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ORIGINAL PAPER

Investigation on the Anticonvulsant Potential of Luteolin and Micronized Luteolin in Adult Zebrafish (*Danio rerio*)

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Abstract

Epilepsy affects around 50 million people worldwide, and an important number of patients (30%) fail to respond to any available antiepileptic drug. Previous studies have shown that luteolin presents a promising potential as an anticonvulsant. On the other hand, different studies showed that luteolin does not promote anticonvulsant effects. Therefore, there is a lack of consensus about the use of luteolin for seizure control. Luteolin low bioavailability could be a limiting factor to obtain better results. Attractively, micronization technology has been applied to improve flavonoids bioavailability. Thus, the present study aimed to investigate the effects of luteolin on its raw form and micronized luteolin in a PTZ-induced seizure model in adult zebrafish (*Danio rerio*). Our results demonstrate that luteolin and micronized luteolin did not block PTZ-induced seizures in adult zebrafish. Also, luteolin and micronized luteolin did not provoke behavioral changes. Finally, our results show that 24 h after seizure occurrence, no changes were detected for *p70S6Kb*, *interleukin 1β*, and *caspase-3* transcript levels. Altogether, we failed to observe an anticonvulsant potential of luteolin in adult zebrafish, even in its micronized form. However, we recommend new studies to investigate luteolin benefits in epilepsy.

Keywords Epilepsy · Luteolin · Micronization · Seizure · Zebrafish

Introduction

Epilepsy is a neurological disorder characterized by one patient's chronic predisposition to have epileptic seizures [1, 2]. The recurrent and unpredictable seizure occurrence impairs the patients' social life and may induce cognitive and psychological consequences [1, 2]. Epilepsy affects around 50 million people in the world, and a notable quantity of patients (30%) fail to respond to any available antiepileptic drug (AED) [1, 3]. Furthermore, in numerous cases, pharmacotherapy is not well tolerated due to the side effects of the AEDs [4]. Then, the search and development of new drugs useful to control epileptic seizures are compulsory.

As one of the major phytoconstituents of *Eclipta alba* (L.) Hassk. (Asteraceae), polyphenol luteolin has antioxidant, neuroprotective, anxiolytic, and anti-inflammatory properties [5]. Therefore, luteolin therapeutic properties suggest a potential application in seizure control [5]. Luteolin single-dose pretreatment (10 and 20 mg kg^{-1}) did not show an anticonvulsant effect in an acute PTZ model in mice but promoted 100% protection from mortality [5]. Besides, PTZ-induced kindling in mice was significantly prevented by luteolin (5, 10, and 20 mg kg^{-1} intraperitoneal (i.p.) injection) in a dose-dependent manner [5]. On the other hand, in a study investigating the effects of acute and chronic luteolin

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administration in a various mouse model of seizure, luteolin did not exhibit anti- or pro-convulsant effects after single dosing in the 6 Hz (0.3–10 mg kg^{-1}, i.p.), maximal electroshock (0.3–20 mg kg^{-1}), and PTZ (3 mg kg^{-1}) seizure models [6]. Also, luteolin did not present anti- or pro-convulsant effects after repeated daily dosing (10 mg kg^{-1}, i.p.) in the 6 Hz model, and no effect was reported after repeated luteolin administration (10 mg kg^{-1}) in the second hit PTZ test [6]. Therefore, there is a lack of consensus about the use of luteolin for seizure control.

Different polyphenols have shown promising pharmacological potential in seizure control [7–9]. However, for this class of organic compounds, the pharmacological potential is impaired by its low bioavailability [10]. To elude this barrier, micronization technology has been applied. Compared to the starting material, micronization technology allows obtaining significantly reduced particle size with a fair size distribution [11, 12]. Consequently, the pharmaceutical industry has used this approach to increase drug solubility and bioavailability [11, 12].

Studies show that luteolin presents a promising potential as an anticonvulsant. On the other hand, different studies showed that luteolin does not promote anticonvulsant effects. The low bioavailability of luteolin could be a limiting factor to obtain better results. Attractively, micronization technology has been applied to improve the bioavailability of flavonoids and consequently pharmacological potential. Thus, the present study aimed at investigating the effects of luteolin (raw) and micronized luteolin in PTZ induced seizures in adult zebrafish.

Materials and Methods

Drugs

Luteolin (98%, Kingherbs, China) was used in its raw and micronized forms. Acetone (99.5%, Vetec, Brazil) and CO_2 (99.9% in a liquid phase, White Martins S.A., Brazil) were used in the micronization process. Tricaine methanesulfonate (MS-222, Sigma-Aldrich, Germany), pentylenetetrazole (PTZ, Sigma-Aldrich, Germany), and diazepam (Fagron Pharmaceuticals, Brazil) were used as an anesthetic, seizure inducer, and AED (positive control), respectively. Commercial kits for RNA extraction (Purelink RNA Mini Kit), RNA, cDNA quantification (Qubit RNA BR Assay Kit and Qubit dsDNA Broad-Range Assay kit), and cDNA synthesis (High Capacity cDNA Reverse Transcription kit) were supplied by Thermo Fisher Scientific Inc, USA. Power UP SYBR Green kit (Invitrogen, USA) was used for quantitative polymerase chain reactions (qPCR). Primers were synthesized by Invitrogen.

