Protective effect of rutin on cognitive impairment caused by phenytoin

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Introduction

Phenytoin (PHT) is one of the extensively used low-cost antiepileptic drugs (AED) which is recognized to cause cognitive impairment. Many studies have interrogated the side effects of PHT. PHT has major adverse effects on memory, learning, and psychomotor functions, whereby, PHT, in both acute and chronic administration, has been found to coherently impair both memory and learning.\(^1\) To achieve an effective therapy for convulsions, it is expected to get complete seizure control without interrupting any cognitive effects. For the attainment of minimal/no memory deficit, adjuvant therapy of an antiepileptic drugs (AEDs) with a known nootropic agents seems to be beneficial. A better approach shall be opted to use an agent that not only treats the cognitive disturbances but also provides seizure protection. In this context, one of the established agents is piracetam (PIM) which is also known for its antimyoclonus activity\(^3\) and specific antiamnesic activity in many experimental exemplars.\(^4\) In addition to this, it has also been proven as protective against pentylenetetrazol (PTZ) kindling-induced neuronal loss and learning deficit\(^5\) and is also useful in patients with stroke.\(^6\) However, in the maximal electroshock model (MES) model, it lacks anticonvulsant activity.\(^4\) Flavonoids are the diverse class of plant-derived molecules that have been domain of interest for research scholars. Rutin, a naturally occurring

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ABSTRACT

Objective: To study the effect of the co-administration of phenytoin (PHT) and rutin in comparison with PHT and piracetam (PIM) on seizure control, cognitive, and motor functions in mice.

Materials and Methods: Increasing current electroshock seizure (ICES) test was used to evaluate the effect of the co-administration of PHT and PIM on convulsions. Cognitive functions in mice were assessed by a 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 acetyl-cholinesterase (AChE) activity was also estimated.

Statistical Analysis: The expression of data was done as mean ± standard error of the mean. The normally distributed data were subjected to one-way ANOVA followed by Dunnett’s test. \(P < 0.05\) was considered significant.

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

Conclusion: Rutin alleviated the PHT-induced cognitive impairment without compromising its antiepileptic efficacy.

KEY WORDS: Acetyl-cholinesterase, cognitive functions, diphenylhydantoin, rutin
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flavonoid, is helpful in maintaining homeostasis in organisms by scavenging free radicals, suppressing cellular immunity, by anti-inflammatory effect as well as anticarcinogenic, antiulcer and antimicrobial potential. Apart from these, it has also proven to be an effective nootropic and neuroprotective agent. Thus, the aim of the present study was to determine the effect of co-administration of rutin and PHT on brain cholinergic system in comparison with the effect seen by the co-administration of PIM and PHT.

Materials and Methods

Animals

Swiss albino mice weighing 30–45 g were used. The experimental protocol was approved by the Institutional Animal Ethics Committee constituted as per the rules of the committee for the Purpose of Control and Supervision of Experiments on Animals, India. They had free access to a standard pellet diet and tap water.

Drugs and Dosing Schedules

PHT marketed as “Dilantin” in the form of suspension was administered intravenously (i.v.) in doses of 8, 12, and 22 mg/kg 2 h prior to each observation. PIM the nootropic standard (“Nootropil” syrup) was given orally in a volume of 125, 250, and 500 mg/kg body weight 1 h prior to each experiment. The Same procedure was followed for rutin. Control groups were administered distilled water (10 ml/kg). All observations were made on the day 21 after 2 h of PHT and 1 h of PIM administration. During these studies, drugs were administered between 10 am and 12 am.

Grouping of Animals

Group I served as normal control. Group II, III, and IV received PHT in dose of 8, 12, and 22 mg/kg; Group V, VI, and VII received PIM in dose of 125, 250, and 500 mg/kg; Group VIII, IX, and X received rutin in the dose of 125, 250, and 500 mg/kg, respectively. Group XI received PHT (12 mg/kg) and PIM (250 mg/kg) while Group XII received PHT (12 mg/kg) and rutin (250 mg/kg).

Increasing Current Electroshock Seizures

For evaluation of the anticonvulsant effect of the drugs, increasing current electroshock seizure (ICES). A current of 2 mA electroshock to each mouse via ear electrodes as a single train of pulses (for 0.2 s) was given with linearly increasing intensity of 2 mA/2 s using an electroconvulsiometer. The current at which the appearance of tonic hind limb extension (HLE) occurred was recorded as the seizure threshold current. When no tonic HLE was observed by a current of 30 mA, electroshock was terminated.

