Protective effect of rutin on impairment of cognitive functions of due to antiepileptic drugs on zebrafish model

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ABSTRACT

Aim: The severity of adverse reactions due to antiepileptics is observed during initiation and early treatment in which impairment of cognitive effects are common. Since long time, herbal medicine is a natural remedy to treat neural symptoms. Phytochemicals have been proven to be potent neuro-protective agents. Rutin, a bioflavonoid is established to be nootropic in many studies. In this study, we aimed to determine the protective effect of rutin in zebrafish against the side effects produced by AEDs.

Materials and Methods: Seizures were induced in zebrafish by phenylenetetrazole. Rutin pretreatment (50 mg/kg, single injection, i.p.) was done before commencement of the study. Behavioral studies were performed as: latency to move high in the tank, locomotion effects, color effect, shoal cohesion, light/dark test on Zebrafish.

Results: Treatment with rutin reverted the locomotor behavior to normal. Treatment with AEDs caused fishes to move in all regions while, in case of treatment with rutin, the response reverted to normal. Treatment with AEDs altered swimming behavior of zebrafish, however, rutin showed a positive effect over this behavior. Treatment with AEDs resulted in restricted movement of zebrafish to the dark zone. Treatment with rutin caused increased latency of zebrafish to move in the light compartment. Similarly, time spent in the light compartment by zebrafish treated with rutin was significantly (P < 0.01) higher as compared to zebrafish treated with AEDs.

Conclusion: The results suggest a protective role of rutin on cognition impaired by AEDs.

KEY WORDS: Cognition, epilepsy, rutin, zebrafish

Introduction

Adverse effects may be mild, acute serious or chronic. The severity of adverse reactions due to antiepileptic drugs (AEDs) is mostly seen during initiation and early treatment. The most common side effects observed with AEDs therapy are CNS adverse effects. These include cognitive effects and psychiatric effects. Cognitive effects typically include diminished memory, attention, intelligence, executive function, language skills, and processing speed. The psychiatric effects include mood disorders, notably anxiety and depression, which are commonly observed. The most severe side effect is diminished memory, attention, intelligence, executive function, language skills and processing speed. Nootropic agents may be used to treat these disorders related to learning abilities and memory. Thus, there is a need to explore treatments for memory dysfunctions. Herbal medicine have been used as a natural remedy to treat neural symptoms. Phytochemicals have been proven to be potent neuro-protective agents. In previous studies, researchers found that phytochemicals including sterols, alkaloids, flavonoids, glycosides, saponins, tannins and terpenes are neuroprotective. Rutin, a well-known flavonoid has been proven to be nootropic in a number of studies. The present study aimed to determine the neuroprotective effect of rutin with reference to the adverse effects produced by AEDs.

Materials and Methods

Chemicals

Potassium chloride, sodium chloride, sodium bicarbonate, sodium phosphate, calcium chloride were purchased from Central Drug House (CDH), India; glucose were purchased from Fischer Scientific (India). Trichloroacetic acid and
the PTZ-treated groups. Before the PTZ exposure, the animals were captured gently from the treatment beaker and were placed in drug beaker used to perform biochemical and molecular analyses. Control group animals were maintained in a 250 mL distilled water was used in the experiment. Drugs

The following drugs were used: Phenytoin (Samarth Pharma, India), Valproic acid (VPA) (Sun Pharma, India), Levetiracetam (Lupin, India). Rutin was purchased from CDH, India.

PTZ model seizures were induced individually in each zebrafish. For this, each fish was exposed to 7.5 mM PTZ in a 250 mL beaker. The seizure-like behavior was assessed according to the stage. These were divided into three stages, stage I; stage II; stage III; clonus like seizures followed by loss of posture i.e. complete immobilization for about 1–3 s.[6,7] The fish was given PTZ treatment until the commencement of stage III. After stage III has reached, each fish was captured gently from the treatment beaker and were placed in drug beaker used to perform biochemical and molecular analyses. Control group animals were maintained in a 250 mL beaker with tank water for the same period and conditions as the PTZ-treated groups. Before the PTZ exposure, the animals remained exposed to AED treatments for 1 h, enough time for all drugs to achieve seizure suppressor effect. Phenytoin sodium (PHT) (450 μM), VPA (50 mM) and levetiracetam (3 mM) concentrations were chosen based on previous studies.[6,7]