Micronization Process

The micronization process occurred by the gas antisolvent technique [13]. Briefly, luteolin was mixed in acetone and heated while being stirred into complete dissolution. Following, the solution was cooled and then stirred for 2 min for complete homogenization. The obtained solution was placed in a chamber, and CO_2 was added to increase the ideal pressure to complete the process. When the pressure was reached, the antisolvent flow was stopped, and the chamber continued to agitate the solution for 10 min at 300 rpm [14]. After this, the washing stage began with the antisolvent flow rate of 10 mL min^{-1}, and the pressure was isobaric by 60 min. The operating conditions were pressure 80 bar and temperature 35 °C. Finally, the material was stored at 4 °C.

Raw and Micronized Luteolin Characterization

In the view of characterizing the non-micronized (raw) and micronized particles, images were obtained by scanning electron microscopy and the particle size was determined by using the software Meter Size (version 1.1) [7, 11]. The melting points of the raw and micronized compounds were verified by using a differential scanning calorimeter (Jade-DSC, Perkin Elmer, USA). Samples (5–10 mg) measurements were performed by heating the compounds from 30 to 350 °C, at a heating speed of 20 °C min^{-1} in an inert atmosphere (N_2 flow: 20 mL min^{-1}) [7, 11].

Animals

Eighty adult (3 months) zebrafish (*Danio rerio*) of both sexes (50:50 sex ratio) were obtained from a local supplier and were acclimated for four weeks before the experiments. The fish were kept in tanks with unchlorinated water at 26 ± 2 °C, maintained under a 14/10 h light/dark cycle photoperiod, and fed twice a day with flake fish food (Alcon Basic, Brazil). A previous study presented specific information about housing conditions [15]. All experimental practices were approved by the Ethics Committee for Animal Use (CEUA Unochapecó Protocol, #009/2019). The trials also respected the National Council for Control of Animal Experimentation recommendations [16]. In the experiments, fish were randomly allocated into the experimental groups using a computerized random number generator.

Drug Pretreatments

Each animal received its respective pretreatment 30 min before the PTZ exposure [7, 9]. Diazepam, luteolin, and micronized luteolin were dissolved in 0.9% saline solution. Saline solution, diazepam at 5 mg kg^{-1}, luteolin at

0.5 mg kg^{-1}, and micronized luteolin at 0.5 mg kg^{-1} were administered by i.p. injection [6, 7]. Previous study presents a detailed description of anesthesia and injection procedures [9]. The sham group corresponded to fish that were not exposed to any drugs or PTZ. The experiments were performed in two rounds, totaling 16 animals per experimental group.

Novel Tank Test

To verify the effects of the different pretreatments on zebrafish behavior, all the animals were individually submitted to the novel tank test [7, 9]. Briefly, zebrafish were individually placed in glass tanks (24 × 8 × 20 cm, length × width × height) containing unchlorinated water, which are virtually divided into three equal horizontal areas. Each animal had its locomotion and exploratory activities recorded for 30 min following drug pretreatment. Each video was further analyzed using the ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA). The elemental endpoints analyzed were: total distance traveled, distance traveled and time spent in the top area, line crossings number (transitions between the areas of the tank), and average speed. The experiments were performed in two rounds, totaling 16 animals per experimental group.

PTZ-Induced Seizure

The animals were individually exposed to 5 mM PTZ during 600 s to induce seizures. Fish were exposed to PTZ solution in glass tanks (8 × 6 × 10 cm, length × width × height) and had their behavior recorded during the whole exposition time. Each recorded video was analyzed by two qualified observers blinded to the pretreatments. Then, the occurrence of each seizure stage and the latency to reach the first behavioral signal of each seizure stage were scored [7, 9]. The seizure-like behavior was classified according to each stage: stage I—dramatically increased swimming activity; stage II—whirlpool swimming behavior; and stage III—clonus-like seizures followed by loss of posture, when the animal falls to one side and remains immobile for 1–3 s, as earlier described for zebrafish [17]. The experiments were performed in two rounds, totaling 16 animals per experimental group.

Real-Time PCR

Twenty-four hours after the PTZ-induced seizures protocol, fish were cryoanesthetized and euthanized by decapitation. The brains were dissected and used for molecular analyses. Total RNA was isolated from samples using the Purelink RNA Mini Kit following the manufacturer's recommendations. Each sample consisted of a pool of 4 brains from zebrafish submitted to the same pretreatment.

The total RNA was quantified by using the Qubit RNA BR Assay Kit. The cDNA was synthesized using the High Capacity cDNA Reverse Transcription kit. An average of 0.5 µg of extracted RNA in a reaction with a final volume of 20 µL was used to synthesize cDNA. cDNA quantification was performed by using the Qubit dsDNA Broad-Range Assay kit, and the samples were subsequently diluted to a final concentration of 5 ng μL^{-1}.

