Original Article

Neuroprotective Efficacy of Quercetin with Lamotrigine and Gabapentin Against Pentylenetetrazole-induced Kindling and Associated Behavioral Comorbidities in Mice

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ABSTRACT

Aim: Epileptic seizure is a widespread neurological condition with multifaceted physiology. Numerous scientific data advise a therapeutic efficacy of bioflavonoids or bioactive compounds in epilepsy against oxidative stress pathway. Thus, the main objective of this investigation has been undertaken to explore the potential neuroprotective efficacy of Quercetin (Que) bioflavonoid with selected anti-epileptic drugs (AEDs) against pentylenetetrazole (PTZ) induced kindled model of convulsions in mice. **Materials and Methods:** Swiss albino mice (20-30 g) were individually separated into seven groups (n = 6). Before PTZ administration, quercetin was dissolved in 0.6% w/v carboxymethylcellulose (CMC) sodium and served for 7 days orally. On the seventh day, 30 minutes before PTZ administration, Lamotrigine (Lmt) and Gabapentin (Gbp) were solubilized with saline and given as single intraperitoneal (i.p.) injections. PTZ (30 mg/kg, i.p.) was given in a sub convulsive dose on alternate days for 12 days until the mice seemed to have complete motor seizures. **Results:** PTZ dosage in the sub-convulsive range (30 mg/kg, i.p., every other day for 12 days) resulting in a progressively rise in convulsive behavior (seizure score). Que (20 mg/kg) + Lmt (15 mg/kg, i.p.) administration for 12 days exhibited improvement in transfer latencies. In a histopathological study, microphotographs (×40) of brain tissue of mice showed cell morphology in the different experimental treated groups. **Conclusion:** Quercetin has been reported to provide antioxidant efficacy as a potent bioactive compound when treated with the combination of quercetin and lamotrigine, which exhibited significant modulation in their neuroprotective efficacy.

Keywords: Lamotrigine, Neuroprotective effect, Anticonvulsant activity, Kindling model, Quercetin, Histopathological study.

INTRODUCTION

Epilepsy is a serious neurological condition of the nervous system. Quercetin is a flavonoid found in a range of fruits and vegetables. Quercetin is a natural antioxidant and scavenger featuring anti-inflammatory, astringent, anti-ischemic, neuroprotective, and anti-epileptic properties. The impact of quercetin on visual memory impairment and neurotoxicity caused by repeated ischemic injury was significantly improved.[1] Flavonoid have been found to exhibit favourable effects on patients with diseases of the cardiovascular and cancer. Moreover, these bioflavonoids have antitumoral, anti-inflammatory, antioxidant, and antiviral effects. Kindling models have been suggested as potentially important and useful method for the identification of anti-epileptogenic therapies.[2] Previous research investigations have elaborated lamotrigine efficacy in the kindling model induced electrically. Another chemical kindling model, such as the pentylenetetrazole (PTZ) model.[3] Mostly Flavonoids are polyphenols that can be present in a variety of natural vegetables, including dietary foods, fruits, and some

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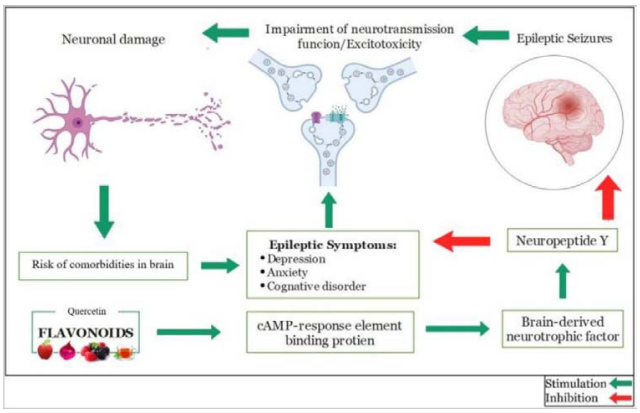


Figure 1: Graphical representation of the neuroprotective potential of flavonoid in epilepsy: Bioflavonoid directly acts on binding protein and influence the neurotrophic factor that helps to prevent the neurodegeneration of neuronal cells.[13]

