UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE FARMÁCIA

DISCIPLINA DE TRABALHO DE CONCLUSÃO DE CURSO DE FARMÁCIA

Estudo do efeito do tipo ansiolítico do flavonoide hiperosídeo em peixes-zebra (*Danio rerio)*

Vivian Herzfeldt

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Porto Alegre, 2016.

Aos meus pais, pelas suas incansáveis

forças de vencer todos os dias.

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Anxiolytic-like effect of hyperoside in zebrafish

Author names and affiliations

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Abstract

The zebrafish is widely used for assessing anxiety-like behaviors since it presents the evolutionarily conserved nature of many behaviors across species. Hyperoside (HYP) is a flavonoid that has been shown as a bioactive molecule with different activities, including antidepressant-like and anxiolytic-like effects in rodents. The aim of this study was to evaluate the effects of repeated administration of HYP on novel tank, light/dark and social interaction tests in zebrafish. The animals were treated for 7 days in beakers with one exposure per day of fluoxetine (FLU) (32 μM) or HYP (0.1, 0.3 and 1 μM). After 6 days of treatment, they were subjected to novel tank test, on subsequent days light/dark test and social interaction. Both HYP and FLU showed an anxiolytic profile in the novel tank test, whereas in the light/dark test only fluoxetine was effective. None of the compounds affected the social interaction behavior. Overall, these data consolidate HYP action in the central nervous system with anxiolytic-like effects.

Highlights

The effects of hyperoside were evaluated in behavioral models in zebrafish.

Hyperoside had anxiolytic-like effects in the novel tank test.

Hyperoside did not alter preference for dark side in the light/dark test.

Hyperoside did not change the social preference.

Keywords

Hyperoside

Anxiety

Zebrafish

Conflicts of interest

The authors have no conflicts of interest to disclose

1. Introduction

Compounds isolated from plants stand out in the search for treating diseases that affect society [1]. Among them, the glycosylated flavonoid hyperoside (HYP) has been studied regarding its anti-inflammatory [2], hepatoprotective [3] and antioxidant [4] antidepressant-like [5,6] activities in rodents and cell cultures. Butterweck and coworkers [5] showed for the first time the antidepressant-like effect of natural flavonoids isolated from *Hypericum perforatum* (HYP, isoquercitrin and miquelianin) in rats. Later, our group isolated HYP from *H. caprifoliatum* and demonstrated the antidepressant-like effect of HYP in the mice forced swimming test, which was prevented by the pretreatment with sulpiride, a dopamine D2 antagonist, indicating that D2-type receptors are involved in the mechanism of action of this flavonoid [6]. In another study, HYP blocked the stress-induced hyperthermia in mice, as well as buspirone, a 5HT1a partial agonist [7]. It is important to point out that the paradigm of stress-induced hyperthermia did not respond to antidepressants, and it has been used to study potential anxiolytics and anti-stress drug candidates [7].

Anxiety disorders are the most common mental disorders [8–10]. Among anxiolytic drugs, the agents of first choice are selective serotonin reuptake inhibitors and serotonin/norepinephrine reuptake inhibitors. Drug treatment should be continued for 6 to 12 months after remission [11]. However, frequently these drugs produce side effects that interfere with the patient's quality of life and medication adherence [12,13]. Thus, there is still a need for new treatments for anxiety disorders.

In addition, there are evidences that dopamine plays an important role in anxiety modulation in different parts of the brain, and dopaminergic agonists, such as apomorphine and quinpirole, as well as dopaminergic antagonists, such as sulpiride and

raclopride, have shown anxiolytic effects in rodent models of anxiety [14]. Thus, dopaminergic mesolimbic circuits have been proposed as novel target for the pharmacological treatment of anxiety[14]. In line with this assumption, reports have suggested that bupropion, a dual dopaminergic/noradrenergic antidepressant, may be effective for panic/anxiety disorders [15–17].

Zebrafish (*Danio rerio*) has dawn as an important new animal model in neuroscience research, especially to study anxiety disorders, for which it has been extensively studied and consolidated [18–24]. Therefore, the aim of this study was to evaluate the effects of repeated administration of HYP on novel tank, light/dark and social interaction in zebrafish.