Spontaneous Alternation Behavior on a Plus Maze

Alternation is the naturally occurring tendency of rodents. The drugs causing amnesia for secure cause impairment of this behavior and vice versa with nootropics. Therefore, an accrual in alternation involves enhanced cognitive ability. Assessment of cognitive functions was made by using a plus maze apparatus. Spontaneous alternation behavior (SAB) was also noted. The maze of height 50 cm was made of wood, painted gray, and consisted a central platform (8 cm × 8 cm) with four symmetrical arms (23.5 cm × 8 cm) having 10 cm walls. Mice were allowed to move freely after being placed in the central platform. The sequence and number of entries in each arm were recorded during an observation period of 5 min. Entry into four different arms on overlapping quintuple sets was defined as alternation. Choice of five consecutive arms within the total set of arm choices make up a quintuple set, e.g., a quintuple set consisting of arms choices A, B, A, C, B was not considered an alternation.

Following the above procedure percentage alternation was calculated as follows:

Percentage alternation = (Actual number of alternation/possible number of alternation) × 100

Rolling Roller Apparatus

Rolling roller studies was used to assess the neurological deficit caused by the drugs. The roller was set to make five revolutions/minute using speed selector. The testing time was taken as 1 min, and the animals were placed on the roller. A normal animal can counterbalance itself throughout the period. Hence, the inability of the animal to maintain equilibrium on the roller for a 1-min test period indicated the neurological deficit.

Estimation of Brain Acetyl-cholinesterase Activity

The method proposed by Hestrin was used for the estimation of whole brain acetyl-cholinesterase (AChE) activity. The method was based on the formation of yellow color resulting due to the reaction of thiocholine with dithiobisnitrobenzoic acid. Spectrophotometric measurements were made for the measurement of rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase. The treatment of sample was first done with 5,5’-dithiobisnitrobenzoic acid followed by determination of optical density (at 412 nm) of the yellow color compound formed during the reaction every minute for a period of 3 min was measured. Folin’s method was used for protein estimation. AChE activity was calculated using the following formula:

\[ R = \frac{\Delta O.D \times Volume \ of \ assay \ (3 \ ml)}{E \times mg \ of \ protein} \]

Where \( R \) = Rate of enzyme activity in “n” mole of acetylthiocholine iodide hydrolyzed/minute/mg protein

\( \delta \ O.D = \) Change in absorbance/minutes

\( E = \) Extinction coefficient = 13600/M/cm

Statistical Analysis

The expression of data was done as mean ± standard error of the mean. The normally distributed data were subjected to one-way ANOVA followed by Dunnett’s test. \( P < 0.05 \) was considered significant.

Results

Increasing Current Electroshock Seizures

In acute studies, for PHT (22 mg/kg, i.v.) 100% protection was observed against ICES by complete abolition of HLE. PHT (12 mg/kg, i.v.) showed 50% protection while at lower doses of PHT (8 mg/kg, p.o.), no protection was seen [Table 1]. PIM and rutin at memory improving doses [Table 1] was found ineffective on ICES [Table 1].
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Table 1:
Effect of acute PHT, acute PIM and its combination on ICES and SAB in mice