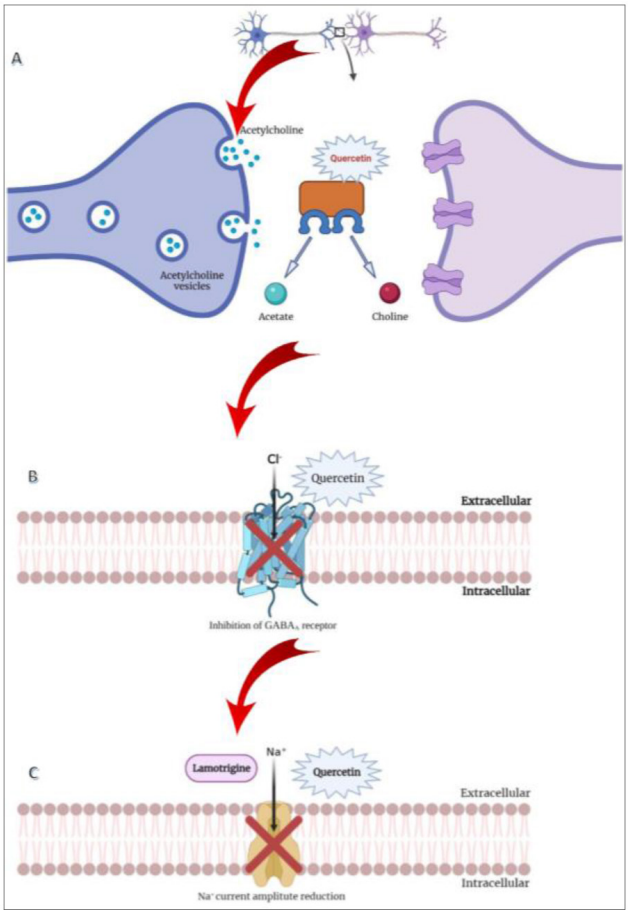


Figure 2: Graphical representation of specific mechanisms of epileptic seizures and the impact of quercetin with lamotrigine on neuronal activity. A) Quercetin leads to inhibition of choline and acetate by blocking acetylcholine enzymatic action. B) Here, the restriction of intracellular Cl^- efflux is triggered by quercetin suppression of $GABA^A$ receptors. C) suppression of the Na^+ efflux leads to reduction of amplitude current by the effect of quercetin and lamotrigine.

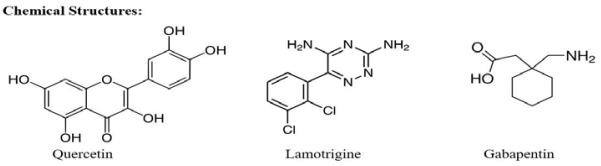
beverages, as shown in Figure 1. Various recent studies have discovered that certain flavonoids have a variety of therapeutic properties for epileptic patients without any adverse effects linked to traditional health systems.[4] The involvement of free radicals in refractory epilepsy has been emphasized by epileptic seizure oxidative stress, neurotoxicity, and alterations in neurochemicals (GABA and glutamate), which are correlated to seizure development, transmission, and regulation.[5] Furthermore, an inequality of excitatory and inhibitory amino acids in the brain is one of the pathophysiological processes of neurological problems.[6] As a consequence, various antiepileptic drugs are intended to restore this imbalance. The inhibitory and excitatory chemicals in the nervous system are glutamate and aminobutyric acid (GABA), accordingly. It has been specified that quercetin may have a potential effect on GABA and glutamate receptors are shown In Figure 2.[7-8] In mice, the excitatory effects of pentylenetetrazole and picrotoxin are associated with GABA antagonism, inducing seizures. Quick or lengthy procedures for PTZ kindling can be used, every day, or alternative treatment sub-threshold dose injections for fifteen or thirty-six days, respectively.[9] The optimal PTZ dose for tonic-clonic convulsions after intraperitoneal administration by the kindling protocol is 30 to 50 mg per kg. In particular, the PTZ kindling model of epilepsy may be used for refractory or drug-resistant epilepsy in which the treatment of drug compounds is used for a long period of time in a particular manner.[10-11] The Amygdala kindling model is also responsible for the evaluation of drug-resistant epilepsy in mice.[12]

Biochemical mechanism of PTZ on GABA receptor

The kindling seizures model focused on PTZ administrations is remarkably frequently used for the evaluation of mechanisms of refractory epilepsy, despite the reality that the actual mechanism by which PTZ induces pharmacological response is still unknown.[14] The PTZ targets have always been the intense priority of many research studies over the last four decades. PTZ has been shown to associate with the benzodiazepine (BZ) promoter regions of the GABA receptors.[15] The hypothesis has been connected to prolonged cyclic nucleotide decomposition and alterations in ion concentration. Flavonoids are a wide collection of natural ingredients with numerous therapeutic properties. Due to their antioxidant potential, flavonoids have achieved such a good effect on disease management, either alone or in combination with other therapeutic drugs.[16-17] Scientific research articles on flavonoid diets supplemented with AEDs highlight the beneficial

potency of bioflavonoids on epileptic seizures, kindling development, and AED-induced comorbidities.[18]

Chemical Structures



Materials and Experimental Design

Animal

Healthy Swiss albino mice with an initial body weight of between (20 and 30 g) aged 14 weeks were used and procured (12:12 hr light-dark schedule) in the animal house facility of Pinnacle Biomedical Research Institute, Bhopal, M.P. with maintaining a consistent pelleted diet and *ad libitum* water with appropriate ventilation and proper hygiene. The mice were housed in polypropylene cages with steel wire mesh over just a paddy husk surface. All animal investigations were carried out following the standards of CPCSEA as well as after gaining prior permission from the IAEC protocol approval no. PBRI/IAEC/22-10-21/008.

