**PROTECTIVE EFFECT OF RUTIN ON COGNITIVE IMPAIRMENT CAUSED BY LEVETIRACETAM**

**ABSTRACT**

Objective: To determine the protective effect Rutin on combined treatment with Levetiracetam in comparison with Levetiracetam and piracetam on seizure control, cognitive and motor functions in mice.

Material and Methods: By increasing current electroshock seizure (ICES), the impact of levetiracetam and piracetam on convulsions was assessed. While motor capabilities were screened using rolling roller apparatus and counting the number of arm entries on a plus maze, cognitive functions in mice were assessed by observing spontaneous behavioral alternation on a plus maze. The Ellman et al. method was used to quantify the activity of brain acetylcholinesterase (AChE).

Results: Without affecting Levetiracetam's ability to prevent ICES in both acute and long-term trials, the study demonstrated that when rutin was given in conjunction with Levetiracetam, it considerably reversed the decrease in spontaneous alternation caused by Levetiracetam. Additionally, it counteracted the rise in AChE activity brought on by levetiracetam.

Conclusion: Without losing its antiepileptic properties, rutin minimized the cognitive impairment caused on by levetiracetam.

Key words: Acetylcholinesterase, cognitive functions, diphenylhydantoin, Rutin.

**INTRODUCTION**

Levetiracetam is a newer drug used as anticonvulsant having various benefits over the accessible drugs. It is extensively used in monotherapy treatment for epilepsy in the case of partial seizures, or as an adjunctive therapy for partial, myoclonic and tonic-clonic seizures [1]. The drug is also known for its numerous plausible benefits for various psychiatric and neurologic conditions such as Tourette syndrome, autism, bipolar disorder and anxiety disorder,[2] as well as Alzheimer's disease[3]. Although,with the drug the most serious adverse effects encountered are behavioral deficit produced by it [3]. Having total seizure control without interfering with cognitive functions is commendable for effective convulsion treatment. To obtain a nominal or nonexistent memory deficit with AED therapy, it may be advantageous to combine adjuvant nootropic compound use with antiepileptic medication therapy. It is necessary to opt for an improved approach that not only treats the cognitive turmoil but shall also endow with seizure protection. One of the established agents piracetam (PIM), PIM (2-oxo-1-pyrolidone acetamide) a know nootropic also known for its antimyoclonic activity(4-6) and specific antiamnesic activity (in many experimental exemplar)(7-9) also it has been proven to be protective effect against pentylenetetrazol (PTZ) (kindling-induced neuronal loss and learning deficit)(10,12). Though, it lacks anticonvulsant activity in the MES model (7). However, notable neuroprotection is witnessed experimentally (11, 12). Thus by various experimental procedures it revealed that PIM is an effective nootropic agent that counteracts impairment caused by ADEs. Among the all occurring flavonoids phtochemicals have been the main area of interest for the research scholars. Rutin is a naturally occurring flavonoids having various pharmacological activity like Studies have shown that rutin scavenge free radicals, (13, 14) suppresses cellular immunity, (15) anti-inflammatory effect (16) as well as anti-carcinogenic (16, 17) and antimicrobial [18] potential. Apart from these it has also proven to be nootropic in n-number of studies (19-25).Thus it would be remunerative to appraise the use of rutin with PHT on seizure and cognitive functions. Thus the aim of present study was study the effect of combination of rutin and Levetiracetam on brain cholinergic system in comparison with the effect seen by the combination of piracetam and Levetiracetam.

**MATERIAL AND METHOD**

**Animals**

Swiss albino mice weighing 24–34 g were kept in cages in groups of 10 at 23–300 C with a natural light-dark cycle, and they were given free access to tap water and a standard pellet diet.

**Drugs and dosing schedules**

Levetiracetam, marketed as "Levroxa" (injection), was given intravenously two hours prior to each observation at doses of 8, 12, and 22 mg/kg body weight in a volume of 10 ml/kg body weight (26). One hour prior to each trial, piracetam, the nootropic standard (often referred to as "Nootropic" syrup), was taken orally at doses of 125, 250, and 500 mg/kg body weight in a volume of 10 ml/kg body weight. For rutin, the same procedure was followed. The control groups were given 10 milliliters of distilled water per kilogram of body weight. Chronic studies were carried out for 21 days. All observations were made on day twenty-one following the administration of piracetam for one hour and levetiracetam for two hours. In long-term studies, drugs were administered given between 10 and 12 a.m. (26).

