Pharmacology, Biochemistry and Behavior 179 (2019) 63–72

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Lutein prevents corticosterone-induced depressive-like behavior in mice with the involvement of antioxidant and neuroprotective activities

Ana Lúcia Bertarello Zeni*, Anderson Camargo, Ana Paula Dalmagro

Laboratório de Avaliação de Substâncias Bioativas, Departamento de Ciências Naturais, Universidade Regional de Blumenau, CEP 89030-903 Blumenau, Santa Catarina, Brazil

ARTICLE INFO

Keywords:
Depression
Corticosterone
Lutein
Oxidative stress
Glutamate

ABSTRACT

Depression is a neuropsychiatry medical condition with high prevalence, in which the hypothalamic-pituitary-adrenal axis dysfunction has been postulated as the main cause. The glucocorticoids can be harmful to the brain, particularly by induction of oxidative stress and glutamatergic damage, therefore antioxidants or neuroprotective agents could have beneficial effects. Lutein (LUT) is a dietary xanthophyll able to arrive in the brain that has been used for therapy of macular degeneration. In this sense, several studies pointed beneficial effects of LUT in the brain, particularly in the hippocampus and prefrontal cortex, key regions in mood regulation. Thus, this study sought to evaluate antidepressant-like, antioxidant and neuroprotective effects of LUT (0.1, 1 and 10 mg/kg) and fluoxetine (10 mg/kg) given orally (*p.o.*), acute, 7 or 21 days, once a day, in combination or not with corticosterone (20 mg/kg) in mice. After behavioral evaluation, the hippocampus, prefrontal cortex, and plasma were collected to assess the oxidative stress markers. And the neuroprotection against glutamate was developed through prefrontal cortex and hippocampal slices. LUT and fluoxetine in acute or subchronic treatment decreased immobility time at the dose 10 mg/kg. Furthermore, corticosterone was effective to induce depressive-like behavior accompanied by an increase of the oxidative stress. Conversely, LUT and fluoxetine were able to counteract the behavioral changes displayed by corticosterone showing antidepressant-like effect. In addition, both LUT and fluoxetine presented antioxidant effect in the hippocampus, prefrontal cortex and plasma of mice, and exhibited a capability to protect hippocampal and prefrontal cortex slices against glutamatergic toxicity. Our results demonstrated that LUT treatment presented an antidepressant-like effect with the involvement of oxidative stress and neurochemical abnormalities amelioration. Therefore, LUT, widely used for therapy of macular degeneration emerge as a promising agent useful in the management of depression.

1. Introduction

Depression is a chronic, multifactorial and potentially life-threatening mental disorder (Nemeroff, 2007; Malinowski et al., 2017). The World Health Organization (WHO, 2017) mentioned that depression is currently the leading reason of disability worldwide affecting > 300 million people. However, less than half of the affected people in the world, in some countries, < 10%, have received medical treatment. The current barriers include (i) lack of resources since the treatment is expensive; (ii) the need of trained professionals and (iii) social stigma associated with the mental disorders (WHO, 2017). Because heterogeneous nature, the pathophysiology of depression has not been fully understood, although, the disease might involve malfunctioning of monoaminergic system, excessive stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, increasing of neurodegeneration and oxidative stress (Sheline et al., 2003; Pytka et al., 2016a, 2016b).

The HPA axis hyperactivity is a common finding in depression leading to an increase of the glucocorticoid level (Myers et al., 2014), and consequently leads to oxidative stress (Zafir et al., 2009; Spiers et al., 2015; Stanić et al., 2016) and glutamatergic excitotoxicity (Lau and Tymianski, 2010; Freitas et al., 2016). These events can be harmful to the brain inducing neuronal cell death particularly in the hippocampus, a key region implicated in mood regulation (Lee et al., 2002; Freitas et al., 2015). In fact, oxidative stress and overstimulation of *N*-methyl-D-aspartate (NMDA) receptors play a fundamental role in the depression onset, thus antioxidant molecules or NMDA receptor antagonists have been exerted antidepressant-like and neuroprotective activities (Zeni et al., 2011; Sałat et al., 2015; Zeni et al., 2017). Recently, the notion that antioxidants exert a neuroprotective effect attached to their ability to repair the central nervous system preventing

