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EVALUATION OF ANTIEPILEPTIC POTENTIAL OF RUTIN AGAINST PENTYLENETETRAZOLE (PTZ) INDUCED SEIZURES AND NEUROBEHAVIOURAL COMORBIDITIES IN SWISS ALBINO MICE

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ABSTRACT

Recurrent, unprovoked seizures are hallmark of epilepsy, a chronic neurological condition that is frequently linked to cognitive and neurobehavioral comorbidities including depression and memory loss. Aim of current study was to assess antiepileptic effects of Rutin, a naturally occurring flavonoid, against seizures in Swiss albino mice caused by pentylenetetrazole (PTZ). For predetermined treatment period of 14 days prior to PTZ challenge, Rutin was given orally at two dosage levels, 50 mg/kg (low dose) and 100 mg/kg (high dose). Modified Racine's scale was used to gauge the intensity of seizures, and Novel Object Recognition Test (NORT), Forced Swim Test (FST) were used to gauge cognitive and emotional behaviors, respectively. Findings showed that Rutin at 100 mg/kg considerably reduced the intensity of seizures and had an antiepileptic effect similar to that of standard valproic acid (VPA). Additionally, in PTZ-induced mice, high-dose Rutin significantly enhanced memory function and decreased depressive-like behavior, whereas the low-dose group just slightly benefited. According to these results, Rutin at a high dose of 100 mg/kg may be a viable option for treating epilepsy and its associated comorbidities.

Keywords: Pentylenetetrazole (PTZ), Seizures, Rutin, Valproic acid (VPA), Modified racine's scale

INTRODUCTION

One of the most prevalent neurological conditions is epilepsy, which is characterized by paroxysmal cerebral dysrhythmia, brief episodes of loss or disruption of consciousness, with or without convulsions, and sensory or behavioral abnormalities **[1]**. Numerous evaluations of the impact of epilepsy on cognition indicate that children and adults with epilepsy may have at least a slight impairment in their intellectual function. However, a thorough examination of seizure kinds and frequencies is necessary to link seizures to mental impairment. One example of how individuals with localized epilepsies typically exhibit particular abnormalities in cognitive skills regulated by corresponding regions is memory impairment associated with temporal lobe epilepsy **[2, 3]**. Ability of PTZ-induced seizure model to produce generalized seizures, assess post-seizure functioning, and screen different therapies for cognitive and affective impairments seen in human epilepsy has been well verified.

Numerous studies have demonstrated the positive benefits of flavonoids and other phytochemicals produced from fruits and vegetables on memory and learning. Rodents neurocognitive function has been demonstrated to be improved by foods high in flavonoids. In order to forecast the effects of flavonoids on human cognitive function, human declarative memory has also been studied using rodent models. Furthermore, earlier studies have shown that flavonoids can help prevent dementia in people **[4-6]**.

A significant nutritious component of meals and plant-based drinks is Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside), a flavonoid of the flavonol family. Antioxidant, anticarcinogenic, cytoprotective, antiplatelet, antithrombic, vasoprotective, cardioprotective, and neuroprotective actions are some of the notable pharmacological characteristics of Rutin. It also reduces ischemia reperfusion damage in the brain and functions as an anticonvulsant in mice given PTZ **[7]**. Recent research has shown that long-term Rutin supplementation dramatically reverses tri-methyltin (TMT)-induced spatial memory deficits and damage to pyramidal neurons in the hippocampus CA3b area, and that Rutin may improve memory retrieval in healthy mice **[8]**. These advantages may be linked to pro-inflammatory cytokines, decreased microglial activation, and Rutin's antioxidative properties. In order to stop PTZ-induced seizures in mice, we focused on Rutin's possible effects on memory recall.

MATERIALS AND METHODS

Materials

Animals

For 14-day trial, there were total of 30 animals used during experiment, which were procured from Jeeva Life Sciences.

They have been kept in polypropylene cages at conditions of 45–64% humidity, 24±2^{0}C and 12/12-hour day/night cycle. Animals were given commercially balanced meal and unlimited water during testing period. Experiments on animals were conducted in compliance with standard set of rules established by CCSEA, and protocol was approved by IAEC, RBVRRWCOP (Protocol No. RBVRR1328/07/2025).

Drugs and Chemicals

Rutin, and PTZ were purchased from LOBA CHEMIE PVT.LTD., and VPA was gifted by DRILS institute for experimental purpose. PTZ, and VPA were dissolved in saline, while Rutin was administered as suspension by dissolving sodium CMC.