Following the manufacturer's recommendations, quantitative polymerase chain reactions (qPCR) were performed by using the PowerUP SYBR Green kit. We used as genes of interest the indirect markers of cell apoptosis (*caspase-3*), inflammatory response (*interleukin 1β*), and mTOR pathway (*p70S6K*). *β-actin* was used as an internal control to normalize the expression of genes of interest. Based on our data, the expression of β-actin was not altered by pretreatment, validating its use as an appropriate housekeeping gene for normalization in this study. All primer sequences are pointed out in Table 1.

Each reaction contained 10 ng of cDNA and 0.5 mM of each primer in a final volume of 10 µL. Each sample was analyzed in triplicate. The PCR cycles had the following conditions: 50 °C for 2 min, 95 °C for 10 min, 40 cycles at 95 °C for 15 s, 60 °C for 1 min. The dissociation occurred at 95 °C (1.6 °C s^{-1}) for 15 s, then 60 °C (1.6 °C s^{-1}) for 1 min, and, finally 95 °C (1.6 °C s^{-1}) for 15 s. The equipment used to perform qPCRs was QuantStudio 3 (Thermo Fisher Scientific). Relative gene expression levels were determined applying the RQ = 2 ^ ΔΔCt method [18].

Statistical Analysis

First, the normality of the data was analyzed by the Shapiro–Wilk test. To investigate if the saline pretreatment

Table 1 Quantitative RT-PCR primers sequences

Proteins	Primer sequence (5′-3′)
ß-actin[a]	F-CGAGCTGTCTTCCCATCCA R-TCACCAACGTAGCTGCTTTCTG
Caspase-3[b]	F-TAGTGTGTGTGTTGCTCAGTC R-CTCGACAAGCCTGAATAAAG
IL-1 ß[c]	F-GAACAGAATGAAGCACATCAAACC R-ACGGCACTGAATCCACCAC
rps6kb1b[d]	F-TGACTGATTTCGGGCTGTGT R-CGATTGTGTCCGCTCCTCAT

[a][19]
[b][20]
[c][21]
[d][22]

had any effects on the animals, we used Welch's t-test to perform sham and saline comparisons (for behavioral and molecular data). The Kruskal–Wallis test followed by Dunn's post hoc test was implemented to investigate the influence of the pretreatments (saline, diazepam, luteolin, and micronized luteolin; independent variable) on behavioral parameters (total distance traveled, distance traveled in the top area, time spent in the top area, line crossings number, and average speed; dependent variable), and on the latency to reach each seizure stage (dependent variable). Kruskal–Wallis test was implemented once the data did not meet the assumption of normality. To investigate the influence of the pretreatments (saline, diazepam, luteolin, and micronized luteolin; independent variable) on the occurrence of each seizure stage, we used the Fisher's exact test (two-tailed) to carry out pairwise comparisons of all pretreatments in PAST v.4.03 [23]. Pairwise *p*-values were adjusted for multiple comparisons using Bonferroni correction with $\alpha = 0.05$ [24]. Results were considered significant at a $p < 0.05$ level.

GraphPad Prism 6.0 was used to produce graphs, in which seizure occurrence data was expressed as the percentage of animals that reached each seizure stage. Results were expressed as a mean ± S.D. or median with interquartile range. Medians are used in conditions when the average is misled due to outliers or distorted distribution [25]. As described previously, the experiments were performed using n = 16 per group. Molecular experiments were performed using n = 4 (4 pools of 4 brains) per group.

Results

Particle Characterization

Luteolin (raw) presented an irregular particle size, in the form of needles or rods, and with relevant agglomeration, while micronized luteolin presented a homogeneous structure (Fig. 1). Table 2 presents the values of the average particle diameter (PD), standard deviation (σ), and the coefficient of variation (CV). Luteolin has an average particle of 22.75 µm size, and micronized luteolin showed an average size of 2.31 µm. Therefore, a tenfold reduction in particle size is observed.

The differential scanning calorimeter permits to see (Supplementary Fig. 1) that luteolin showed an endothermic peak at 286.10 °C with $\Delta H = 38.956$ J g^{-1} which is a characteristic of its melting point. A change in the melting point can be seen in micronized luteolin with the appearance of two endothermic peaks, the first at 245.39 °C with $\Delta H = 3.153$ J g^{-1} and the second peak at 322.27 °C with $\Delta H = 30.774$ J g^{-1}, which indicates a change in the crystalline structure, represented by the change in the melting point of the compound.