Drug and Treatments Schedule

In this study, Quercetin (CAS Number: 117-39-5, Sigma, USA, Pentylenetetrazole (CAS Number: 54-95-5, Sigma, USA), Lamotrigine (CAS Number: 84057-84-1, Sigma, USA), and Gabapentin (CAS Number: 60142-96-3, Sigma, USA) were used. The amounts of these drug compounds were designated on the evidence of past research. Before PTZ administration, quercetin was dissolved in 0.6% w/v carboxymethylcellulose (CMC) sodium and served orally for 7 days. On the seventh day, Lmt and Gbp were dissolved with normal saline and given as a single (i.p.) intraperitoneal injection 30 minutes prior to PTZ treatment. The following groups were included in the research protocol (n = 6):

- Group 1 (Naive): Healthy animals (no treatment)
- Group 2 (PTZ): Vehicle (0.6% w/v Sod. CMC) + PTZ (30 mg/kg, i.p.) every other day for duration of 12 days.
- Group 3 (Lmt + PTZ): Lmt (15 mg/kg, i.p.) + PTZ (30mg/kg, i.p.) every other day for duration of 12 days.
- Group 4 (Gbp + PTZ): Gbp (20mg/kg, i.p.) + PTZ (30mg/kg, i.p.) every other day for duration of 12 days.
- Group 5 (Que + PTZ): Que (20mg/kg, i.p.) + PTZ (30mg/kg, i.p.) every other day for duration of 12 days.

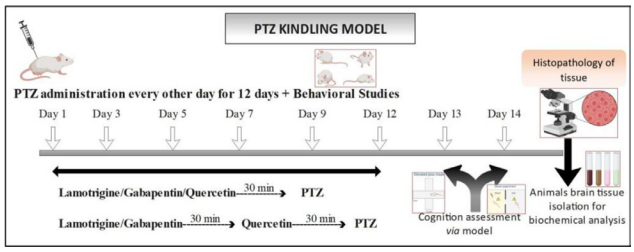


Figure 3: Graphical representation of treatment of groups against PTZ kindling model.

- Group 6 (Lmt + Que + PTZ): Que (20mg/kg, i.p.) + Lmt (15 mg/kg, i.p.) + PTZ (30mg/kg, i.p.) every other day for duration of 12 days.
- Group 7 (Gbp + Que + PTZ): Que (20mg/kg, i.p.) + Gbp (20mg/kg, i.p.) + PTZ (30mg/kg, i.p.) every other day for duration of 12 days.

Pentylenetetrazole-Induced Kindled Seizures

PTZ (30 mg/kg, i.p.) was given in sub-convulsive doses on alternate days for 12 days until the animal seemed to have complete motor seizures. Animals were kept in a plexiglass chamber (40 × 23 × 23 cm) with partitions in between after each injection of PTZ, and seizure intensity was monitored for 30 minutes.[19-20] The frequency of convulsion was assessed using a 4 point scoring scale: 0 = no response, 1 = myoclonic jerks, 2 = Straub's tail with an upright position, and 3 = clonus with head jerks, total responses of mean were evaluated for the period of treatment.[21] After the last PTZ administration, the cognitive behaviour of the animal was examined for 24 hr (13th and 14th days) are shown in Figure 3.

Behavioural Study

The Racine scale was used to observe the severity of behavioural response in PTZ-induced seizures: Stages 0: No response, Stage 1: Facial and ear twitching, Stage 2: Myoclonic involuntary jerks, Stage 3: Myoclonic sudden jerks, Stage 4: Tonic-clonic seizure, Stage 5: Generalized clonic-tonic convulsion.[21]

a) Passive avoidance (punishment) reinforcement (step-down)

A specially modified, two-different training model that has been validated in our laboratory was used to assess recall and learning processes. The device consists of a plexiglass chamber (40 × 23 × 23 cm) with a metallic grid-bottom and wooden platform (12 x 8 cm) attached in the centre, which provides a safe zone. The animals were used for training trials in which they were separated on an SFZ platform and each fall toward the grid bottom was punished with a 2-sec electric foot shock (50 V a.c.). After that, the mice were permitted to stay

in the safe zone for 60 sec. Following the last training session without shock, the step-down delay was observed in seconds [acquisition latency (AL)]. After 24 hr, the mice were observed again, but this time no sudden shock was applied, and the time it took the mice to step down was recorded. [retention latency (RL)]. The animal that didn't escape from SFZ within this timeframe was given a 600-sec cut-off time.[22]

b) Elevated plus-maze model

Elevated model is made up of two uplifted long open arms (18 cm × 7 cm) and, two long-closed arms of similar sizes joined by a main rectangular size - 6 cm × 5 cm, which is another model for assessing cognitive dysfunction. The model is elevated to a height of 28 cm above the ground, and each arm has 12-cm-high sidewalls. Individual animals were placed on the edge of one open arm, facing a distance from the mid-center, and the period it took the mice to walk from the open arm to either of the closed (elevated) arms of the apparatus was observed to determine the initial transfer latency (ITL). The mice were lightly pushed in the direction of a closed arm if they did not reach either of the closed arms within 120 sec (cut-off time).[22] The mice were permitted to observe the response for 20 sec before finally being returned to their main cage. After 24 hr, time spent by the mice to reached in the closed arm was observed [retention transfer latency] (RTL).

