**PROTECTIVE EFFECT OF RUTIN ON COGNITION IMPAIRMENT CAUSED BY SODIUM VALPROATE.**

**ABSTRACT**

Purpose: The goal is to compare the effects of sod valproate and rutin vs sod valproate and piracetam on seizure control, cognitive function, and motor function in mice.

Methods: A technique called increasing current electroshock seizure (ICES) was used to assess how sod valproate and piracetam together affected convulsions. While motor abilities were screened utilizing rolling roller apparatus and counting the number of arm entries on a plus maze, cognitive functions were evaluated by observing spontaneous behavioral alternation on a plus maze. The Ellman et al. method was used to evaluate the activity of brain acetylcholinesterase (AChE).

Results: Without affecting sod valproate's ability to prevent ICES in both acute and long-term trials, the study demonstrated that rutin, when taken in conjunction with sod valproate, dramatically reversed the phenytoin-induced decrease in spontaneous alternation. Additionally, it counteracted the rise in AChE activity brought on by sod valproate.

Conclusion: Without compromising its antiepileptic properties, rutin reduced the cognitive damage caused by sod valproate.

**KEY WORDS**: Acetylcholinesterase, cognitive functions, sodium valproate, rutin.

**INTRODUCTION**

One of the key anticonvulsant medications that is frequently used to treat epilepsy is sodium valproate. The medication is known to impair cognition, though. Numerous research on sodium valproate have shown that it has cognitive adverse effects, such as behavioral changes including aggression, hyperactivity, and enhanced alertness, as well as the ability to detect depressive symptoms or emotional shifts. Sodium valproate has been found to have significant negative impacts on psychomotor functions, such as memory and learning, in numerous investigations (1-5). Complete seizure control without interfering with cognitive consequences is essential for an anticonvulsive treatment to be effective. Adjuvant therapy of antiepileptic medications (AEDs) with well-known nootropic substances appears to be advantageous in every way for achieving a small or nonexistent memory loss. Piracetam (PIM) is one of the well-known agents in this situation. Numerous experimental exemplars have shown that PIM (2-oxo-1-pyrolidone acetamide), a nootropic also renowned for its antimyoclonic activity (4-6), has particular antiamnesic efficacy (7-10). In addition to these activities, it has been demonstrated to have a protective effect against learning deficit and neuronal death caused by pentylenetetrazol (PTZ) kindling (11,12). It does not, however, have anticonvulsant properties in the MES model (7). Important neuroprotective effects have been observed in experiments (13, 12). Consequently, it was discovered through a variety of experimental techniques that PIM is a potent nootropic that reverses the impairment brought on by PTZ. Flavonoids are the primary focus of research among the different phytoconstituents, attracting the attention of scientists. A naturally occurring flavonoid, rutin has been tested for a variety of pharmacological properties. Rutin has been shown in studies to have anti-inflammatory (17), anti-carcinogenic (17, 18), antimicrobial (19), suppressor of cellular immunity (16), and scavenger of free radicals (14, 15). In addition to these benefits, numerous investigations have shown that it has nootropic properties (20–26). Therefore, the purpose of this study was to compare the effects of piracetam and sodium valproate versus the combination of rutin and sodium valproate on the cholinergic system of the brain.

**MATERIAL AND METHODS**

**Animals**

Swiss albino mice, weighing between 24 and 34 grams, were kept in cages with a natural light-dark cycle in groups of ten at 23 to 30 degrees Celsius. They have free access to tap water and a typical pellet diet. The following study has been authorized by the CPCSEA Institutional Animal Ethics Committee (Project No. 64).

**Drugs and dosing schedules**

The control groups received 10 milliliters of distilled water per kilogram of body weight. Sodium valproate, which is sold under the brand name "Dilantin," was utilized as a suspension and given intravenously in doses of 8, 12, and 22 mg/kg body weight two hours before each observation (27). One hour before each trial, piracetam (also known as "Nootropil" syrup) was administered orally at doses of 125, 250, and 500 mg/kg body weight in a volume of 10 ml/kg body weight. The process for rutin was the same. For 21 days, chronic studies were conducted. After administering sodium valproate for two hours and piracetam for one hour, all observations were conducted on day 21. In long-term research, medications were given between 10 and 12 a.m. (28).

**Increasing Current Electroshock Seizures (ICES)**

ICES (29) was used to assess the medications' anticonvulsant impact, and Marwahet al. (30) made modifications. An electroconvulsometer was used to administer a single train of pulses (for 0.2 seconds) with a linearly rising intensity of 2 mA / 2 sec in order to begin electroshocking each mouse with a current of 2 mA via ear electrodes. 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 revealed no tonic HLE (34).