**Increasing Current Electroshock Seizures (ICES)**

The approach described by Kitano et al. (27) and modified by Marwah et al. (28) was used to assess the anticonvulsant effect of the drugs ICES. A single train of pulses with a linearly increasing strength of 2 mA / 2 sec was first given to each mouse via ear electrodes at a current of 2 mA using an electroconvulsometer. It took 0.2 seconds to complete. The current at which tonic Hind Limb Extension (HLE) occurred was identified as the seizure threshold current (STC). When a current of 30 mA revealed no tonic HLE, electroshock was halted.

**Spontaneous Alteration Behavior (SAB) on a plus maze**

Spontaneous alteration behavior (SAB) is the propensity of animals, even single-celled organisms, to alternate between their non-reinforced (Dember & Richman, 1989) selections of T- or Y-maze arms on subsequent trials following an initial trial or turn. In rodents, alternation is a natural tendency. Medication-induced behavioral impairment is altered by nootropics, and vice versa. Better cognition is thus indicated by a change in vacillation, and vice versa. Cognitive functioning was assessed using a plus maze, following the guidelines recommended by Itoh et al. (29) and Ragozzino et al. (30) for SAB. The maze was 50 cm tall, made of wood, painted gray, and included four symmetrical arms (23.5 x 8 cm) with walls of 10 cm and a central platform (8 x 8 cm). Mice were put on the center platform and given free reign. The amount of entries in each arm and their order were recorded during the five minutes of observation. The entry into four different arms on overlapping quintuple sets was defined as alternation.Within the total set of arm choices, a quintuple set consists of five consecutive arm alternatives; for instance, a quintuple set with the choices A, B, C, and B for arms was not considered an alternation (26).

Following the above procedure percentage alternation was calculated as follows:

Percentage alternation = Actual no. of alternation/Possible no. of alternation X 100

Possible alternation = no. of arms entries –4

The number of arm entries was also recorded separately to determine the motor influence on the observed effects. (26)

**Rolling roller apparatus**

The approach outlined by Dunham et al. (31) was used to evaluate the neurological impairment caused by the drugs. The testing session lasted one minute, during which the animals were placed on the roller, which was set to rotate at a speed of five revolutions per minute. The animal can counterpoise itself throughout the duration under normal conditions. Consequently, the animal's incapacity to sustain balance on the roller for a duration of one minute served as evidence of its neurological deficit.

**Estimation of brain acetylcholinesterase (AChE) activity**

Ache activity throughout the entire brain was measured using the Ellman et al. technique (32). This approach is based on the fact that thiocholine reacts with dithiobisnitrobenzoate ions to generate a yellow hue. Using a spectrophotometer, the rate at which thiocholine was formed from acetylcholine iodide in the presence of tissue cholinesterase was determined. After treating the sample with 5, 5'-dithionitrobenzoic acid (DTNB), the optical density (OD) of the yellow chemical produced during the reaction was measured at 412 nm every minute for three minutes (26). Folin's approach was applied for protein estimation. The Ache activity was calculated using the following formula (26).

R = δ O.D X Volume of Assay (3 ml)/ E X mg of protein

Where R= rate of enzyme activity in ‘n’ mole of acetylthiocholine iodide hydrolyzed / minute / mg protein

δ O.D = Change in absorbance / minutes

E = Extinction coefficient = 13600 /M/cm Statistical analysis

The expression of data was done as mean ± SEM. P values <0.05 were considered significant.

**RESULTS**

**INCREASING CURRENT ELECTROSHOCK SEIZURES (ICES)**

By totally eradicating HLE, levetiracetam administered intravenously at a dose of 22 mg/kg showed 100% protection against ICES in acute tests. At far lower dosages (8 mg/kg, p.o.), no protection was seen, while 50% protection was demonstrated at a lower dose of 12 mg/kg, i.v. (Table 1). PIM and rutin did not have a significant effect on ICES at memory-improving doses calculated from Table 1.