Biochemical Assay

Animals were sacrificed in order by decapitation following behavioural assessments; The brains were washed with isotonic saline, then noted the weight, and cut into two similar halves. The other half part was used to make biochemical calculations. For biochemical analysis, 10% (w/v) homogenates tissues were added to 0.1 M phosphate (pH 7.4) buffer. Then centrifugated homogenates at 10,000 g for 15 min at 4°C. Supernatants were split into aliquots and were used for biochemical assessment.[23-24] For biochemical analyses, a UV spectrophotometer 2202 [systronics] was used.

a) Estimation of nitrite level

The amount of nitrite in the precipitate was measured as a marker of nitric oxide (NO) synthesis in which the colorimetric method was used with Greiss reagent (0.2% N-[1-naphthyl] ethylenediamine di-hydrochloride, 2.6% phosphoric acid, 1% sulfanilamide). The Greiss reagent and supernatant were combined in equal proportions and reserved for 10 min. The absorbance was noted by using a double beam UV–VIS spectrophotometer [UV spectrophotometer 2202 (systronics)] at 560 nm. A sodium nitrite standard calibration curve was used to evaluate the unknown concentration amount of nitrite in the supernatant, which was quantified as micromoles per litre.

b) Estimation of reduced glutathione (GSH) level

Glutathione levels in the animal brain were usually investigated using Ellman and his collaborators' approach. The 1 ml of supernatant was crystallised by cold digested at 4°C for 60 min with 1 ml [5% sulfosalicylic acid]. The homogenate was centrifuged at 1000 rotations per minute for 15 min at 4°C. 2.7 ml of [0.1 M phosphate buffer] at pH 8 and 0.3 ml of 5,5 [dithiobis-2-nitrobenzoic acid] were used for 1 ml of the precipitate. The finding was determined by using the chromophore's molar extinction coefficient (1.38 × l04 M-1 cm-1) and was represented as micromoles of GSH per milligrams of protein. The yellow colour formed was instantly detected at 430 nm using a double beam UV–VIS spectrophotometer. [UV spectrophotometer 2202 (systronics)] was used.

c) Estimation of Protein Level

The protein was also determined and followed by the biuret technique with bovine serum albumin used as the standard.

d) Estimation of Lipid Peroxidation Level

The Wills model was followed to calculate the quantitative level of lipid peroxidation in the mice's brains. The values were determined using the chromophore's molar extinction coefficient (1.58 × 107 M-1 cm-1) and reported in the result as malondialdehyde nanograms per milligram of proteins. The level of malondialdehyde (MDA), which is a major content of lipid peroxidation, was also determined using a double beam UV–VIS spectrophotometer [UV spectrophotometer 2202 (systronics)] in a reaction with thio-barbituric acid at 540 nm.[25]

e) Estimation of Catalase Level

The Luck method was used to detect catalase level, which accurately measures the decomposition of hydrogen peroxides (H_2O_2) at 240 nm. The combination comprised 0.04 ml of homogenate tissue supernatant (10%) and 3 ml of H_2O_2 phosphate buffer.[25] The variation in the value of absorbance was noted at 240 nm by using a [UV spectrophotometer 2202 (systronics)]. The Kono method was used to measure the activity of superoxide dismutase, which inhibited the decreasing of nitro blue tetrazolium (NBT), which was detected at 540 nm by double beam UV–VIS spectrophotometer [UV Pharmaspec, 1700, Shimadzu (Japan)]. The results were reported in micromoles of H_2O_2 disintegrated per milligrams of protein/min. In a sense, the reaction was started by the pouring of hydroxylamine HCl into a solution of Nitro-blue tetrazolium chloride. The

Table 1: Pharmacological effect of quercetin with selected AEDs against PTZ induced kindled mice.

Sl. No.	Groups	Treatment, dose mg/kg body weight i.p.	No. of animal convulsed/no. of animal used (on 12th Day)	Percentage % of protection	Duration of latency of convulsion (s) mean ± SEM
1	Group I: Control	Naive (no treatment)	0/6	100	-
2	Group II: Negative	PTZ+NS	6/6	0	60.26±2.45
3	Group III: Standard	Lmt + PTZ	1/6	83.33	1570.34±5.6*
4	Group IV: Standard	Gbp + PTZ	2/6	66.66	1260.42±4.7*
5	Group V: Test I	Que + PTZ	4/6	33.33	216.56±8.4*
6	Group VI: Test II	Que + Lmt + PTZ	0/6	100	-
7	Group VII: Test III	Que + Gbp+ PTZ	1/6	83.33	1480.48±5.7**

One-way ANOVA was used to analyze the data, followed by Dunnett's multiple comparison values against the control group. *$p < 0.5$; **$p < 0.01$.

findings were represented as units per mg of protein, with one part of the enzyme matching the amount of enzymes that completely inhibits the rate of the reaction.

Histopathology

After receiving an overdose of anesthesia, the animals were decapitated and sacrificed. The brain was carefully removed from the dead animals and then a histopathological study was conducted. Other characteristics were measured, and calculated per milligramme of protein of tissue by using the entire brain. The tissue was dissected out and mixed with 10% formalin before being stained with eosin and hematoxylin.[26]

a) Statistical analysis

The data obtained were assessed using Version 8.0, Graph-Pad Prism software. All experimental and biochemical estimation values were determined as mean ± SEM. Statistically, results were examined by using an (ANOVA) analysis of variance by Dunnett's test multiple comparison tests, $p<0.5$; $p<0.01$ was measured to be as significant.