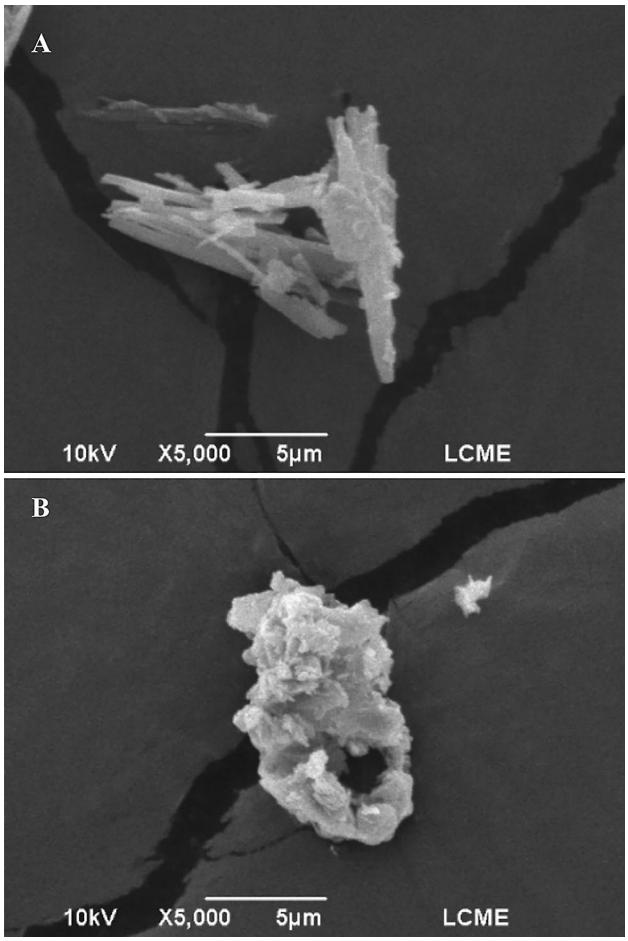

Fig. 1 Results of scanning electron microscopy of the raw (**A**) and micronized (**B**) luteolin

Table 2 Particle diameter (PD) in micrometers (µm) and respective coefficient of variation (CV) of luteolin and luteolin micronized by gas antisolvent technique (GAS)

Compound	T (°C)	Pressure (bar)	PD (µm)	CV
Luteolin	–	–	22.75 ± 15.77	0.69
Micronized luteolin	35	80	2.31 ± 1.04	0.45

The operating conditions of GAS, the temperature in celsius degrees T (°C) and pressure in bars, were also given

Behavioral Parameters in the Novel Tank Test

There were no behavioral differences between the sham and saline groups (supplementary Fig. 2) for total traveled distance (Welch corrected $t = 0.914$; df = 29.60; $p = 0.368$), distance traveled in the top area (Welch corrected $t = 0.025$;