**SPONTANEOUS ALTERATION BEHAVIOR**

**Acute studies**

The annihilation of the percentage alternation on the plus maze showed a cognitive benefit at an intravenous dose of 12–22 mg/kg. At lower concentrations (8 mg/kg, i.van, and 125 mg/kg, p.o., respectively), neither Levetiracetam nor PIM exhibited much of a reaction (Table 1), but at higher doses, both PIM and Levetiracetam demonstrated encouraging results. Furthermore, there was no confounding effect on memory without altering any effect on ICES, as indicated by the findings of the combined effects of Levetiracetam (12 mg/kg, p.o.) and PIM (250 mg/kg), which were fairly equal to those of the control group (Table 1).

**Chronic studies**

The percentage alternation is decreased by levetiracetam (12 mg/kg, i.v. X 21 days), which considerably decreases function, according to the chronic studies. When PIM (125 mg/kg, p.o. X 21 days) and Levetiracetam (125 mg/kg, p.o. X 21 days) and Rutin (125 mg/kg, i.v. X 21 days) were combined, on the other hand, the deterioration was reversed. (Table 2).

**Rolling roller apparatus**:

In both acute and chronic tests, as well as when used in combination, no motor deficit was observed at any dose of Levetiracetam plus PIM or Rutin.

**Whole brain AChE activity**

Levetiracetam (8 mg/kg, p.o.) did not significantly alter the total brain AChE activity compared to the control. However, when the dose was increased to 12 mg/kg p.o., a significant increase in AChE activity in comparison to control was seen. At lesser doses (125 mg/kg, p.o.), neither PIM nor Rutin appreciably altered brain AChE activity. However, at a dose of 250 mg/kg, p.o. significantly decreased AChE levels. When Levetiracetam (12 mg/kg, i.v.) was paired with PIM (250 mg/kg, p.o.) and Levetiracetam (12 mg/kg, i.v.) with Rutin (250 mg/kg, p.o.), the AChE levels were similar to the control (Table 3).

**DISCUSSION AND CONCLUSION**

Levetiracetam (12–22 mg/kg, i.v.) was found to have a negative impact on cognitive performance in both acute and long-term investigations. However, it was discovered that the doses were ED50 and ED100 against ICES. These findings corroborated those found in the cognitive function investigations of PHT and Sod.valproate (33–39). PIM's nootropic property (7-9) and antimyoclonic activity (4-6) are well-established facts. In numerous studies, it has also shown remarkable outcomes compared to spontaneous alternation behavior. Increased PIM dosages have shown measurable effects as a nootropic on the MES model and a potent antiepileptic effect on ICES (26) as well (7). The flavonoid rutin is also well-known for its nootropic properties (19–25). Therefore, the goal of this study was to show how co-administration of PIM and rutin with a clinically validated AED can benefit antiepileptic treatment. Levetiracetam caused cognitive impairment without interfering with its effectiveness against ICES when PIM and Rutin were administered together, according to the current study's findings. Additionally, this study indicated that Rutin was pillared for the findings when accessed with PIM. The percentage alternation increased when a lower dose of levetiracetam was given, but no significant effects were observed. (10, 26). The rolling roller apparatus was used to assess the impact of motor influences for PIM and Rutin both separately and in combination with Levetiracetam, where no discernible impact on motor functions was seen. Researchers are currently debating the mechanism underlying PIM's nootropic effect, as well as that of rutin. PIM has been explained by a number of mechanisms, including increased oxidative glycolysis, effects on the cholinergic system (24), and effects on Ca2+ channels (23). In contrast, Rutin has no supporting evidence other than oxidative glycolysis (25), and some have also suggested that it is involved in the cholinergic system. PIM and Rutin induced a decrease in "brain AChE activity," but levetiracetam considerably raised it in the current study, validating the effects of these drugs on the cholinergic system. Learning and memory are also impacted by Levetiracetam’s disruption of the cholinergic system, which lowers brain ACh levels (2, 16, and 17). In this sense, our results were consistent with a consistent report. Interestingly, at lesser dosages, levetiracetam shows no impairment or aChE levels. PIM is a member of the class of substances known as pyrrolidones, most of which affect the cholinergic system (8,11,24). Rutin is a member of the flavonoid family, and many of its members have different effects on the cholinergic system. PIM and rutin reduced the brain's AChE activity in our investigation. An intriguing detail to note in this context is that co-administration of Levetiracetam and PIM Rutin reportedly increased the Levetiracetam-induced abrupt increase in total brain AChE level, demonstrating the opposing effects of Levetiracetam and PIM/Rutin on the cholinergic system. To sum up the study, When taken as adjuvant therapy with levetiracetam, PIM and rutin reversed the adverse effects on the cholinergic system. However, it is essential to explore the full potential of rutin in order to ameliorate the cognitive deficits induced by levetiracetam and secure the optimum position in the current AED therapy.