RESULTS

Pharmacological efficacy of quercetin, lamotrigine, gabapentin and their combinations against PTZ-induced kindled seizure in mice

The repeated treatment of PTZ every other day for 12 days *via* sub convulsive dose showed a progressively rise in sudden convulsive behavior (seizure score) in kindled mice, concluding in generalized clonic–tonic convulsions in comparison to the naive group. When compared to the control (PTZ-kindled) group, the treatment of Que (20 mg/kg) with Lmt (15 mg/kg) for the period of 12 days substantially decreased the duration of kindling and delivered 100% protection in seizures induced by PTZ kindling. However, when Gbp

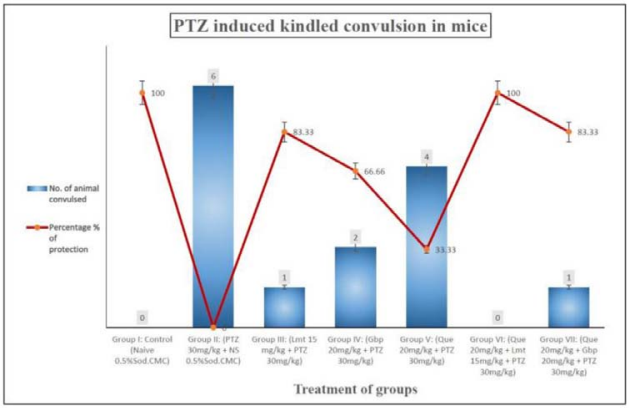


Figure 4: Pharmacological efficacy of quercetin with lamotrigine and gabapentin against PTZ induced kindled mice.

(20 mg/kg) was administered for 12 days, it suggestively increased the duration of latency of convulsive action when compared to a control group is shown in Figure 4, although the combination action with the quercetin minimized the intensity of seizure activity expressed in Table 1.

Modulatory potential action of quercetin, lamotrigine, gabapentin, and its combinations on the behavioral response (score) against PTZ-induced kindled mice

Numerous behavioral responses in PTZ-induced kindling mice were observed is shown in Figure 5, followed by a Racine scale, a known method for categorizing seizure intensity into 5 stages. PTZ treated animals experienced 100% score from stage 1 to stage 5; Clonic-tonic phase, as well as an 80% response at stage 5. on the other hand, Lmt-treated mice, only exhibited a sedative effect in 80% of the animals. Que treated mice evolved from stage 1 to stage 4. Animals treated with a combination of drugs (Que + Lmt) were recognized from stage 3 to stage 4 in 10–20% of cases. Except

Table 2: Behavioral response of mice on PTZ induced kindled seizure.

Behavioral response	Naive	PTZ	Lmt + PTZ	Gbp + PTZ	Que + PTZ	Que +Lmt + PTZ	Que + Gbp + PTZ
Stage - 0	100%	0	0	0	0	0	0
Stage - 1	0	100%	0	10%	20%	0	20%
Stage - 2	0	100%	0	20%	100%	0	0
Stage - 3	0	80%	20%	0	50%	10%	10%
Stage - 4	0	100%	50%	40%	80%	20%	50%
Stage - 5	0	100%	0	0	0	0	0
Mortality	0	0	0	0	0	0	0
Sedation	0	0	80%	50%	0	0	0

for the controls, none of the mice were treated with numerous medication regimens are shown in Table 2.

Stages 0: no response; Stage 1: facial and ear twitching; Stage 2: myoclonic involuntary jerks, lacking upright posture; Stage 3: myoclonic sudden jerks, with upright bilateral clonus forelimb; Stage 4: Tonic-clonic seizure; Stage 5: generalized clonic-tonic convulsion, with lacking postural change.[27]

Modulatory Potent action of lamotrigine and gabapentin on the neuroprotective efficacy of quercetin on passive avoidance task on PTZ induced kindled mice

Chronic PTZ administered animals exhibited a significant impairment in memory and learning that was confirmed by a significant fall in latency of step-down [AL and RL] when compared to the naive group. In contrast, PTZ treatment with Lmt (15 mg/kg, i.p.) of 12 days significantly decreased the latency of step-down in both AL and RL periods are shown in Figure 6. Additionally, Lmt (15 mg/kg) was administered for 12 days and resulted in better latencies of step-down through acquisition and retention phases (AL and RL) as compared to the control animal group. In comparison with the control group, Gbp (20 mg/kg, i.p.) developed a substantial drop in the level in AL and RL and showed significant memory and cognitive impairment. But, when as compared to the naive animal group, administration with Que (20 mg/kg) showed a minor but significant effect.

Modulatory effect of lamotrigine and gabapentin on the neuroprotective potential of quercetin in elevated plus-maze model on kindled mice.