Rutin Dosing

Rutin pretreatment (50 mg/kg, single injection, i.p.) was done before commencement of the study.[8]

Behavioral Apparatus

The procedures for group behavior task (GBT) were performed in an isolated room. Twenty-Four hours prior to the experiments, both male and female fish (approximately 1:1 distribution) were moved to the experimental room in order to reduce the variance in environment during the behavioral assay. The GBT was performed according to Platto et al.[9] For the simultaneous evaluation of height in the tank, locomotion, color, and shoal cohesion the test tanks (26 cm × 10 cm × 22 cm, length × width × height) were recorded for 5 min: (1) Latency to the first entry in the dark compartment; (2) time spent in the light compartment; and (3) number of crossings between compartments. The apparatus was filled with 3 cm of water. This shallow tank restricts bottom-dwelling, which is a well-established anxiety behavior in a new environment. Thus, the main protective strategy is seen to be effective against predators in several fish species.[10] Shoal cohesion was measured as an individual parameter for each fish by comparing to “internal control” fish, taking following scores: (1) Complete lack of group cohesion or fish interaction; (2) loose or partial shoaling behavior; (3) normal distance and shoaling behavior compared to ‘internal control’; and (4) increased shoal cohesion.

Behavioral Scores in the Group Behavior Task

Height in the tank

The position (bottom × middle × upper levels) was taken as an index of anxiety, as taken in case of rodents that is, the position near the wall versus the position in center of an open field.[11] Fish were observed for a minute and noted according to the following scores during 1 min observations: (1) Movement restricted to the bottom third of the tank; (2) preference for the lower two-thirds of the tank; (3) similar times exploring the three thirds; (4) preference for the upper two-thirds; and 5-only in the upper third.

Locomotion

Locomotion was considered as a general index of behavioral excitation/inhibition. This was evaluated by comparing to “internal control” fish, taking following scores: (1) Virtually immobile; (2) slower than normal; (3) normal; (4) increased locomotion; and (5) intense locomotion.

Color

Zebrafish show their response toward different colors. Their movement to a specific color is observed. The red region of the tank was frequently visited while the yellow region was almost neglected in normal condition while in diseased conditions this condition was reverted. Fish response to the color region was rated visually and scored as: (1) No movement to yellow region; (2) movement to yellow region 5–10 times in 1 min; (3) normal swim; (4) movement to yellow region more than 20 times in 1 min.

Shoal cohesion

Zebrafish generally prefer to swim in groups and their group aggregation is termed shoal cohesion.[12] This behavior strategy is seen to be effective against predators in several fish species.[13] Shoal cohesion was measured as an individual parameter for each fish by comparing to “internal control” fish (i.e. a group of three untreated fish habituated in an independent tank) and were scored as following: (1) Complete lack of group cohesion or fish interaction; (2) loose or partial shoaling behavior; (3) normal distance and shoaling behavior compared to ‘internal control’; and (4) increased shoal cohesion.

Light/dark task

It has been shown that zebrafish show a marked preference for dark zones.[14] Based on similar results shown by rodents toward brightly illuminated areas,[15] this test in particular is used light/dark test is typically used for the evaluation of anxiolytics effect in rodents. Zebrafish were placed in the light zone of the apparatus with drug-free water and the following measures were recorded for 5 min: (1) Latency to the first entry in the dark compartment; (2) time spent in the light compartment; and (3) number of crossings between compartments. The apparatus was filled with 3 cm of water. This shallow tank restricts bottom-dwelling, which is a well-established anxiety behavior in a new environment. Thus, the main protective strategy is black preference, which is the measure used in this task.

Statistical Analysis

The results are expressed as mean. For color affected locomotory analysis, data were analyzed with a two-tailed t-test for the preference of one color over another in each combination. The Wilcoxon matched-pairs signed-rank test was used to determine the differences between the time spent in the black and white compartments. Statistical comparison was performed to analyze time spent in light compartment (ANOVA) followed by Bonferroni’s test (P < 0.05 was considered as statistically significant).
**Result**

*Behavioral Scores in the Group Behavior Task*

**Height in the tank**

The height up to which the fish travelled was taken as an index of anxiety, as it is considered in the case of rodents that is, the position near the wall versus the position in center of an open field.[12] Results were obtained by observing the fishes for a 5 min. In case of phenytoin and gabapentin the movement was almost restricted to the bottom third of the tank for the first min while in case of VPA preference was given for the lower two-thirds of the tank. As time increased gradually, the movement shifted toward the normal conditions. In case of treatment with rutin results revealed that the movement was like the normal conditions [Figure 2].