COMPETING INTERESTS:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL APPROVAL

The study was approved by the CPCSEA Ethics Committee (project no. 64, Nov. 2001), and all experimental procedures were conducted strictly in accordance with ethical standards.

**References**

1. Bhatt A, Chamola K, Rawat SS. Evaluating The Impact of Tourism in Giving Uttarakhand’s Villagers an Employment. NOLEGEIN-Journal of Global Marketing. 2024 Apr 16;7(1):9-14.
2. Sen A, Toniolo S, Tai XY, Akinola M, Symmonds M, Mura S, Galloway J, Hallam A, Chan JY, Koychev I, Butler C. Safety, tolerability and efficacy outcomes of the Investigation of Levetiracetam in Alzheimer's Disease (ILiAD) trial. medRxiv. 2024:2024-05.
3. Andrei G. Malykh and M. Reza Sadaie, ‘Piracetam and Piracetam-like Drugs: From Basic Science to Novel Clinical Applications to CNS Disorders’, *Drugs*, 2010, 287–312.
4. Krishna KV, Rasheed AR, Kumar S, Charhate KB, Tathe PR, Malik A. Assesment of polyherbal formulation for nootropic potential
5. Tan JK, Nazar FH, Makpol S, Teoh SL. Zebrafish: A pharmacological model for learning and memory research. Molecules. 2022 Oct 30;27(21):7374.
6. Fatahinezhad N, Lorigooini Z, Arabi M, Rabiei Z, Sheykhshabani SK, Rafieian-Kopaei M. Effects of Hyssopus Officinalis Hydroalcoholic Extract on Pentylenetetrazol-Induced Convulsive Seizures in Rat. Neurochemical Research. 2022 Dec;47(12):3792-804.
7. Hu T, Zhao Y, Hu Z, Zuo C. Clinical Effects of Piracetam Plus Mannitol on Cerebral Edema, Neurological Function, and Vascular Endothelin-1 Levels Following Cerebral Hemorrhage. Neurochemical Journal. 2023 Mar;17(1):156-62.
8. Dubey S, Ganeshpurkar A, Bansal D, Dubey N. Experimental studies on bioactive potential of rutin. Chronicles of Young scientists. 2013 Jul 1;4(2):153-.
9. Heilman KM, Nadeau SE. Emotional and neuropsychiatric disorders associated with Alzheimer's disease. Neurotherapeutics. 2022 Jan 1;19(1):99-116.
10. Dubey S, Ganeshpurkar A, Shrivastava A, Bansal D, Dubey N. Rutin exerts antiulcer effect by inhibiting the gastric proton pump. Indian journal of pharmacology. 2013 Jul 1;45(4):415-7.
11. Williams RG, Li KH, Phillips PE. The influence of stress on decision-making: Effects of CRF and dopamine antagonism in the nucleus Accumbens. Frontiers in Psychiatry. 2022 Jan 25;12:814218.
12. Sreelatha I, Choi GY, Lee IS, Inturu O, Lee HS, Park YN, Lee CW, Maeng S, Park JH. Neuroprotective Properties of Rutin Hydrate against Scopolamine-Induced Learning and Memory Deficits in Rats.
13. Dubey S, Ganeshpurkar A, Bansal D, Dubey N. Experimental studies on bioactive potential of rutin. Chronicles of Young scientists. 2013 Jul 1;4(2):153-.