The value of ITL as well as RTL, which both reflect task acquisition and retrieval, were found to be significantly different in groups. When compared to the naive mice group, the control mice seemed to have a significant rise in both (ITL) as well as (RTL), which showed severe cognitive impairments in Figure 7. Moreover, associated

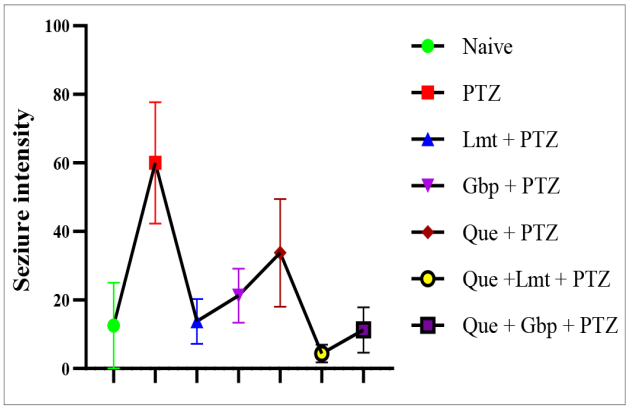


Figure 5: Behavioral responses observed by Seizure intensity of individual groups on PTZ-induced kindled mice.

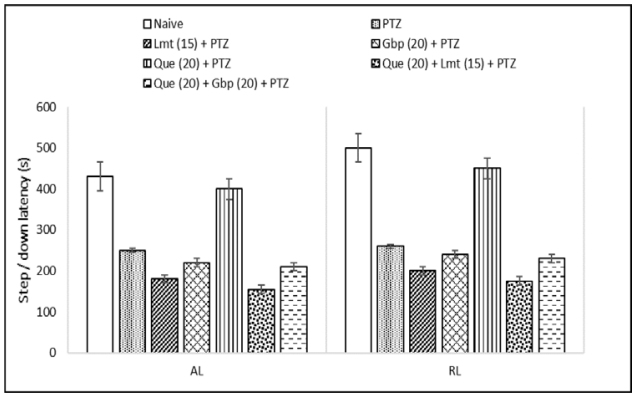


Figure 6: Modulatory effect of lamotrigine and gabapentin on the neuroprotective efficacy of quercetin on passive avoidance task on PTZ-induced kindled animal. All values of mean expressed ± SEM. $p < 0.05$ when compared to AL and RL of naive.

with the PTZ-treated group, administration of 20 mg/kg quercetin improved RTL as well as a significant reduction in ITL, confirming its neuroprotective potential. In addition, when correlated to the control, treatment of Lmt (15 mg/kg, i.p.) with Gbp (20 mg/kg, i.p.) outcome in significant delays in ITL and RTL, indicating substantial memory and learning

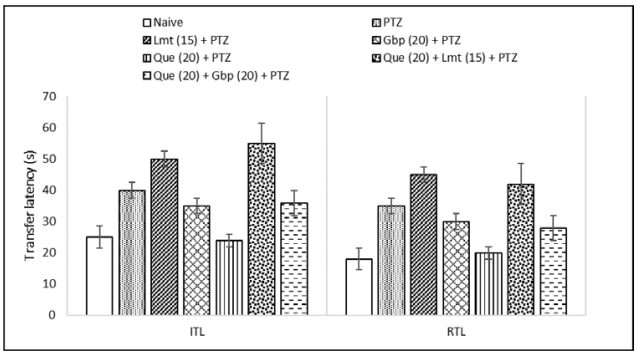


Figure 7: The modulatory potent effect of lamotrigine and gabapentin on the neuroprotective efficacy of quercetin in elevated plus maze task on kindled mice. All values of mean expressed ± SEM. $p < 0.05$ when compared to ITL and RTL of naive.

cognition effects. In contrast, when compared to the control, Que (20 mg/kg) + Lmt (15 mg/kg, i.p.) treatment of 12 days lead to given enrichment in transfer latencies (ITL and RTL).

Neuroprotective efficacy of quercetin, lamotrigine, gabapentin and its combinations on nitrite, glutathione, catalase, and lipid peroxidation levels in kindled mice induced by PTZ

PTZ (30 mg/kg) chronic treatment resulted in a massive reduction of brain GSH, catalase, and SOD concentrations when compared to the naive animal. In contrast, Que (20 mg/kg) for 12 days, Gbp (20 mg/kg) provided significant disruption of brain catalase and GSH concentrations, whereas PTZ (control) induced significant damage to all these antioxidant enzymes. Treatment with lamotrigine (15 mg/kg) for 12 days preserved the antioxidant enzymes against exhaustion. Furthermore, when quercetin (20 mg/kg) was treated with lamotrigine (15 mg/kg), these major antioxidant enzyme levels were significantly restored when compared to the naive group, as shown in Figure 8. When quercetin (20 mg/kg) was treated with gabapentin, the outcomes were observed to be non-significant when compared to the naive.