Methods

Experimental design

Table 1: Experimental design

SI.no.	Groups	Treatment
1.	Vehicle control	Normal saline
2.	PTZ control	Normal saline + PTZ [45 mg/kg, i.p]
3.	Standard group	VPA[100 mg/kg, p.o] + PTZ[45 mg/kg, i.p]
4.	Rutin low dose	Rutin[50 mg/kg, p.o] + PTZ[45 mg/kg, i.p]
5.	Rutin High dose	Rutin[100 mg/kg, p.o] + PTZ[45 mg/kg, i.p]

Experimental procedure

Induction of seizures in Swiss albino mice

For 14 days, each experimental group, apart from vehicle control group, received corresponding pretreatments. During subsequent duration, vehicle group was given normal saline. To ensure consistency, treatments were given at same time every day. All animals, with exception of vehicle control group, received PTZ at 45 mg/kg dose, intraperitoneally [i.p.] on final day, past thirty minutes of last pretreatment dose, in order to induce seizures. Each animal was then monitored for 30 minutes to evaluate seizure behaviour.

Seizure behaviour assessment in Swiss albino mice

Each animal was placed in a separate transparent observation cage as soon as PTZ was administered, and for 30 minutes, they were monitored closely to evaluate seizure behaviour. To reduce outside stimuli that can affect seizure manifestation, observation area was kept silent. Modified Racine's Scale for seizures induced by PTZ in mice was used to classify seizure behaviour.

Figure 1: PTZ induced seizure behaviour assessment in Swiss albino mice

Table 2: Modified Racine's Scale for seizure behaviour assessment

Stage	Seizure behaviour	Seizure score
Satge-0	No changes in behaviour	0
Stage-1	When an animal experiences absence seizures, it stops moving and typically sits in the corner of the observation chamber, with mild ear and facial twitch	1
Stage-2	Small twitches throughout the body, with the possibility of an animal becoming immobile in myoclonic seizures	2
Stage-3	Forelimb expansions that are bilateral, and or, unilateral	3
Stage-4	Forelimb clonic seizures on both sides	4
Stage-5	Tonic-clonic seizures that are generalized, accompanied by a loss of posture and erratic running and leaping	5
Stage-6	Extension of hind limb, with or, without death	6

Further, to evaluate the seizure induced behavioural impairments, like memory and cognition deficits, and depression, the following behavioural tests were conducted after the PTZ administration.

Novel Object Recognition Test [NORT]

NORT is a popular behavioural test for evaluating memory and cognition in mice. It has three phases. During habituation stage, mice were placed in an empty arena without any objects and were permitted to wander freely for fifteen minutes each day for two consecutive days. Training session involved placing two identical objects, A and A', into arena where mice freely explored for ten minutes. Test session, involved exploration by mice for five minutes, and one of just-familiar objects (A) were swapped with a new different one (B). To reduce scent cues, arena and objects were sanitized using 75% ethanol. Time spent investigating new and familiar items is denoted by TN and TF, respectively **[9]**. Discrimination index (DI) is computed as per formula,

$$DI = T_N - T_F / T_N + T_F$$

Forced Swim Test [FST]

Behavioural despair is a behavioural paradigm used to assess for anti-depressant activity, evaluated by FST. A clear cylindrical container of twenty-five centimetres in length, and by ten centimetres in width, filled with water at a level where mice could keep their head above the surface level maintained at optimum temperature was used in which mice were forced to swim. Final four minutes of the six-minute test session were used to monitor the amount of immobility observed. Each mouse was deemed immobile when it floated in the water without moving, only making small movements to maintain its head above the surface **[10]**.