| Group | Treatment  | Dose (mg/kg, p.o.) | Seizure threshold current (mA) | Percentage of protection | ICES | Percentage of alteration | SAB | Number of arm entries |
|-------|------------|-------------------|------------------------------|--------------------------|-----|-------------------------|-----|------------------------|
| I     | Distilled water | 10 mg/kg | 16.1±0.41 | 0 | 62.1±3.92 | 71.1±3.14 | 15±1.51 |
| II    | PHT        | 8      | 21.4±1.39 | 0 | 0       | 68.6±3.60 | 17±1.37 |
| III   | PHT        | 12     | 29.4±2.16 | 50 | 86.9±6.91 | 57.5±3.92 | 19±1.23 |
| IV    | PHT        | 22     | 40±0.0 | 100 | 0       | 49.3±4.01 | 21±1.17 |
| V     | PIM        | 125    | 15.8±0.85 | 0 | 0       | 79.3±6.19 | 20.0±2.86 |
| VI    | PIM        | 250    | 15.9±0.42 | 0 | 0       | 84.7±6.27 | 16.8±2.42 |
| VII   | PIM        | 500    | 16.6±1.74 | 0 | 0       | 86.9±6.91 | 20±1.900 |
| VIII  | Rutin      | 125    | 15.0±0.16 | 0 | 0       | 74.2±3.91 | 19±1.24 |
| IX    | Rutin      | 250    | 15.1±0.45 | 0 | 0       | 77.1±3.92 | 20.9±2.76 |
| X     | Rutin      | 500    | 15.7±0.89 | 0 | 0       | 80.5±4.07 | 21.8±2.85 |
| III   | PHT        | 12     | 29.4±2.16 | 50 | 0       | 62.1±3.92 | 19±1.23 |
| VI    | PIM        | 250    | 15.3±0.42 | 0 | 0       | 86.1±4.72 | 16.8±2.40 |
| XI    | PHT + PIM  | 12+250 | 31.0±1.06 | 50 | 0       | 71.4±6.46 | 21.6±1.9 |
| XII   | PHT + rutin| 12+250 | 30.9±1.02 | 50 | 0       | 69.4±4.61 | 22±1.84 |

Seizure threshold current values were analyzed using one-way ANOVA followed by Dunnett’s test and alteration values by Kruskal-Wallis H-test followed by a multiple range test. ICES=Increasing current electroshock seizure, SAB=Spontaneous alternation behavior, PHT=Phenytoin, PIM=Piracetam, SAB=Spontaneous alternation behavior, ICES=Increasing current electroshock seizure.

Spontaneous Alteration Behavior

Acute studies

PHT at a dose of 12–22 mg/kg, i.v. significantly abolished the percentage alternation on plus maze, which explains the improvement in cognitive function. At lower doses 8 mg/kg, i.v. no significant effect was observed [Table 1]. PIM at doses 250 mg/kg, p.o. and above demonstrated a significant reduction in percentage alternation [Table 1]. Co-administration of PHT (12 mg/kg, i.v.) and PIM (250 mg/kg) given as such no deterioration was seen i.e., the results were somewhat similar to the control group. Similarly, in case of combined treatment of PHT (12 mg/kg, p.o.) and Rutin (250 mg/kg) positive results were seen, i.e. no debilitating effects were seen on memory without altering any effect on ICES [Table 1].

Chronic Studies

The chronic studies revealed that PHT (12 mg/kg, i.v. × 21 days) caused a significant impairment leading to reduction in the percentage alternation. Treatment with combination of PIM (125 mg/kg, p.o. × 21 days) and Rutin (125 mg/kg, i.v. × 21 days) with PHT (125 mg/kg, i.v. × 21 days) reverted the impairment [Table 2].

Rolling Roller Apparatus

No dose of PHT and PIM or rutin in both acute and chronic studies, as well as in combination, showed any motor deficit.

Whole Brain Acetyl-cholinesterase Activity

The whole brain AChE activity with PHT (8 mg/kg, p.o.) did not differ from the control. However, PHT (12 mg/kg, p.o.) demonstrated a significant rise in AChE activity as compared to control. At lower doses (125 mg/kg, p.o.), neither PIM nor rutin altered brain AChE activity significantly. However, at a dose of 250 mg/kg, p.o. lowered AChE levels significantly. A combination of PHT (12 mg/kg, i.v.) with PIM (250 mg/kg, p.o.) and PHT (12 mg/kg, i.v.) with rutin (250 mg/kg, p.o.) exhibited AChE levels similar to control [Table 3].

