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: The effect of Levetiracetam in combination with piracetam on convulsions was evaluated by increasing current electroshock seizure (ICES). The Cognitive functions in mice were evaluated by spontaneous alternation in behavior on a plus maze while motor functions were screened using rolling roller apparatus and by counting the number of arms entries on a plus maze. Brain acetylcholinesterase (AChE) activity was measured using the Ellman et al method.

Results: The study showed that rutin when co-administered with Levetiracetam, significantly reversed Levetiracetam -induced reduction in spontaneous alternation without altering the efficacy of Levetiracetam against ICES in both acute and chronic studies. Further, it also reversed Levetiracetam induced increase in AChE activity.

Conclusion: Rutin alleviated the Levetiracetam –induced cognitive impairment without compromising its antiepileptic efficacy.

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]. For efficient treatment for convulsions it is praiseworthy to have complete seizure control without interrupting any cognitive effects. It can be beneficial to go for the antiepileptic drugs therapy with adjuvant use of nootropic agents for achievement of nominal/no memory deficit with AED 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 METHODS**

**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. 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.

**Drugs and dosing schedules**

Two hours before each observation, levetiracetam, which is branded as "Levroxa" (injection), was administered intravenously in doses of 8, 12, and 22 mg/kg body weight in a volume of 10 ml/kg body weight (26). Piracetam, the nootropic standard (also known as "Nootropic" syrup), was administered orally in doses of 125, 250, and 500 mg/kg body weight in a volume of 10 ml/kg body weight one hour before each trial. The same protocol was used for rutin. Distilled water in a volume of 10 milliliters per kilogram of body weight was administered to control groups. For 21 days, chronic studies were conducted. After administering levetiracetam for two hours and piracetam for one hour, all observations were made on day twenty-one. In long-term research, medications were given between 10 and 12 a.m. (26).

**Increasing Current Electroshock Seizures (ICES)**

To evaluate the anticonvulsant impact of the medications ICES, the methodology presented by Kitano et al. (27) and modified by Marwah et al. (28) was employed. Using an electroconvulsometer, a single train of pulses with a linearly rising strength of 2 mA / 2 sec was first administered to each mouse via ear electrodes at a current of 2 mA. This was done for 0.2 seconds. The seizure threshold current (STC) was defined as the current at which tonic Hind Limb Extension (HLE) appeared. Electroshock was stopped when a current of 30 mA showed no tonic HLE.

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

The tendency of animals, even single-celled organisms, to switch between their non-reinforced (Dember & Richman, 1989) choices of T- or Y-maze arms on successive trials after an initial trial or turn is known as spontaneous alteration behavior (SAB). Alternation is a natural inclination in rodents. Nootropics change the behaviour impairment brought on by medications, and vice versa. Consequently, a shift in vacillation indicates better cognition, and vice versa. A plus maze was utilized to evaluate cognitive functioning in accordance with the protocol suggested by Itoh et al. (29) and Ragozzino et al. (30) for SAB. Made of wood, painted grey, and standing 50 cm tall, the maze had four symmetrical arms (23.5 x 8 cm) with 10 centimetre walls and a central platform (8 x 8 cm). Mice were placed on the middle platform and allowed to roam freely. Over the course of five minutes of observation, the number of entries in each arm and their sequence were noted. Alternation was described as the entry into four distinct arms on overlapping quintuple sets.A quintuple set is made up of five consecutive arm options within the overall set of arm choices; for example, a quintuple set with the choices A, B, C, and B for arms was not regarded as 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 neurological impairment brought on by the medications was assessed using the methodology described by Dunham et al. (31). The animals were put on the roller, which was programmed to rotate at a rate of five revolutions per minute, and the testing period lasted one minute. Under typical circumstances, the animal can counterpoise itself for the duration. Therefore, the animal's neurological deficiency was demonstrated by its inability to maintain equilibrium on the roller for a one-minute test period.

 **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)**

In acute tests, levetiracetam at a dose of 22 mg/kg, intravenously, demonstrated 100% protection against ICES by completely eliminating HLE. 50% protection was shown at a lower dose of 12 mg/kg, i.v., however no protection was observed at considerably lower doses (8 mg/kg, p.o.) (Table 1). ICES did not respond well to PIM and rutin at memory-improving dosages estimated from Table 1.

**SPONTANEOUS ALTERATION BEHAVIOR**

**Acute studies**

At a dose of 12–22 mg/kg, intravenously, the annihilation of the percentage alternation on the plus maze demonstrated a cognitive benefit. Both Levetiracetam and PIM showed little reaction at lower dosages (8 mg/kg, i.van, and 125 mg/kg, p.o., respectively) (Table 1), while both PIM and Levetiracetam showed promising outcomes at higher doses (Table 1). Additionally, the results of the combined impact of Levetiracetam (12 mg/kg, p.o.) and PIM (250 mg/kg) were somewhat comparable to those of the control group, meaning that there was no confounding influence on memory without changing any effect on ICES (Table 1).