2. Materials and methods

2.1. *Animals*

Adult wild-type zebrafish from both sexes (50:50, 12 months old) weighing approximately 200 mg were obtained from the heterogeneous breeding stock of Federal University of Rio Grande do Sul. The fish were acclimatized for at least 2 weeks in 40-L tanks before onset of experiments. All animals were kept in tanks with water (2.5 fish per liter) and controlled physicochemical parameters (pH 7.0 ± 0.3, 7.0 ± dissolved oxygen 0.4 mg / L; total ammonia <0.01 mg / L; hardness 5.8 mg / L alkalinity, and 22 mg / L $CaCO_3$). These parameters were evaluated daily. Sodium thiosulfate (1% solution) was used to neutralize chlorine in the proportion of 3 drops for each liter of water. The temperature was maintained at 25 ± 2 ° C. The light/dark cycle complied with the conditions stablished as ideal for these animals (14/10 h). The fish were fed with feed Tetramin® twice daily. All protocols of this study were approved by the Ethics Commission on Animal Use of Federal University of Rio Grande do Sul under process number 30746.

2.2. *Treatments*

The treatments consisted of fluoxetine (FLU) (Daforin®, EMS, Brazil) or hyperoside (HWI Analytik GMBH) at concentrations of 32 μM and 0.1 μM, 0.3 μM and 1 μM, respectively. We exposed the zebrafish in groups of 5 animals for 10 minutes in 600-mL beakers containing the solutions of treatments for 7 days. Control group was kept

under the same conditions and was submitted to the same treatment schedule but without drugs.

2.3. *Experimental Design*

The experimental protocol was carried out according to Fig 1. After the habituation time (15 days) , different groups of the animals were transferred and maintained in 5-L tanks. The animals were treated for 5 consecutive days, always at the same time of day (11:00 a.m.). On day 6, the animals were subjected to the novel tank test (8:00-11:00 a.m.) and after that, the animals were exposed to the treatments. On day 7, the animals were subjected to the light/dark test (8:00-11:00 a.m.) and were also treated after the behavioral protocol. On day 8, the animals were subjected to the social interaction test and after that, they were euthanized. All experiments were recorded using a webcam (Logitech®). For test apparatuses, the water was adjusted to hometank conditions and the tank water was changed after each group trial.

[Fig. 1 here]

2.4 *Behavioral tests*

2.4.1 *Novel tank*

The test was performed according to [25]. Animals were transferred individually to the test aquarium (24 × 8 × 20 cm, width x depth x height), which was virtually divided into three equal horizontal sections (top, middle and bottom) and recorded for 6 minutes. The videos were later analyzed with ANY-Maze® program (Stoelting CO, USA). The following parameters were evaluated for 6 min: total distance traveled, distance in the upper zone, time in the upper zone and entries in the upper zone of the tank.

2.4.2 *Light/dark*

The apparatus consists of a glass tank (18 × 9 × 7 cm) divided by a sliding guillotine-type partition (9 × 7 cm) in two equally sized dark and white compartments [25]. The water level was 3 cm and the partition was raised 1 cm above the tank floor to allow zebrafish to swim freely between the two sides of the tank. Fish were individually placed in the light zone of the apparatus and the time spent in the light compartment and the number of crossings between compartments were recorded for 5 min.

2.4.3 *Social interaction*

It was performed according to [26]. The animal was placed into a test tank (30 × 15 × 10 cm, length x height x width) virtually divided into two vertical sections, between two identical aquaria, one with a group of 15 fish and another empty with only water. After 30 s of adaptation in the test tank, the animal behavior was recorded for 10 s. The time the animal spent in the section closer to the tank with the shoal was calculated with a response to social stimuli.

2.5. *Statistical analysis*

Data were analyzed by one-way ANOVA followed by Tukey post hoc test. Values of $p < 0.05$ were considered significant. We used the GraphPad Prism 6.0 software for Windows.