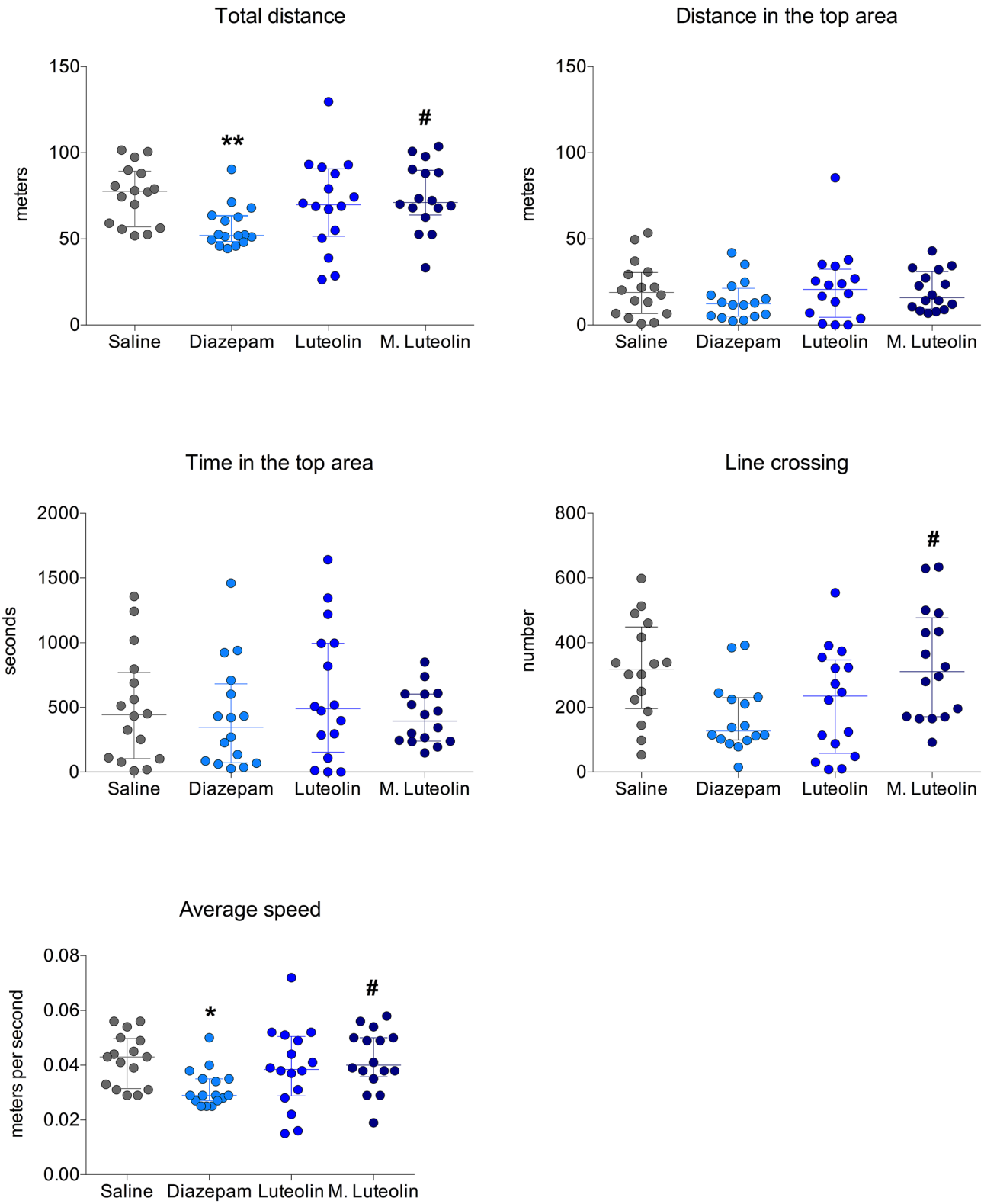

Fig. 2 Effects of pretreatment with saline (0.9%), diazepam (5 mg kg^{-1}), luteolin (0.5 mg kg^{-1}), and micronized luteolin (0.5 mg kg^{-1}) on the locomotor and exploratory activity of zebrafish in the novel tank test before PTZ-induced seizures. Data are expressed as median with interquartile range. Data were analyzed by Kruskal–Wallis test (considering treatment as the independent variable) followed by the Dunn's post hoc test (n = 16). *$p < 0.05$ vs. saline; **$p < 0.01$ vs. saline and $^{\#}p < 0.05$ vs. diazepam

df = 26.69; $p = 0.980$), time spent in the top area (Welch corrected $t = 0.987$; df = 29.73; $p = 0.331$), line crossings (Welch corrected $t = 1.553$; df = 24.12; $p = 0.133$), and average speed (Welch corrected $t = 0.840$; df = 29.53; $p = 0.407$).

We observed an influence of pretreatments on three behavioral parameters (Fig. 2): total traveled distance (Kruskal–Wallis statistic = 12.41; $p < 0.01$), line crossings (Kruskal–Wallis statistic = 11.25; $p < 0.05$), and average speed (Kruskal–Wallis statistic = 12.13; $p < 0.01$) parameters. In summary, animals pretreated with diazepam traveled a lower distance when compared to saline (Mean rank difference = 20.75; $p < 0.01$) and micronized luteolin groups (Mean rank difference = 19.25; $p < 0.05$). Also, animals from the diazepam group showed a lower crossing number (Mean rank difference = 17.28; $p < 0.05$) than micronized luteolin group. Additionally, animals pretreated with diazepam showed lower average speed than saline (Mean rank difference = 20.22; $p < 0.05$) and micronized luteolin (Mean rank difference = -19.34; $p < 0.05$) groups.

Seizure Occurrence and Development

The occurrence of the tonic–clonic seizure stage III (Fig. 3) was reduced by diazepam pretreatment (Bonferroni corrected significance level = 0.00017088). Also, the occurrence of stage III was fewer in the diazepam group than in the luteolin group (Bonferroni corrected significance level = 0.00017088).

The seizure development results (latency) are shown in Fig. 4. No difference among the different pretreatments was observed for stage I latency (Kruskal–Wallis statistic = 4.06; $p = 0.254$). Differences among the different pretreatments were observed for stage II (Kruskal–Wallis statistic = 11.03; $p = 0.011$) since diazepam (Mean rank difference = − 18.94; $p < 0.05$) and micronized luteolin (Mean rank difference = − 18.72; $p < 0.05$) increased the latency. Finally, the obtained data (Kruskal–Wallis statistic = 25.460; $p < 0.0001$) showed that animals pretreated with diazepam took longer to reach tonic–clonic seizure stage III in comparison with saline and luteolin groups (Mean rank difference = − 29.31; $p < 0.0001$ and Mean rank difference = 26.34; $p < 0.001$, respectively).

Molecular Parameters

The expression level of genes related to neurogenesis (*p70S6Kb*), inflammatory response (*interleukin 1β*) and cell apoptosis (*caspase-3*), 24 h after PTZ exposure, was investigated by quantitatively qPCRs. Our data show that there were no changes in the *p70S6Kb* (Welch corrected $t = 1.874$; df = 3.729; $p = 0.139$), *interleukin 1β* (Welch corrected $t = 0.714$; df = 5.646; $p = 0.503$) and *caspase-3* (Welch corrected $t = 1.651$; df = 5.613; $p = 0.153$)