**Chronic studies**

According to the chronic trials, Levetiracetam (12 mg/kg, i.v. X 21 days) significantly impairs function, which lowers the percentage alternation. Conversely, the deterioration was reversed by the combination of 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) with Levetiracetam (125 mg/kg, i.v. X 21 days) (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**

There was no discernible change in the entire brain AChE activity with Levetiracetam (8 mg/kg, p.o.) and the control. However, a notable increase in AChE activity relative to control was noted when the dose was raised to 12 mg/kg p.o. Neither PIM nor Rutin significantly changed brain AChE activity at lower doses (125 mg/kg, p.o.). However, p.o. dramatically reduced AChE levels at a dose of 250 mg/kg. AChE levels were comparable to control when Levetiracetam (12 mg/kg, i.v.) was combined with PIM (250 mg/kg, p.o.) and Levetiracetam (12 mg/kg, i.v.) with Rutin (250 mg/kg, p.o.) (Table 3).

**DISCUSSION**

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. It has also demonstrated impressive results over spontaneous alternation behavior in a variety of investigations. Higher dosages of PIM have demonstrated a strong antiepileptic impact against ICES (26) as well as noticeable effects as a nootropic on the MES model (7). The flavonoid rutin is also well-known for its nootropic properties (19–25). Therefore, the purpose of this study was to demonstrate the antiepileptic treatment benefits of co-administration of PIM and rutin with a clinically proven AED. 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. There were no noteworthy results when a lesser dose of Levetiracetam was administered; however, there was an increase in the percentage alternation (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 (40), and effects on Ca2+ channels (41). 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. Levetiracetam significantly increased "brain AChE activity" in the current investigation, whereas PIM and Rutin caused a decrease in "brain AChE activity," confirming the effects of these medications 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). Therefore, our findings supported a consistent report in this regard. Notably, Levetiracetam does not exhibit any impairment or aChE levels at lower doses. PIM belongs to the group of compounds called pyrrolidones, the majority of which have an effect on 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, PIM and Rutin reversed the negative effects on the cholinergic system when used as adjuvant therapy with Levetiracetam. However, in order to gain the best position in the current AED therapy and improve the cognitive deficits caused by levetiracetam, it is vital to investigate the full potential of rutin.

COMPETING INTERESTS DISCLAIMER:

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.

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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 | 16.1 ±0.41 | 0 | 71.1±3.14 | 15±1.51 |
| II | Leve | 8 | 21.4 ± 1.39 | 0 | 63.1±2.4 | 14±1.01 |
| III | Leve | 12 | 29.4 ±2.16 | 50 | 53.7±2.8 | 15±1.10 |
| IV | Leve | 22 | 40 ±0.0 | 100 | 44.2±3.9 | 18±1.02 |
|  F 35.1104 H 11.07 df 3 df 3 p < 0.01 p < 0.01 |
| I(control) | Distilled water | 10 ml/kg | 15.2 + 0.33  | 0 | 79.0+ 6.04 | 16.2 + 1.75 |
| V | PIM | 125 | 15.8 + 0.85 | 0 | 79.3 ± 6.19 | 20.0 ± 2.860 |
| VI | PIM | 250 | 15.9 + 0.42 | 0 | 84.7 ± 6.27 | 16.8 ± 2.420 |
| VII | PIM | 500 | 16.6 + 1.74 | 0 | 86.9 ± 6.91 | 20.1 ± 1.900 |
|  H 8.64  df 3  P < 0.05   |
| VIII | Rutin  | 125 | 15.0±0.16 | 0 | 74.2±3.91 | 19.1±2.40 |
|  IX |  Rutin |  250 | 15.1±0.45 | 0 | 77.1±3.92 |  20.9±2.76 |
|  X |  Rutin |  500 | 15.7±0.89 | 0 | 80.5±4.07 |  21.8±2.85 |
|  H 7.49 Df 3 P <0.05  |
| III | Leve | 12 | 29.4 ±2.16 | 50 | 53.7±2.8 | 15±1.10 |
| VI | PIM | 250 | 15.3 + 0.42 | 0 | 86.1 ± 4.72 | 16.8 ± 2.40 |
| XI | leve + PIM  | 12+ 250 | 31.0± 1.06 | 50 | 71.4 ± 6.46 | 21.6 ± 1.9  |
| XII | leve + Rutin  | 12 +250 | 30.9± 1.02 | 50 | 69.4± 4.61 | 22.6 ± 1.84 |
|  F 39.70 H 8.46 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 | 69.32 ± 4.14 (9) | 11.25 ±0.75 |
| LEVE |  8 | 63.1±2.4 | 14±1.01 |
| LEVE |  12 | 53.7±2.8 | 15±1.10 |
| PIM |  125 | 76.2 ± 2.19 | 20.0 ± 2.860 |
| Rutin  |  125 | 79.3 ± 2.06 | 21.7 ±2.09 |
| LEVE+ PIM |  12+ 125 | 80.1 + 3.09 | 17.9 ± 1.16 |
| LEVE + Rutin |  12+125 | 82.9 ± 3.06 | 19.9 ± 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) LEVELEVE PIM PIM LEVE+PIM LEVE + Rutin LEVE + Gal | 10 ml/kg 8 12125 250 12+250 12+25012+250 | 107.1 + 6.19 109.0 + 7.16 187.6 + 11.06\* 111.4 + 9.04 96.3 + 7.41\* 121.1 + 5.03 122.8+ 4.86120.6+4.70 |

 H 17.17

 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) |