3. Results

[Fig. 2 here]

[Fig. 3 here]

[Fig. 4 here]

Fig. 2 shows the effects of FLU (32 µM) and HYP (0.1, 0.3 and 1 µM) in the novel tank test. HYP was significantly different from the control on distance in upper zone (Fig. 2B, $F_{(4, 64)} = 12{,}90$, $p<0.05$), time spent in upper zone (Fig 2C, $F_{(4, 64)} = 24{,}78$, $p<0.05$) and number of entries in the upper zone (Fig. 2D, $F_{(4, 64)} = 6{,}92$, $p<0.05$). As expected, FLU significantly increased the distance (Fig 2B), time (Fig 2C) and number of entries in the upper zone (Fig. 2D). Neither the FLU nor the HYP treatment affected the total distance traveled.

Fig. 3 shows the effects of FLU (32 µM) and HYP (0.1, 0.3 and 1 µM) in the light/dark test. FLU significantly increased the time spent in the lit side of the tank ($F_{(4, 62)} = 2{,}91$) when compared to control. HYP had no effect. The treatments showed no effects on the number of crossings between compartments.

Fig. 4 shows the effects of FLU (32 µM) and HYP (0.1, 0.3 and 1 µM) in the social interaction test in zebrafish. Both treatments were devoid of effect in this test.

4. Discussion

Herein we demonstrated for the first time the effects of repeated hyperoside (HYP) and fluoxetine (FLU) administration (7 days) on behavioral parameters in zebrafish. Both HYP and FLU have shown an anxiolytic profile in the novel tank test whereas in the light/dark test only fluoxetine was effective. None of the compounds affected the social interaction behavior.

The zebrafish has a rich behavioral repertoire and the usefulness of this specie in neuroscience studies has grown in recent decades due to its physiological and genetic homology to humans, low cost, high throughput and similarity with mammalian central nervous system (CNS) morphology [19,20].

The zebrafish lives in shoals and under normal conditions, it prefers company. This normal behavior may be altered by MK-801, a non-competitive NMDA-R antagonist [27] and restored by antipsychotic drugs [28]. HYP did not change the social interaction behavior, suggesting that this drug has not propensity to induce cognitive and social interaction deficits.

Exposure to novelty in novel tank test evokes a robust anxiety response in zebrafish [21]. When the zebrafish is placed in a new environment, it has a natural tendency to explore deeper areas, which is considered an anxiogenic behavior [29,30] that may be modulated by anxiolytics and antidepressants. For example, fluoxetine [21,25], buspirone and diazepam [31] increase the time in the upper zone. In the current study, we demonstrated that HYP, like fluoxetine, increased the time spent in the novel tank upper zones as well as the number of entries, which indicates an anxiolytic effect. However, HYP was not effective in the light/dark test at the tested concentrations.

The light/dark preference is a behavioral paradigm widely used to evaluate candidates to anxiolytic drugs in rodents, and more recently in zebrafish. Zebrafish treated with benzodiazepines, buspirone, ethanol, as well as N-acetylcysteine, increased the time spent in the lit side [22,25]. Nevertheless, the results of this test are conflicting. Differences between age, room light and configurations of the tank have resulted in different behaviors [32]. Furthermore, pharmacological evidence suggested that this assay is sensitive to anxiolytic, but not panicolytic, drugs [33].

Of note, HYP appears to have dopaminergic action since our group has demonstrated that its antidepressant like-effect in rodents is impaired by sulpiride, a D2 receptor antagonist [6]. Brandão and coworkers (2015) demonstrated a dual role of dopamine D2-like receptors in the mediation of conditioned and unconditioned fear.

These authors postulated that dopamine D2 receptor inhibition in the terminal fields of the mesolimbic dopamine system provokes anxiolytic-like effects whereas the activity of midbrain substrates of unconditioned fear are enhanced by D2 antagonists, suggesting that D2 receptor-mediated mechanisms play opposing roles in fear/anxiety processes, depending on the brain region under study. Dopamine seems to mediate conditioned fear by acting at rostral levels of the brain and modulate unconditioned fear at the midbrain level, possibly by reducing the sensorimotor gating of aversive events [34].