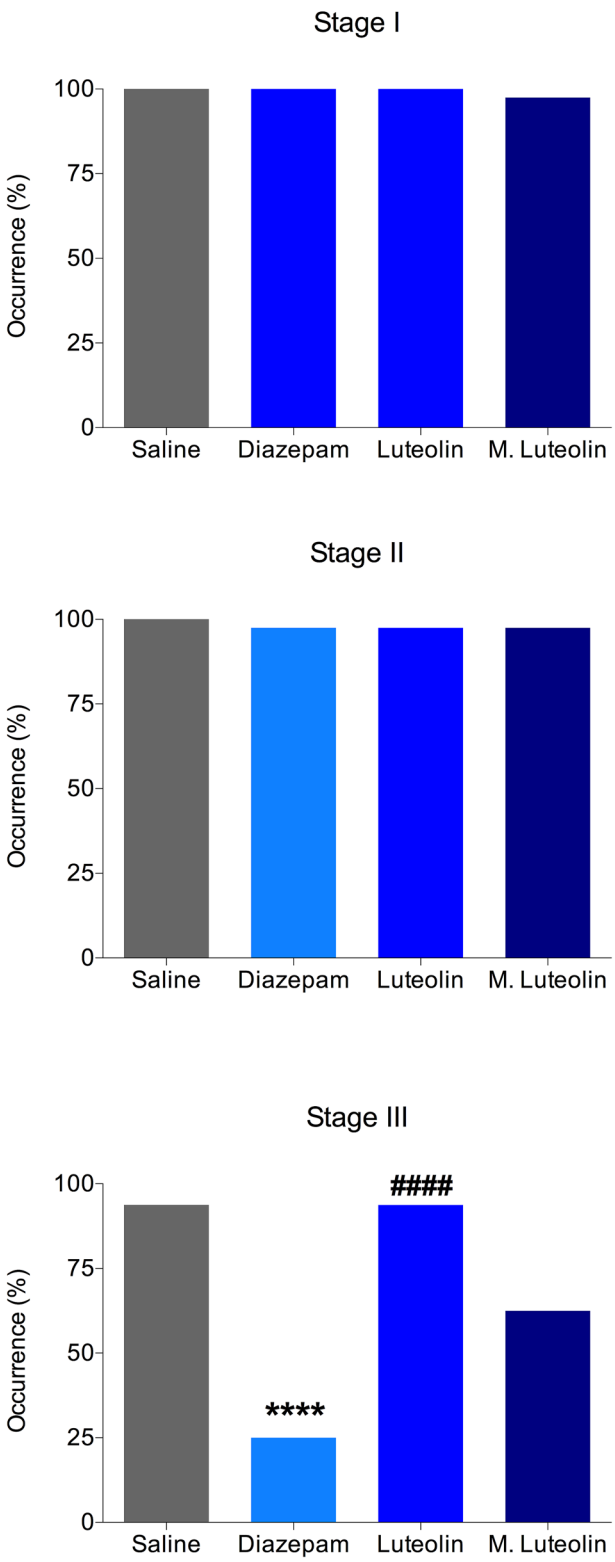

Fig. 3 Effects of pretreatment with saline (0.9%), diazepam (5 mg kg^{-1}), luteolin (0.5 mg kg^{-1}), and micronized luteolin (0.5 mg kg^{-1}) on the occurrence of each seizure stage (I, II and III) in zebrafish. Zebrafish were submitted to pretreatments 30 min before the PTZ-induced seizures. Data are expressed as the percentage of animals that reached each seizure stage. Obtained data were analyzed using Fisher's exact test (n = 16). $^{****}p < 0.0001$ vs. saline and $^{\#\#\#\#}p < 0.0001$ vs. diazepam