14. Contreras-García IJ, Cárdenas-Rodríguez N, Romo-Mancillas A, Bandala C, Zamudio SR, Gómez-Manzo S, Hernández-Ochoa B, Mendoza-Torreblanca JG, Pichardo-Macías LA. Levetiracetam mechanisms of action: from molecules to systems. Pharmaceuticals. 2022 Apr 13;15(4):475.
15. Upadhyay SD, Ahmad Y, Kohli S, Sharma RK. Evaluation of acetylcholinesterase and butyrylcholinesterase inhibitory activity of Huperzine-A; in silico and in vitro studies. Indian Journal of Biochemistry and Biophysics (IJBB). 2019 Aug 23;56(3):224-9.
16. Fang T, Valdes E, Frontera JA. Levetiracetam for seizure prophylaxis in neurocritical care: a systematic review and meta-analysis. Neurocritical care. 2022 Feb 1:1-1.
17. Upadhyay SD, Ahmad Y, Kohli S, Sharma RK. Synergistic effect of folic acid and galantamine against experimentally induced oxidative stress in IMR 32 cells. Indian Journal of Experimental Biology (IJEB). 2022 Apr 1;60(04):286-92.
18. Dubey S, Ahmad Y, Kohli S. Protective effect of huperzine A on phenytoin induced cognition impairment: Behavioral and biochemical study. Indian Journal of Biochemistry and Biophysics (IJBB). 2022;59(2):205-13.
19. Ganeshpurkar A, Saluja AK. Protective effect of rutin on humoral and cell mediated immunity in rat model. Chemico-Biological Interactions. 2017 Aug 1;273:154-9.
20. Aranda-Abreu GE, Rojas-Durán F, Hernández-Aguilar ME, Herrera-Covarrubias D, Chí-Castañeda LD, Toledo-Cárdenas MR, Suárez-Medellín JM. Alzheimer’s Disease: Cellular and Pharmacological Aspects. Geriatrics. 2024 Jun 22;9(4):86.
21. Upadhyay SD, Lodha S, Ahmad Y, Kohli S, Sharma RK. A Review on Medhya Rasayanas: A Brain Bracer. Pharmacognosy Communications. 2021 Oct 1;11(4).
22. Halder S, Anand U, Nandy S, Oleksak P, Qusti S, Alshammari EM, Batiha GE, Koshy EP, Dey A. Herbal drugs and natural bioactive products as potential therapeutics: A review on pro-cognitives and brain boosters perspectives. Saudi Pharmaceutical Journal. 2021 Aug 1;29(8):879-907.
23. Dubey S, Ganeshpurkar A, Bansal D, Dubey N. Protective effect of rutin on cognitive impairment caused by phenytoin. Indian Journal of Pharmacology. 2015 Nov 1;47(6):627-31.
24. Lin CY, Chang MC, Jhou HJ. Effect of Levetiracetam on Cognition: A Systematic Review and Meta-analysis of Double-Blind Randomized Placebo-Controlled Trials. CNS drugs. 2024 Jan;38(1):1-4.
25. Gongora M, Nicoliche E, Magalhães J, Vicente R, Teixeira S, Bastos VH, Bittencourt J, Cagy M, Basile LF, Budde H, Velasques B. Event-related potential (P300): the effects of levetiracetam in cognitive performance. Neurological Sciences. 2021 Jun;42:2309-16.