5. Conclusion

For the first time, the effects of HYP in zebrafish behavior have been shown. Once again, the importance of zebrafish in cross-species behavioral analyzes was strengthened. Therefore, considering the results obtained in this study, consolidate HYP action in the CNS with anxiolytic effects and more studies will be performed to characterize concentration-response, as well as the related action mechanism.

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Legends for figures

Fig 1. Experimental timeline and design.

Fig 2. Effects of fluoxetine (32 µM) and hyperoside (0.1, 0.3 and 1 µM) on distance (A), distance in the upper zone (B), time in the upper zone (C) and number of entries in the upper zone (D) in the novel tank test. Data are expressed as mean + standard error of mean (S.E.M). n=12-17. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 Vs. control group, respectively. #p<0.05 Vs. Fluoxetine group. One-way ANOVA followed by Tukey post hoc test.

Fig 3. Effects of fluoxetine (32 µM) and hyperoside (0.1, 0.3 and 1 µM) on time in the lit side of the tank (A) and number of crossings (B) in the light/dark test. Data are expressed as mean + standard error of mean (S.E.M). n=12-17. *p<0.05 Vs. control group. One-way ANOVA followed by Tukey post hoc test.

Fig 4. Effects of fluoxetine (32 µM) and hyperoside (0.1, 0.3 and 1 µM) in the social interaction in zebrafish. Data are expressed as mean + standard error of mean (S.E.M). n=12-17. One-way ANOVA followed by Tukey post hoc test.

Figures

Figure 1.

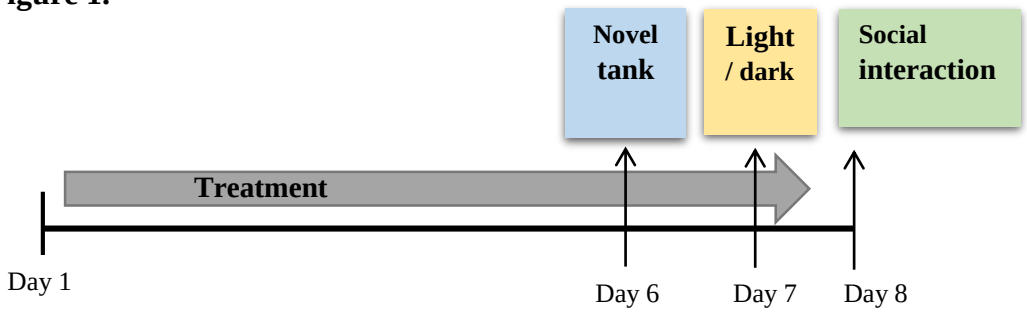

Figure 2.