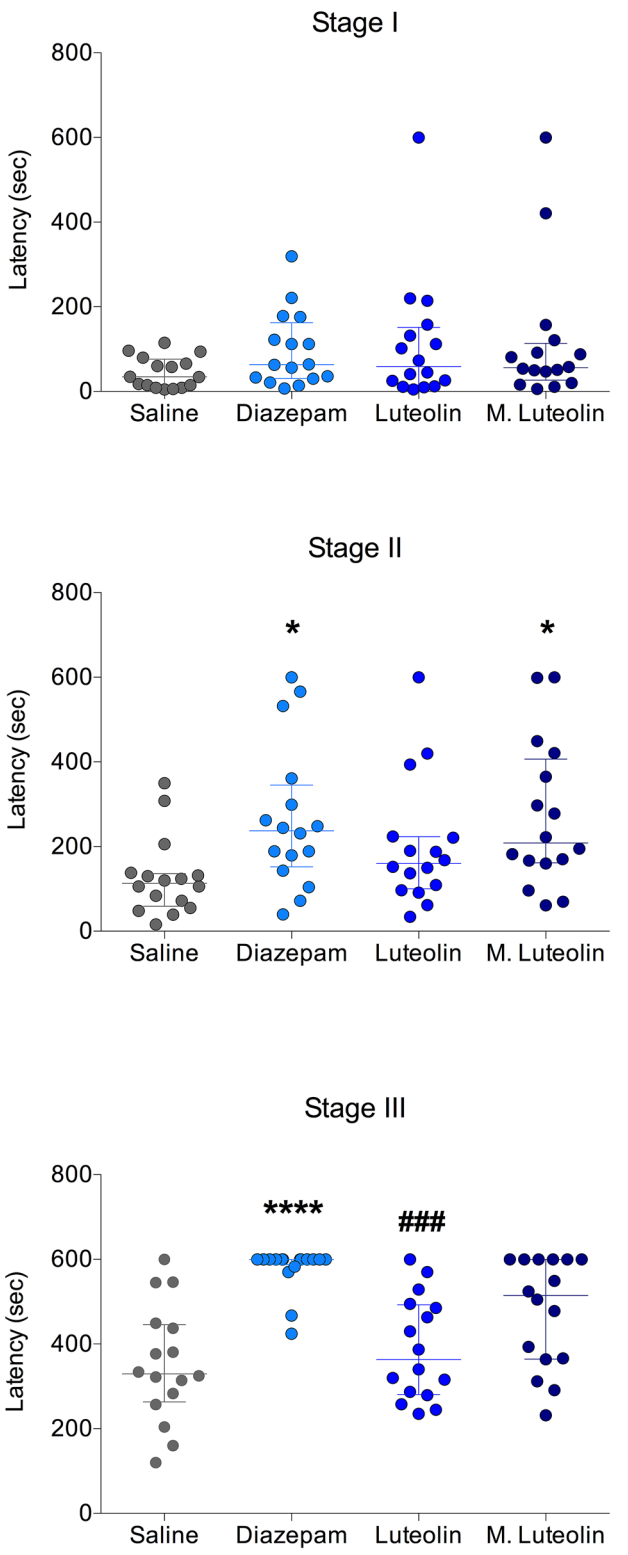

Fig. 4 Effects of pretreatment with saline (0.9%), diazepam (5 mg kg^{-1}), luteolin (0.5 mg kg^{-1}), and micronized luteolin (0.5 mg kg^{-1}) on the latency to reach each seizure stage (I, II and III) in zebrafish. Zebrafish were submitted to pretreatments 30 min before the PTZ-induced seizures. Data are expressed as median with interquartile range. Data were analyzed by Kruskal–Wallis test (considering treatment as the independent variable) followed by the Dunn's post hoc test (n = 16). *$p < 0.05$ vs. saline; ****$p < 0.0001$ vs. saline and $^{###}p < 0.001$ vs. diazepam

transcript levels in zebrafish brain 24 h after PTZ-induced seizures (Fig. 5).

Discussion

Here, we investigated for the first time the effects of luteolin and micronized luteolin on behavior and PTZ-induced seizures in adult zebrafish. Differently from diazepam, luteolin and micronized luteolin did not induce changes in zebrafish locomotion and exploratory behavior. Considering the data about PTZ-induced seizures, we showed that the positive control diazepam reduced tonic–clonic seizure stage occurrence and slowed the seizure development. Luteolin and micronized luteolin did not change the occurrence of any seizure stage (I, II, or III). Additionally, luteolin and micronized luteolin did not slow the seizure development.

The in vitro results observed here could suggest an increased luteolin bioavailability induced by particle size reduction after the micronization process. According to [26, 27] a change in the melting point of a compound, as observed by comparing luteolin and micronized luteolin, can alter its dissolution and solubility properties. The micronized compound also showed an exothermic peak at 255 °C, which may be due to instability in the particle structure formed after processing [28]. A peak close to 100 °C may result from the hygroscopicity of luteolin, and it has also been observed by other authors [29, 30]. Polyphenols' pharmacological potential could be enhanced by increasing their bioavailability, through the micronization process. Therefore, the micronization process could increment luteolin potential in seizure control [7, 11]. A limitation of our study is that we cannot demonstrate the ability of luteolin and micronized luteolin to penetrate the zebrafish brain. Despite the previous reports showing the central effects of this compound in rodents [5, 31–35], additional studies are needed to verify this issue in zebrafish.

The results from novel tank test exposed here show luteolin and micronized luteolin did not induce changes in zebrafish locomotion and exploratory behavior. Diazepam reduced the traveled distance and mean speed. Therefore, in the novel tank test, diazepam induced clear alteration on motor coordination/exploration without anxiolytic effects. This result corroborates with a previous study showing this drug did not present anxiolytic effects in this test [36].

At the moment, there is a lack of consensus about the use of luteolin for seizure control. A previous study has