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

The natural inclination that rodents exhibit is alternation. This behavior is impaired by medications that create amnesia for secure causes, and vice versa with nootropics. As a result, improved cognition follows an accretion in alternation, and vice versa. A plus maze suggested by Itoh et al. (31) was used to assess cognitive processes, and SAB was recorded using the methodology suggested by Ragozzino et al.., (32). This 50-cm-tall maze was constructed of wood, painted gray, and featured a center platform (8 x 8 cm) with four symmetrical arms (23.5 x 8 cm) with walls that were 10 cm high. After being positioned in the middle platform, mice were free to roam around. 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 choices within the overall set of arm choices; for example, a quintuple set with the choices A, B, A, C, and B 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 Dunham et al. (33) approach was applied to evaluate the neurological impairment brought on by the medications. Using a speed selector, the roller was set to rotate at a rate of five revolutions per minute. The animals were put on the roller for the one-minute test period. A typical animal can maintain its equilibrium throughout time. Therefore, the animal's neurological deficiency was demonstrated by its inability to keep balance on the roller for a one-minute test period (34).

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

The whole brain AChE activity was estimated using the Ellman et al. approach (33) that was suggested. The technique was based on the fact that thiocholine reacts with di-thio-bis-nitrobenzoate ions to generate a yellow color. The rate at which thiocholine is formed from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5,5'-dithionitrobenzoic acid (DTNB), and then the optical density (OD) of the yellow chemical that was generated during the reaction was measured every minute for three minutes (26). Protein estimate was done using Folin's technique. The following formula was used to determine the AChE activity (26).

R = δ O.D.× Volume of Assay (3 ml)/ E × 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, sod valproate (22 mg/kg, i.v.) shown 100% protection against ICES by completely eliminating HLE, whereas at dosage (12 mg/kg, i.v.), sod valproate demonstrated 50% protection. No protection was shown at lower sod valproate dosages (8 mg/kg, p.o.) (Table 1). The memory-improving dosages of PIM and rutin from Table 1 were found to be ineffective on ICES (Table 1).

**Spontaneous Alteration Behavior**

**Acute studies**

The cognitive effect was explained by sodium valproate, which significantly eliminated the percentage alternation on the plus maze at an intravenous dose of 12–22 mg/kg. Insufficient effects were observed at smaller intravenous dosages, such as 8 mg/kg (Table 1). PIM did not show much promise at lower dosages (125 mg/kg, p.o.), but at doses of 250 mg/kg, p.o. and higher, there was a noticeable increase in percentage alternation (Table 1). When PIM (250 mg/kg) and sodium valproate (12 mg/kg, p.o.) were administered together, there was no decline and the outcomes were almost identical to those of the control group. Similarly, when sodium valproate (12 mg/kg, p.o.) and rutin (250 mg/kg) were administered together, good outcomes were observed, meaning that memory impairment was not observed without affecting ICES in any way (Table 1).

**Chronic studies**

The chronic studies revealed that sodium valproate (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. X 21 days) with sod valproate (125 mg/kg, p.o. X 21 days) and rutin (125 mg/kg, i.v X 21 days) with sodium valproate (125 mg/kg, i.v. × 21 days) reverted the impairment (Table 2).

**Rolling roller apparatus**:

In both acute and chronic tests, as well as when taken together, none of the sodium valproate and PIM or rutin dosages resulted in any motor impairment.

**Whole brain AChE activity**

Sodium valproate (8 mg/kg, p.o.) had no effect on the total brain's AChE activity compared to the control. In contrast to control, sodium valproate (12 mg/kg, p.o.) showed a notable increase in AChE activity. Neither PIM nor rutin significantly changed brain AChE activity at lower doses (125 mg/kg, p.o.). However, AChE levels were considerably reduced by a dosage of 250 mg/kg, p.o. AChE levels were comparable to control when sodium valproate (12 mg/kg, i.v.) was combined with PIM (250 mg/kg, p.o.) and sodium valproate (12 mg/kg, i.v.) with rutin (250 mg/kg, p.o.) (Table 3).

**DISCUSSION**

The current study's findings showed that sodium valproate (12–22 mg/kg, i.v.) had a negative impact on cognitive performance in both acute and long-term investigations. Additionally, the dosages against ICES were determined to be ED50 and ED100. These findings also support several earlier findings about the harmful effects of sodium valproate on cognitive processes. (4-5). According to some research, PIM has strong antimyoclonic effects (8–10), nootropic effects (9–11), and the ability to prevent spontaneous alternation behavior. Additionally, PIM has been shown to have a strong nootropic impact on the MES model (8) and an effective antiepileptic effect against ICES (26) at higher dosages. The nootropic properties of rutin, a naturally occurring flavonoid, have been well established (19–25). Therefore, the purpose of the current study was to ascertain the value of co-administration of PIM and rutin with sodium valproate. The findings of this trial demonstrated that PIM and rutin, when taken together with sodium valproate, dramatically reversed the cognitive impairment caused by the drug without impairing its ability to prevent ICES. The findings achieved for the established combination with PIM were corroborated by rutin in this study. There is noteworthy evidence of an increase in the percentage alternation at a lower dosage of 125 mg/kg. However, it was successful in reversing the SAB impairment brought on by sodium valproate. The findings from earlier research, which demonstrated that PIM prevented learning deficits and neuronal death caused by PTZ kindling, further corroborated the data (12,28). Animals were tested on rolling roller apparatus both separately and in combination with sodium valproate to examine the effects of PIM and rutin on motor influences. There was no discernible impact on motor functions in this investigation. Several mechanisms have been proposed for PIM, including modification of the cholinergic system, influence on Ca2+channels, and augmentation of oxidative glycolysis (34) (31). However, other from oxidative glycolysis, no comparable information is available for rutin (26). The study found that sodium valproate (12 mg/kg, p.o.) significantly increased "brain AChE activity," whereas co-administration of PIM and rutin (250 mg/kg, p.o.) decreased "brain AChE activity," confirming the medicines' antagonistic effects on the cholinergic system (37). Learning and memory were hampered by sodium valproate because it interfered with the cholinergic system and the brain's ACh levels (3,17,18). The majority of the pyrrolidones, of which PIM is a member, are known to affect cholinergic activities (9,12,25). Rutin is a flavonoid and many members of these 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 sodium valproate with PIM-rutin apparently increased the sodium valproate-induced sharp rise in total brain AChE level showing the countervailing action of PIM-Rutin and sodium valproate on the cholinergic system. To accomplish the work, it can be concluded that PIM and rutin, when given in adjuvant therapy along with sodium valproate, are capable to minimize the adverse effects caused by sodium valproate. Although, it is necessary to investigate the complete prospective of rutin in improving the sodium valproate induced cognitive impairment in the current AED therapy.