Histopathological microphotographs (×40) of brain tissue of mice showing cell morphology in the different experimental treated groups

In histopathology, cytological tissue photomicrographs were viewed under the biological instrument (Compound Microscope: National 169-PH Trinocular, Tanotis., Pvt., Lmt., Bangalore) by a blinded observer. The following cytological histopathological investigation analyzed progressive abnormalities in cells, cytoplasmic cell shrinkage, nuclear chromatin aggregation, excessive eosinophilia, squeezed cytoplasm, and cell disintegration,

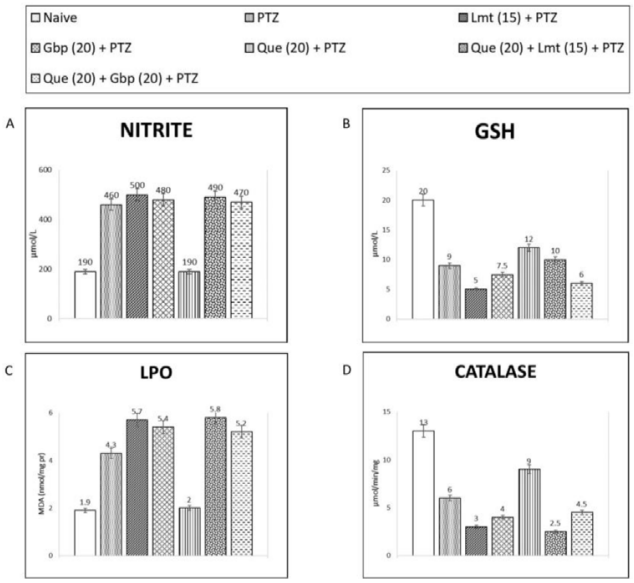


Figure 8: Neuroprotective efficacy of quercetin, lamotrigine, and gabapentin and its combination on Nitrite (A), GSH (B), LPO (C), Catalase (D) concentration in the kindled mice brain. All values of mean expressed ± SEM and ANOVA expressed by Tukey test, $p < 0.05$ significantly as compared to naïve.

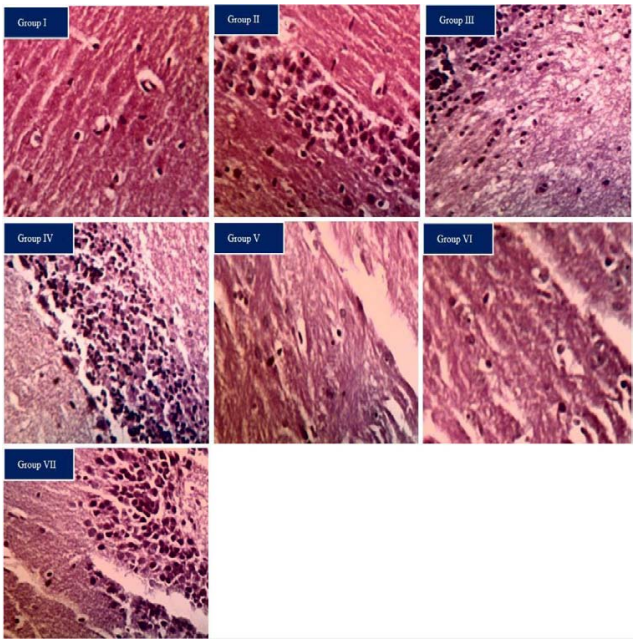


Figure 9: Mice brain photomicrographs (×40) displaying neuronal cell morphology in the different treated groups. Group I: Naive group, displaying normal cells; Group II: Vehicle treated with (0.6% w/v CMC Sod.) + PTZ (30 mg/kg, i.p.), displaying diffuse cell injury; Group III: Lmt (15 mg/kg, i.p.) + PTZ (30mg/kg, i.p.), showing near-normal cell morphology; Group IV: Gbp (20mg/kg, i.p.) + PTZ (30mg/kg), showing less changes than II group; Group V: Que (20mg/kg, i.p.) + PTZ (30mg/kg, i.p.), showing recovery of cell damage; Group VI: Que (20mg/kg, i.p.) + Lmt (15 mg/kg, i.p.) + PTZ (30mg/kg, i.p.) showing normal cell morphology, indicating considerable protection; Group VI: Que (20mg/kg, i.p.) + Gbp (20mg/kg, i.p.) + PTZ (30mg/kg, i.p.), showing less protection than group VI.

to assess the proportional percentage of cell damage, as shown in Figure 9.