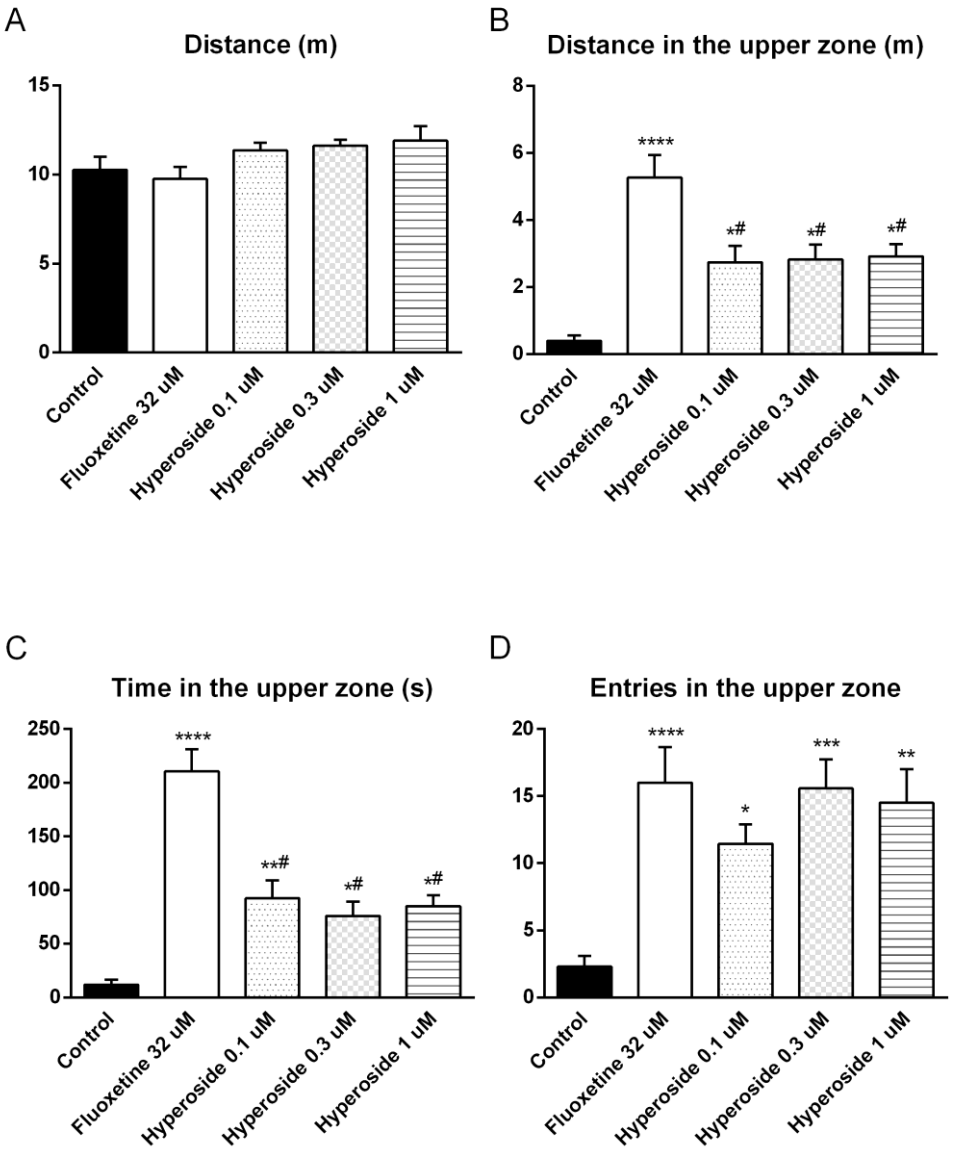

Figure 3.

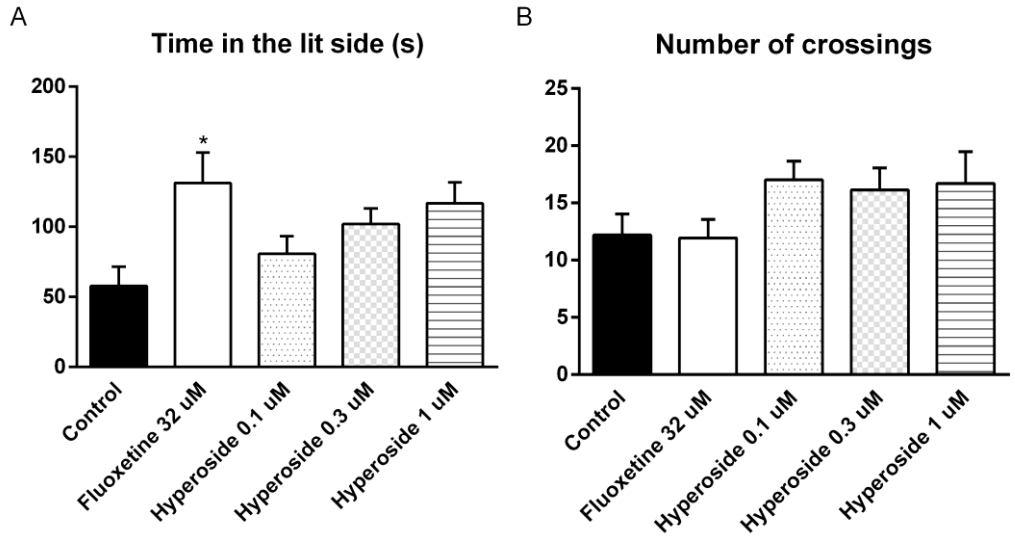

Figure 4.