.

Table 1-Effect of acute levetracetam (leve), acute piracetam (PIM) and its combination on ICES and SAB in mice

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | Treatment | | Dose (mg/kg) | | | ICES | | | | SAB | |
| Seizure threshold current(mA) | | % protection | | % alteration | No. of arm enteries |
| I | Distilled water | | 10 mg//kg | | | 15.1 ±0.31 | | 0 | | 71.4 ± 3.04 | 13 ± 1.41 |
| II | Leve | | 8 | | | 20.4 ± 1.29 | | 0 | | 65.1 ± 2.6 | 14 ± 1.21 |
| III | Leve | | 12 | | | 28.2 ± 2.06 | | 50 | | 52.7± 2.7 | 15 ± 1.10 |
| IV | Leve | | 22 | | | 39 ± 0.0 | | 100 | | 44.2±3.9 | 17.1 ± 1.03 |
| F 34.104 H 12.07  df 3 df 3  p < 0.01 p < 0.01 | | | | | | | | | | | |
| I(control) | Distilled water | | 10 ml/kg | | | 14.2 + 0.33 | | 0 | | 78.0+ 6.04 | 15.2 + 1.75 |
| V | PIM | | 125 | | | 14.8 + 0.85 | | 0 | | 78.3 ± 6.19 | 19.0 ± 2.860 |
| VI | PIM | | 250 | | | 14.9 + 0.42 | | 0 | | 83.7 ± 6.27 | 15.8 ± 2.420 |
| VII | PIM | | 500 | | | 15.6 + 1.74 | | 0 | | 85.9 ± 6.91 | 19.1 ± 1.900 |
| H 8.64  df 3  P < 0.05 | | | | | | | | | | | |
| VIII | | Rutin | | 125 | 14.0 ± 0.16 | | 0 | | 73.2±3.91 | | 18.1±2.40 |
| IX | Rutin | | 250 | | | 14.1 ± 0.45 | | 0 | | 76.1±3.92 | 19.9±2.76 |
| X | Rutin | | 500 | | | 14.7 ± 0.89 | | 0 | | 79.5±4.07 | 20.8±2.85 |
| H 7.49  Df 3  P <0.05 | | | | | | | | | | | |
| III | Leve | | 12 | | | 19.4 ±2.16 | | 50 | | 52.7±2.8 | 14±1.10 |
| VI | PIM | | 250 | | | 16.3 + 0.42 | | 0 | | 85.1 ± 4.72 | 15.8 ± 2.40 |
| XI | leve + PIM | | 12+ 250 | | | 30.0± 1.06 | | 50 | | 70.4 ± 6.46 | 20.6 ± 1.9 |
| XII | leve + Rutin | | 12 +250 | | | 29.9± 1.02 | | 50 | | 68.4± 4.61 | 21.6 ± 1.84 |
| F 38.70 H 7.36  Df 3 df 3  P < 0.01 p < 0.01 | | | | | | | | | | | |

Values are mean + SEM, Values within parentheses are number of animals, ICES- Increasing current electroshock seizure, SAB-Spontaneous alternation behaviour. Seizure threshold current values were analysed using one-way ANOVA followed by Dunnett’s test and alternation values by Kruskal–Wallis H test followed by a multiple range test, \*P<0.05, † P<0.01 Vs control, ‡ P< 0.05 Vs Group III

table 2-Effect of chronic Levetracetam (LEVE) and piracetam (PIM) on SAB

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | Dose | % alternation | No. of arms entries |
| Control | 10 ml/kg | 68.30 ± 4.14 (9) | 10.25 ±0.75 |
| LEVE | 8 | 62.1 ± 2.4 | 13 ± 1.01 |
| LEVE | 12 | 52.7±2.8 | 14 ± 1.10 |
| PIM | 125 | 75.2 ± 2.19 | 19.0 ± 2.860 |
| Rutin | 125 | 78.3 ± 2.06 | 20.7 ±2.09 |
| LEVE+ PIM | 12+ 125 | 79.1 + 3.09 | 16.9 ± 1.16 |
| LEVE + Rutin | 12+125 | 81.9 ± 3.06 | 18.9 ± 1.97 |

Values are mean+SEM, Values within parentheses are number of animals, AChE-whole brain AChE activity. \*P<0.05 Vs control (multiple range test)

table 3- Effect of acute Levitracetam (LEVE), acute piracetam (PIM) and its combination on AChE activity in mice

|  |  |  |
| --- | --- | --- |
| Treatment | Dose  (mg/kg, p.o.) | AChE |
| Control  (distilled water)  LEVE  LEVE  PIM  PIM  LEVE+PIM  LEVE + Rutin  LEVE + Gal | 10 ml/kg  8  12  125  250  12+250  12+250  12+250 | 106.1 + 6.19  108.0 + 7.16  186.6 + 11.06\*  110.4 + 9.04  95.3 + 7.41\*  120.1 + 5.03  121.8+ 4.86  119.6+4.70 |

H 16.17

Df 5

P <0.01

|  |
| --- |
| Values are mean+SEM, Values within parentheses are number of animals, AChE-whole brain AChE activity. \*P<0.05 Vs control (multiple range test) |