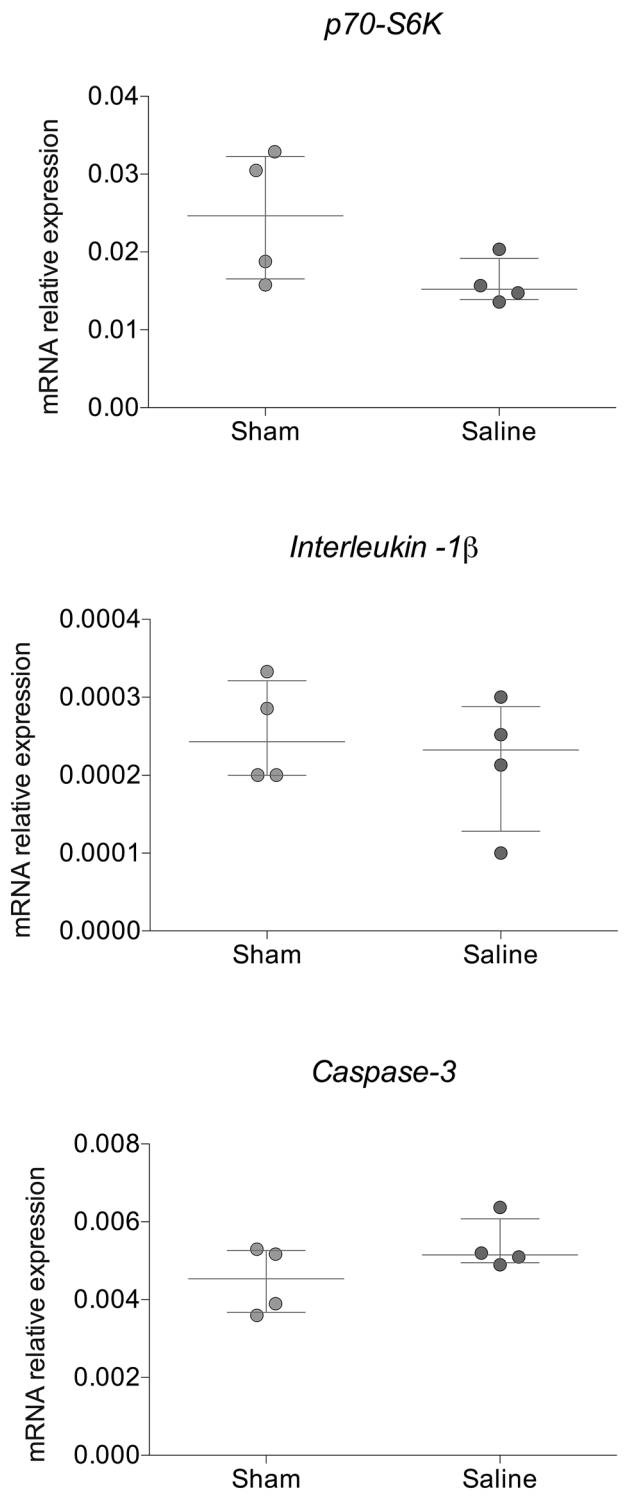

Fig. 5 Comparison of relative expression of *p70S6K*, *interleukin 1β* and *caspase-3*, genes in each zebrafish brain from sham and saline groups**.** Gene expression values were normalized to housekeeping gene *β-actin*. Sham group corresponds to animals manipulated but not exposed to PTZ or drugs, and animals from the saline group were pretreated with 0.9% saline and then exposed to PTZ. Data are expressed as median with interquartile range. Obtained data were analyzed by Welch's t-test (n = 4 brain pools/group)

already shown a potential application of luteolin in seizure control [5]. However, another study showed that luteolin did not exhibit anticonvulsant effects after single dosing in the 6 Hz, maximal electroshock, and PTZ seizure models [6]. Also, luteolin did not present anticonvulsant effects after repeated daily dosing in the 6 Hz model, and no effect was reported after repeated luteolin administration in the second hit PTZ test [6]. Here, we failed to observe a possible anticonvulsant potential of luteolin, even on its micronized form. Our results suggest that micronized luteolin is not efficient like other micronized polyphenols (e.g. curcumin and resveratrol) in acute seizure control [7–9]. Luteolin activates adenosine A_1 and A_{2A} receptors [37]. A_1 and A_{2A} receptors activation hyperpolarizes the postsynaptic cell membrane and inhibits presynaptic excitatory neurotransmitter release, promoting an anticonvulsant effect [20, 38–40]. Luteolin treatment promotes the inhibition of mTOR/4E-BP1 signaling pathway in PC12 neuronal cells [41]. Studies suggest that mTOR/4E-BP1 inhibition can be a good approach to epilepsy management. However, mTOR/4E-BP1 inhibition has been most effective against epileptogenic mechanisms [42]. Therefore, despite its lack of effects on the acute seizure model, luteolin effects on epilepsy are not discharged.

Given identify possible molecular changes induced by acute seizure occurrence, and the luteolin and micronized luteolin potentials to prevent those changes, we analyzed the transcript levels of the markers of mTOR and neurogenesis pathway *p70S6K*, inflammatory response *interleukin 1β,* and cell apoptosis *caspase-3*. Our data show no changes in transcript levels of referred genes in the zebrafish brain 24 h after PTZ-induced seizures (in comparison with a sham group). Previous studies show that *interleukin 1β* and *p70S6K* are upregulated in epilepsy [43]. However, it is not clear if acute seizure episodes could upregulate *interleukin 1β* and *p70S6K*. Our data indicate that acute seizure occurrence is not sufficient to activate *interleukin 1β* and *p70S6K* signaling. Finally, our data showed that *caspase-3* relative expression from animals exposed to PTZ was not different from those animals belonging to the sham group, suggesting that acute seizure did not induce cell apoptosis.

PTZ-induced kindling was significantly prevented by luteolin (5, 10, 20 mg/kg, i.p.) in a dose-dependent manner in mice [5]. Also, luteolin administration inhibited lipid peroxidation, a process that characterizes epileptogenesis [5]. Results presented in this study indicate a lack of effects of luteolin on its raw and micronized forms in acute seizure occurrence and development. Adding the results shown here to those obtained in previous studies permits us to suggest that luteolin, and consequently micronized luteolin, could be more effective in a chronic administration, inhibiting the epileptogenesis process [5].

Conclusions

In conclusion, our results demonstrate that luteolin and micronized luteolin did not block PTZ-induced seizures in adult zebrafish. Adding this study to previous studies is possible to suggest that luteolin, and consequently micronized luteolin, could be more effective during neurodevelopment or in a chronic administration. Therefore luteolin benefits in epilepsy are not discharged, and more studies are necessary.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11064-021-03409-8.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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