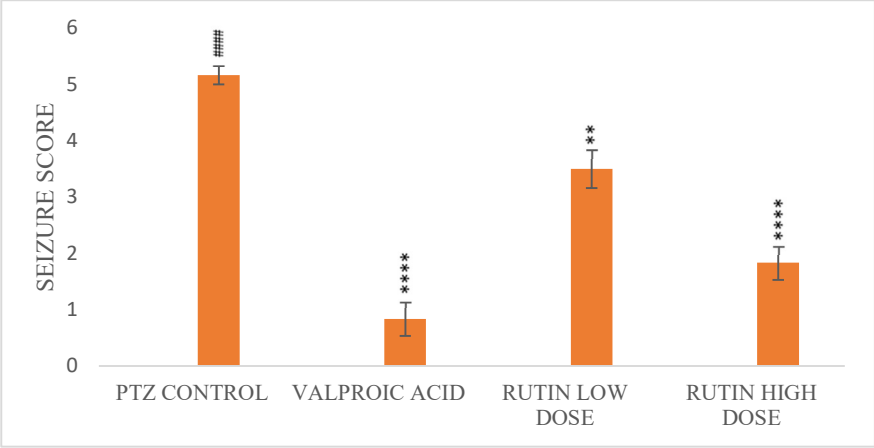
Statistical Analysis

For all values, mean ± SEM (n=6) was employed. For repeated multiple pairwise comparisons between the various groups, data was analysed by graph pad prism software using one-way analysis of variance (ANOVA) and Tukey test. P-values less than 0.005 regarded as statistically significant.

RESULTS

Evaluation of efficacy of Rutin against PTZ induced seizures in Swiss albino mice

Seizure behaviour evaluation of Swiss albino mice in different groups following PTZ administration is represented in below **Figure 2**. PTZ control group had severe seizure phases that were categorized using Racine's scale. PTZ control group has mostly displayed hind limb extension (stage 6), and tonic clonic seizures of generalized type (stage 5).



Values represented by mean ± SEM (n=6), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to PTZ control, #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 compared to vehicle control

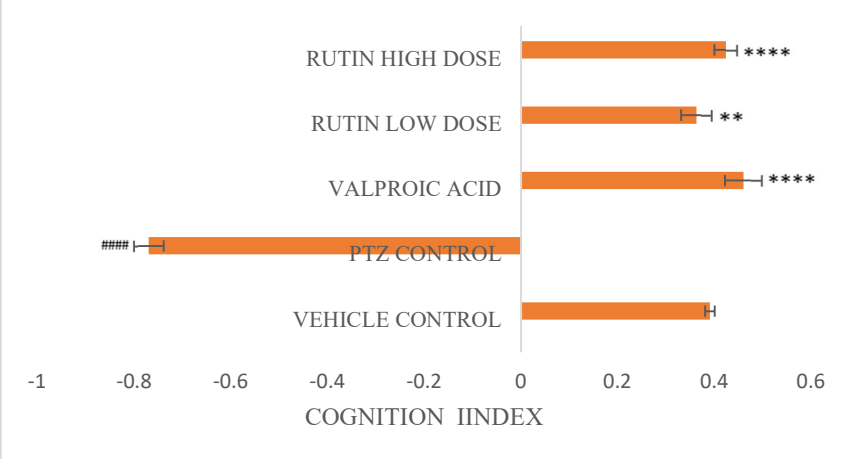
Figure 2: Effect of Rutin against PTZ induced seizure behaviour in Swiss albino mice

When compared by PTZ control group, treatment group that received Rutin low dose (50mg/kg) demonstrated unilateral or bilateral forelimb extensions (stage 3), forelimb clonic seizures both sides (stage 4), myoclonic seizures (stage 2), delay in

seizure start latency. Only absence seizures with modest facial and ear twitching (stage-2) were observed in group receiving Rutin high dose (100 mg/kg) in comparison to PTZ control group. There was also substantial delay in onset of seizures and 50% reduction in seizure severity. Only minor absence seizures and mostly no behavioural alterations (stage-0) were seen in VPA treated group.

Evaluation of efficacy of Rutin on discrimination index in NORT

Average discrimination index of all groups as determined by NORT following seizure induction was represented in **Figure 3**. PTZ control group discrimination index reduced significantly by 297% when compared to vehicle control group. Discrimination index increased by 142.8%, and 154.99% in low dose and high dose Rutin treated groups respectively, when compared to PTZ control group. When compared to PTZ control, discrimination index in VPA treatment group increased by 159.8%.



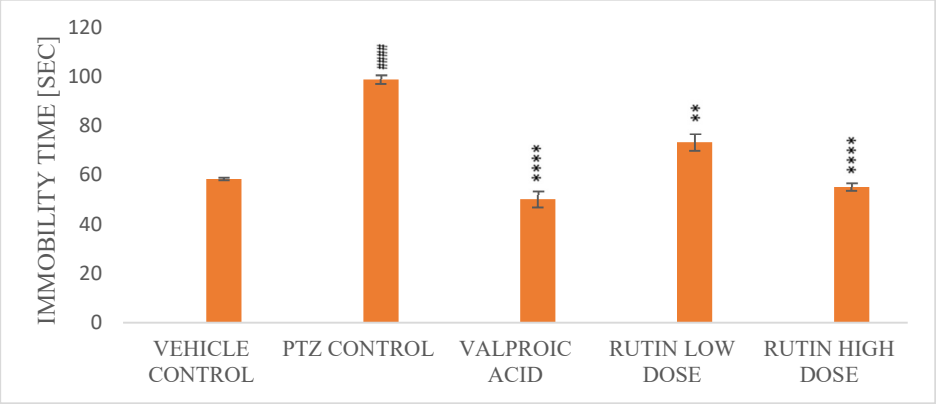
Values represented by mean ± SEM (n=6), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to PTZ control, #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 compared to vehicle control