Discussion

The results showed that PHT (12–22 mg/kg, i.v.) had an adverse effect on the cognitive function both in acute and chronic studies. Indeed, these doses were found to be ED50 and ED100 against ICES. These results support the results obtained in the previous studies of PHT on cognitive functions. It is also well known that PIM is a well-known nootropic with a good antimyoclonic activity and, in various studies, it has shown impressive results over SAB. It has been also seen that, at higher doses PIM, however effective antiepileptic effect has against ICES, also it has shown prominent results as nootropic on the MES model. Rutin, naturally occurring flavonoids, has also been established for its nootropic activity. Thus in the present study, it was aimed to determine the usefulness of co-administration of PIM and rutin with clinically used AED in antiepileptic therapy. Our study results revealed that PIM and rutin when co-administered with PHT impressively reverted the cognitive impairment produced by PHT without hindering the efficacy of PHT against ICES. In this study, Rutin supported the results as obtained for established for the combination with PIM. At the lower dose of 125 mg/kg, the enhancement in the percentage alternation was seen but did not show significant data. However, this dose was effective in reversal of PHT-induced impairment of SAB. This result supported the data in which PIM was shown to prevent PTZ kindling-induced neuronal loss and learning deficits. The rolling roller apparatus was used to
to study the effect of motor influences with PIM and rutin individually alone and also in combination with PHT where no significant effect on motor functions was seen. The mechanism by which PIM shows its nootropic effect is still under research scholars’ consideration and same is the case with rutin. For PIM, a number of mechanisms have been advocated such as an enhancement of oxidative glycolysis,[21] an effect on the Ca\(^{2+}\) channels,[22] and an effect on the cholinergic system.[21] but in case of rutin, no such supportive data is available except for oxidative glycolysis[23] some data also says for its involvement in cholinergic system. The latter is important in the process of learning and memory. In our study, PHT (12 mg/kg, p.o.) prominently increased the "brain AChE activity" while on the other hand PIM and rutin (250 mg/kg, p.o.) lowered the "brain AChE activity" affirming the countervailing action of these drugs on the cholinergic system. The impairment caused due to PHT on learning and memory is due to interference in the cholinergic system also it lowers the brain AChE levels.[23] Thus, our results revealed a persistent report in this context. It is worth noting that PHT at 8 mg/kg, p.o. did not show an impairment and did not affect AChE levels. PIM belongs to pyrrolidones group most of which are known to influence cholinergic functions.[24,25] Along with rutin, flavonoids and many members of this group have varying action on cholinergic system. In this study, AChE activity was reduced by PIM as well as by rutin in brain. It is important to know an interesting fact in this context that co-administration of PHT with PIM rutin apparently increased the PHT-induced sharp rise in total brain AChE level showing the countervailing action of PIM/rutin and PHT on the cholinergic system. The present study confirms our previous findings, where rutin demonstrated the protective effect on impairment of cognitive functions due to antiepileptic drugs on zebrafish model.[26] Thus, it can be said that PIM and rutin when in adjuvant therapy with PHT, it reverted the adverse effect produced on the cholinergic system. It is necessary to explore the complete potential of rutin in improving the PHT-induced cognitive impairment and getting the right stand in the current AED therapy.

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**Conflicts of Interest**

There are no conflicts of interest.

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**Table 2:** Effect of chronic PHT and PIM on SAB

| Treatment          | Dose (ml/kg) | Percentage of alternation | Number of arms entries |
|--------------------|-------------|---------------------------|------------------------|
| Control            | 10          | 69.32±4.14                | 11.25±0.75             |
| PHT                | 8           | 73.21±1.35                | 21.0±1.15              |
| PHT                | 12          | 50.22±4.52*               | 19.4±1.27              |
| PIM                | 125         | 76.05±2.59                | 21.0±2.86              |
| Rutin              | 125         | 79.6±2.46                 | 22.7±2.19              |
| PHT + PIM          | 125         | 81.2±4.09                 | 19.3±1.36              |
| PHT + rutin        | 125         | 83.6±4.16                 | 18.9±1.79              |

*P<0.05 versus control (multiple range test). Values are means±SEM. SEM=Standard error of mean, AChE=Acetyl-cholinesterase, PHT=Phenytoin, PIM=Piracetam, SAB=Spontaneous alternation behavior.

**Table 3:** Effect of acute PHT, acute PIM, and its combination on AChE activity in mice

| Treatment          | Dose (mg/kg, p.o.) | AChE            |
|--------------------|-------------------|-----------------|
| Control (distilled water) | 10 ml/kg          | 107.4±6.19      |
| PHT                | 8                 | 113.0±8.76      |
| PHT                | 12                | 189.3±12.26*    |
| PIM                | 125               | 111.0±9.04      |
| PIM                | 250               | 96.5±7.41*      |
| PHT + PIM          | 12+250            | 126.1±5.23      |
| PHT + rutin        | 12+250            | 127.8±4.71      |

H=18.07, df=5, P<0.01

Values are means±SEM, values within parentheses are number of animals. AChE-whole brain AChE activity. *P<0.05 versus control (multiple range test).

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