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Table 1-Effect of acute Sod valproate (Sod val), acute piracetam (PIM) and its combination on ICES and SAB in mice

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group  | Treatment  | Dose (mg/kg,p.o) |  ICES |  SAB |
| Seizure threshold current(mA) | % protection  | % alteration  | No. of arm enteries  |
| I  | Distilled water  | 10 mg//kg | 17.1 ±0.41 | 0 | 70.1±3.14 | 14±1.51 |
| II | Sod val | 8 | 23.8 ± 1.69 | 0 | 66.6±3.60 | 18±1.37 |
| III | Sod val | 12 | 29.12 ±3.82 | 50 | 58.5±3.82 | 17±1.23 |
| IV | Sod val | 22 | 37 ± 0.0 | 100 | 55.7±3.91 | 20±1.17 |
|  F 37.1007 H 11.36 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 | 17.2 + 1.75 |
| V | PIM | 125 | 14.8 + 0.85 | 0 | 78.3 ± 6.19 | 21.0 ± 2.860 |
| VI | PIM | 250 | 14.9 + 0.42 | 0 | 85.7 ± 6.27 | 17.8 ± 2.40 |
| VII | PIM | 500 | 15.6 + 1.74 | 0 | 89.9 ± 6.91 | 21.1 ± 1.900 |
|  H 8.64 Df 3 P < 0.05 |
| VIII | Rutin  | 125 | 16.0±0.16 | 0 | 75.2±3.91 | 18.1±2.40 |
|  IX |  Rutin |  250 | 16.1±0.45 | 0 | 76.1±3.92 |  21.9±2.76 |
|  X |  Rutin |  500 | 16.7±0.89 | 0 | 81.5±4.07 |  22.8±2.85 |
|  H 7.49 Df 3 P <0.05  |
| III | Sod val | 12 | 29.12 ±3.82 | 50 | 59.5±3.82 | 18±1.2.3 |
| VI | PIM | 250 | 16.9 + 0.42 | 0 | 83.7 ± 6.27 | 17.8 ± 2.40 |
| XI | Sod val + PIM  | 12+ 250 | 32.0± 1.06 | 50 | 72.1 ± 4.93 | 22.60 ± 1.9  |
| XII | Sod val + Rutin  | 12 +250 | 31.9± 1.02 | 50 | 68.0± 4.02 | 22.6 ± 1.48 |
|  F 38.70 H 8.16 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 behavior. Seizure threshold current values were analyzed 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 Sod.valproate (Sod.val) and piracetam (PIM) on SAB

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment  |  Dose  |  % alternation  | No. of arms enteries |
|  Control  | 10 ml/kg | 63.12 ± 4.14  | 08.25 ±0.69 |
| Sod.val |  8 | 68.12 ± 1.05  | 18.0 ± 1.01 |
| Sod.val |  12 | 48.12 ±3.95\* | 18.02 ± 1.17 |
| PIM |  125 | 77.05 ± 2.59 | 22.0 ± 2.86 |
| Rutin  |  125 | 78.6 ± 2.46 | 22.7 ±2.19 |
| Sod.val + PIM |  12+ 125 | 78.2 + 3.09 | 21.1 ± 1.13 |
| Sod.val + Rutin |  12+125 | 80.6 ± 3.16 | 19.8 ± 1.27 |

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 Sod valproate (sod.val), acute piracetam (PIM) and its combination on AChE activity in mice

|  |  |  |
| --- | --- | --- |
|  Treatment | Dose (mg/kg, p.o.) | AChE |
| Control(distilled water) Sod.valSod.valPIM PIM Sod.val +PIM Sod.val + Rutin  | 10 ml/kg 8 12125 250 12+250 12+250 | 104.1 + 3.61 109.0 + 7.61 174.9 + 11.16\* 113.0 + 9.04 97.5 + 7.41\* 122.7 + 5.23 128.8+ 4.17 |

 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) |