Figure 3: Effect of Rutin on discrimination index in NORT

Evaluation of efficacy of Rutin on immobility time in FST

Average immobility time for each group as determined during FST following seizure induction was represented in **Figure 4**. When compared to vehicle control group, PTZ control group immobility time increased by 53.7%. When compared to PTZ control, immobility time following treatment was reduced by 37.04%, 44.08%, in Rutin low dose (50mg/kg) and high (100 mg/kg) treated groups respectively. Immobility period was 47.65% shorter in VPA treated

group than in PTZ control group. Group that received Rutin at 100 mg/kg, showed a significant decrease in immobility time, almost similar as that of VPA treated group.



Values represented by mean ± SEM (n=6), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to PTZ control, $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001, $^{\#\#\#\#}$p<0.0001 compared to vehicle control

Figure 4: Effect of Rutin on immobility time during FST

DISCUSSION

According to the evaluation of seizure behaviour in Swiss albino mice, given in **Figure 1**, group receiving Rutin at 100 mg/kg significantly reduced seizures almost as much as standard valproic acid when compared to low dose treatment group. This is attributed to mechanism of Rutin, a potent antioxidant that reduces inflammation and oxidative stress in neurons, facilitates neuronal stability, is responsible to restore normal locomotor activity post seizures **[11]**. The most common comorbidity among epileptic patients, whether in children and adults, is seizure induced memory and cognitive impairment. Cognitive function, especially recognition memory, is evaluated using NORT. Stress brought on by hippocampus loss of synaptic plasticity and neurotransmitter dysregulation, which impacts learning and memory, is frequently connected to seizure induced memory and cognitive impairment. According to previous studies, Rutin can raise BDNF levels **[12, 13]**. This is supported by fact that groups treated with Rutin, especially at 100 mg/kg showed an increase in discrimination index, as shown in **Figure 3**, that was almost equivalent to that of groups treated with conventional VPA. Rutin promote synaptic plasticity, stabilize the neuronal HPA axis, and improve memory and cognition by raising BDNF levels and restoring GABA in cerebral hippocampus.

Majority of people with epilepsy reported experiencing depression as a result of seizure induced behavioural impairment. Behavioural despair, a characteristic of depressive-like behaviour, is evaluated using FST in order to examine the antidepressant effect of Rutin as given in **Figure 4**. Mechanistically, Rutin's antidepressant and neuroprotective properties are evidently related to its capacity to alter important neurotransmitters implicated in depression, specifically via regulating hypothalamic-pituitary-adrenal (HPA) axis and improving GABAergic

transmission. This mechanism of Rutin contribute to increase stress resistance and lessen depressed behaviour.

CONCLUSION

The present study demonstrates that Rutin exerts significant antiepileptic and neuroprotective effects against PTZ induced seizures in Swiss albino mice. Among tested doses, high-dose Rutin (100 mg/kg) showed pronounced reduction in seizure severity, closely matching efficacy of valproic acid. Behavioral assessments using NORT and FST further revealed that high-dose Rutin effectively alleviated seizure associated cognitive and depressive impairments, indicating its potential role in managing not only seizure activity but also its neurobehavioural comorbidities. These beneficial effects might be attributed to Rutin's multifaceted antioxidant, anti-inflammatory, and neuromodulatory properties, suggesting its mechanism involves mitigation of oxidative stress and neuroinflammation, highlighting its role in epilepsy management. Future research should focus on elucidating the precise molecular pathways underlying Rutin's antiepileptic action and long-term clinical evaluations are warranted to confirm its efficacy, safety, and translational applicability.

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ETHICS AND CONSENT

Experiments on animals were conducted in accordance with the standard guidelines of CCSEA, and the protocol was approved by IAEC, RBVRRWCOP (Protocol No. RBVRR1328/07/2025).

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