DISCUSSION

In the present study, this protocol is used to investigate the pharmacological animal model of epilepsy that is widely accessible. Chemical kindling caused by PTZ has a long tradition and is a popular model for understanding epilepsy behavior, histopathological changes are shown in Figure 9, and cellular abnormalities.[27] Previous research indicates that repetitive sub convulsive injection of PTZ (a $GABA^A$ binding site Cl channel blocker) mimics the onset and broadminded development of the convulsive behavioral activity, resulting in generalized clonic-tonic seizures (i.e., chemical kindling epilepsy).[28] Several scientists have aimed to develop knockout or knock-in animals as epilepsy models and have also flourished in establishing animals that seem to have spontaneous seizures. In addition, pharmacological epileptic seizure induction is still a well-thought-out and effective model for investigating epileptic seizures. Another frequent technique is used for convulsion induction other than genomic alteration requires implanting electrodes into a mice brain and bringing disruptive and destructive epileptic fits. This procedure is expensive, complicated, and demands surgical expertise to implant the electrode in the brain at the exact point. In conclusion, the study on the kindling model found the adjuvant therapeutic potential efficacy of quercetin with the combination of lamotrigine in epileptic conditions.[29] In the PTZ-induced kindling, scores were recorded by the repeated treatment of a sub convulsive dose of PTZ (30 mg/kg, i.p.) every other day, resulting in a progressively rise of jerky seizure score, concluding in generalized convulsions in comparison to the naive animals.[30] Although the combination with the bioflavonoid quercetin minimized the intensity of seizure activity in PTZ induced kindled mice. On behavioral responses were observed, followed by the Racine scale, a known method for categorizing seizure intensity into 5 stages. PTZ treated mice experienced 100% observed from stage 1 to stage 5. Animals treated with a combination of drugs (Que + Lmt) developed from stage 3 to stage 4 in 10-20% of cases. In addition, chronic PTZ-treated animals exhibited a significant impairment in memory and learning that was proved by a significant fall in latency of step-down [AL and RL] when compared to the naive group. Alternatively, the good neuroprotective action of quercetin was exhibited in elevated models in kindled mice. In addition, ITL and RTL values which both reflect task acquisition and retrieval were found to be significantly different in naive animals. Que (20 mg/kg) + Lmt (15 mg/kg, i.p.) administration for 12 days resulted beneficial improvement in transfer latencies (ITL and RTL). In a histopathological study, microphotographs (×40) of brain tissue of mice showed cell morphology in the different treated groups. In conclusion, sixth group showed normal cell morphology and indicated considerable protection against PTZ kindled mice. PTZ caused considerable oxidative stress, as evidenced by elevated nitrite and lipid peroxidation, as well as reduced levels of glutathione and catalase enzymes. Furthermore, In PTZ kindled mice, oxidative stress was reduced by quercetin treatment for 12 days by attenuating the alteration in antioxidative enzyme levels. Treatment with quercetin has been stated to provide a good intracellular antioxidant potent agent against oxidative stress variables, and treatment with the combination of quercetin and lamotrigine exhibited significant modulation in their neuroprotective efficacy.[31]

CONCLUSION

In summary, the findings suggested that quercetin may be neuroprotective in epileptic situations. The investigation further illustrates the participation of the nitric oxide biomechanism in the promising therapeutic outcomes of lamotrigine with quercetin as an adjuvant, which can provide hope that quercetin might be involved as a complement drug with lamotrigine in medicinal therapies of generalized epileptic patients and interrelated issues. Furthermore, considerable research is required to explore the exact mechanism and clinical condition of epileptic seizure situations before any clinical manifestations can be established.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AEDs: Anti-epileptic drugs; **CMC:** Carboxymethylcellulose; **CNS:** Central nervous system; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **GBP:** Gabapentin; **IAEC:** Institute Animal Ethics Committee; **LMT:** Lamotrigine; **OECD:** Organization for Economic Co-operation and Development; **PTZ:** Pentylenetetrazole; **QUE:** Quercetin.

Author Contribution

Data collection, Data analysis, and interpretation, drafting the article- Aman Shrivastava*, Research Scholar, Department of Pharmacology, Institute of Pharmaceutical Research, G.L.A University, Mathura (U.P), INDIA.

Ethical Approval

Institute Animal Ethics Committee (IAEC) protocol approval no. PBRI/IAEC/22-10-21/008

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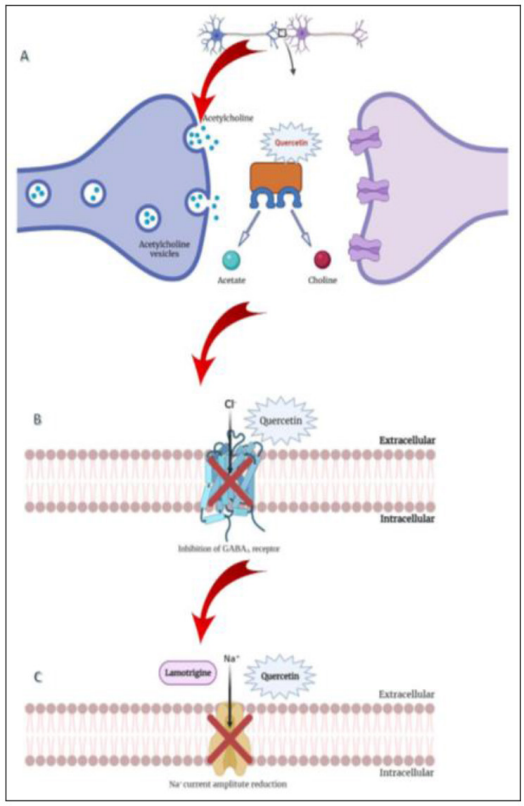
PICTORIAL ABSTRACT



SUMMARY

- Quercetin is a flavonoid found in a range of fruits and vegetables. Quercetin is a natural antioxidant and scavenger featuring anti-inflammatory, astringent, anti-ischemic, neuroprotective, and anti-epileptic properties.
- It has been stated that quercetin provides a good intracellular antioxidant potent agent against oxidative stress variables, and treatment with the combination of quercetin and lamotrigine exhibited significant modulation in their neuroprotective efficacy.
- In PTZ kindled mice, we observed that oxidative stress was reduced by treatment with quercetin for 12 days by attenuating the alteration in antioxidative enzyme levels.

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