flavanones, and anthocyanins) found in fruits and fruit juices can improve memory.[27]

Oxidative stress plays an important role in the pathogenesis of epileptogenesis and related complications. Literature reports indicate that PTZ firing causes elevated levels of MDA, nitric oxide, and decreased levels of GSH, superoxide dismutase, and catalase, which can lead to neurodegeneration and mitochondrial damage was.[28] Chlorogenic acids, the major class of phenolic acids found in coffee, play a central role in enhancing antioxidant capacity by increasing the activity of GSH, superoxide dismutase, and catalase, leading to MDA and monoxidation. It also plays an important role as an anti-inflammatory agent by decreasing nitrogen activity. Acetylcholine plays an important role in memory. Acetylcholine levels in tissues were indirectly estimated using AChE levels. Several studies on PTZ have indicated that elevated AChE levels may contribute to cognitive impairment by lowering brain acetylcholine. Chlorogenic acid lowered her AChE levels and improved her PTZ effect on memory. Thus, chlorogenic acid's antioxidant and AChE-inhibitory capacity attenuated PTZ-induced memory deficits. The progressive nature of epileptogenesis alters the development and function of brain regions through neurodegeneration. There is increasing evidence linking it to increased oxidative stress and reduced antioxidant enzyme activity, and repetitive seizures play an important role in neuronal cell death.[29] In the present study, neurobehavioral changes induced by PTZ kindling are supported by oxidative stress, which mirrors epilepsy-associated neurodegeneration. In experimental models, chlorogenic acid showed dose-dependent effects on acute PTZ-induced seizures and associated oxidative stress, inflammation, and cognitive impairment. This action of chlorogenic acid may contribute to its antispasmodic effect. Chlorogenic acid provided better protection against PTZ-induced inflammation, memory impairment, oxidative stress, and neurodegeneration. This study shows that pretreatment with 10 mg/kg chlorogenic acid reduced seizure score and inflammation, and attenuated PTZ-induced effects such as memory loss, oxidative stress biomarkers, and neurodegeneration. The above results may be due to its antioxidant, anti-inflammatory effects, AChE activity inhibition, and chlorogenic acid neuroprotective effects.

CONCLUSION

The results of our study showed that chlorogenic acid provided better protection against PTZ-induced firing, behavioral disturbances, and oxidative stress. he oxidative biomarker and AChE results strongly suggest that the protective effect is due to a reduction in oxidative stress, an increase in antioxidant enzymes that accompanies this neuroprotection. Our study is a preliminary finding on the anti-epileptic and antioxidant effects of chlorogenic acid on PTZ-induced kindling and thus may be a future candidate in the treatment of epilepsy. Further studies in preclinical and clinical scenarios are needed to provide better scientific support for the development of chlorogenic acid as a

therapeutic regimen to alleviate epilepsy-related comorbidities.

AUTHOR CONTRIBUTIONS

Each author actively contributed to the idea and design, collection of the data, or the analysis and, interpretation of the findings. All authors also made substantial contributions in drafting the article and reviewing.

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