**Locomotion**

Locomotion was considered as a parameter for normal behavior. During the course of treatment with antiepileptics the locomotion was increased (due to impairment) in comparison to the normal while it was toward the normal when treated with rutin which shows that rutin reverts the impairment caused due to AEDs [Figure 3].

**Color**

In the previous studies, it was established that zebrafish shows response to different color. They move toward a specific color. The red region of the tank was frequently visited while the yellow region was almost neglected in normal condition while in diseased conditions this condition was reverted. When the fish were treated with AEDs, they moved in all regions while in case of treated group the response was again reverted to the normal conditions [Figure 4].

**Shoal cohesion**

Shoal cohesion is the phenomena in which fish prefer to swim in groups, and their group aggregation is termed as Shoal Cohesion. Normally it is seen that zebrafish swim in groups. When they were treated with AEDs, a vast difference was seen in their swimming behavior. This result shows that the effects of rutin were positive over the disrupted swimming behavior produced by the administration of AEDs [Figure 5].

**Light/dark test**

Treatment with AEDs caused movement of zebrafish restricted to the dark zone. Treatment with rutin caused increased latency of zebrafish to move in the light compartment. Similarly, time spent in the light compartment by zebrafish treated with Rutin was significantly (P < 0.01) more as compared to zebrafish treated with AEDs [Figure 6].

**Discussion**

Zebrafish have proven to be an useful model for the study of nervous system development compared to rodent models used in pharmacological studies, zebrafish have various advantages for high-throughput screening like they are inexpensive, easier to handle due to small size, produce large numbers of progeny (up to 200 eggs in one mating), and develop rapidly (days as opposed to weeks). These beneficial characteristics of zebrafish, their external fertilization and transparency at embryonic and larval stages, makes it a useful alternative tool for the study of developing vertebrate nervous system. Even the newly developed larval zebrafish have a rich
behavioral response,[19,12] thus the present study was aimed to determine the effect of rutin on brain over the adverse effects produced by AEDs using zebrafish model.

The study demonstrated that all AEDs have specifically decreased shoal cohesion and height in the tank in the GBT. The GBT task performed for these AEDs showed that the administration of these drugs has caused significant behavioral changes in locomotion, height, shoal cohesion and response to color. The light/dark task was sensitive for all antiepileptics. It is well-established that the light/dark task is an interesting screening task for antiepileptics, whereas shoal cohesion and height in the tank could serve as useful endpoints to differentiate the type of antiepileptic response. Zebrafish has a natural tendency to remain initially at the bottom of a novel environment (e.g. a test tank) and then gradually, over a few minutes, explore the higher portions of the test tank.[112] The fear response of zebrafish also includes forming stronger shoal cohesion, freezing and giving response to color.[110] The exposure to the new environment of a tank is not particularly alarming to produce such behaviors, but the effects of AEDs on shoal cohesion became apparent only after a min in the tank. These distinct time courses and sensitivities to different drugs suggest that the neurobiological systems underlying height in the tank and shoal cohesion are quite independent, but differential kinetics of the drugs tested may play a role in their observed behavioral profile. In the present study, AEDs induced an increase in the intensity response to color in zebrafish, but aggression parameters were not evaluated. The light/dark task has been classically used as an anxiety test in rodents. Anxiolytics have been found to increase time in the light zone whereas anxiogenic drugs decrease it. The light/dark task has been classically used as an anxiety screening task for antiepileptics, whereas shoal cohesion and height in the tank could offer the potential for a primary screen to identify a wide variety of potential anticonvulsants. Epilepsy Res 2007;75:18-28.

Our results confirmed this dark preference of zebrafish and showed that AEDs were effective in increasing time in the light zone. It should be noted that in the light/dark task the apparatus was quite different from the GBT and that fish were tested alone. These results suggest that the light/dark task may be useful for behavioral high-throughput screening of side effects produced by AEDs compounds since it is quick and easily. These results suggest that the behavioral changes produced by AEDs can be overcome by treatment with rutin.

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