* Corresponding author at: Departamento de Ciências Naturais, Universidade Regional de Blumenau, Campus I, 89030-903 Blumenau, SC, Brazil.
E-mail address: anazeni@furb.br (A.L.B. Zeni).

https://doi.org/10.1016/j.pbb.2019.02.004
Received 13 January 2019; Received in revised form 12 February 2019; Accepted 14 February 2019
Available online 17 February 2019
0091-3057/

neurodegeneration *via* oxidative stress was evidenced (Wąsik and Antkiewicz-Michaluk, 2017). The antioxidant *N*-acetylcysteine (NAC) has been reported in basic and clinical research of depression affecting redox modulation, neurogenesis and, inflammatory and glutamatergic pathways (Dean et al., 2011; Deepmala et al., 2015).

Considering the high cost of depression to the society there is an urgency to better understand its pathophysiological basis, as well as search for new, cheaper and effective therapies for the management of this condition (Ghasemi et al., 2014). In this regard, natural compounds emerge as innovative therapies offering different perspectives and modes of action deserving special attention to expand options of treatments to depressed patients. Lutein (LUT), a dietary xanthophyll (oxygenated carotenoid) is commonly found in the dark green leaves, for instance, *Spinacia oleracea* L. (spinach), *Brassica oleracea* var. *acephala* DC. (kale) and *Brassica oleracea* var. *italica* Plenck (broccoli - Sommerburg et al., 1998). Moreover, LUT is currently used for therapy of macular degeneration since it is an ocular health enhancer with the ability to cross the blood-retina barrier (Bone et al., 1988; Bone and Landrum, 2010).

Several reports have demonstrated beneficial activities of LUT in different disease models, including neuroprotection against retinal ischemic injury (Choi et al., 2006; Li et al., 2009; Sasaki et al., 2012) and anti-diabetic property (Muriach et al., 2006; Arnal et al., 2010) due to its mechanisms of action involving anti-inflammatory, antioxidant and anti-apoptotic activities. Furthermore, it has been shown that LUT was capable to reduce neuronal injury against stroke (Li et al., 2012) and NMDA-induced retinal neural damage (Zhang et al., 2016). Nonetheless, some authors have observed that LUT protected neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 3-nitropropionic acid (3-NP) inducing damage in Parkinson's and Huntington's disease models, respectively (Binawade and Jagtap, 2013; Nataraj et al., 2016), and also increases mice's declarative memory (Silva et al., 2017).

Importantly, LUT nutritional supplement is recommended (Sasaki et al., 2012) since it has been used aiming at to increase cognitive ability in older adults (Johnson et al., 2008) and brain-derived neurotrophic factor (BDNF) level in youngsters (Stringham et al., 2016). This xanthophyll also decreases reactive oxygen species (ROS) in newborns and non-smokers (Perrone et al., 2009, 2014; Wang et al., 2013). Furthermore, Wang et al. (2008) and Milaneschi et al. (2012) observed low amounts of LUT in the plasma from patients with Alzheimer's disease associated with depressive symptoms and from older people a prediction of development of a new depressive frame.

Recently, Badgujar and Saraf (2015) demonstrated that LUT shows the antinociceptive-antidepressant-like effect by modulating monoamine levels. Furthermore, Jiang et al. (2017) and Sharma et al. (2017) showed that astaxanthin and lutein-zeaxanthin association treatments revealed an antidepressant-like effect with the involvement of the serotonergic system. Considering the aforementioned information, this study aimed to determine the antidepressant-like effect of LUT not only in acute and subchronic treatments but also in a chronic corticosterone model of depression in mice. Additionally, the participation of antioxidant and neuroprotective activities of LUT was also investigated.

2. Material and methods

2.1. Animals

Male Swiss mice (2–3 months old, 30–40 g) were maintained at 21–23 °C, with free access to water and food, under a 12: 12 h light/dark cycle (lights on at 07:00 h). The methods used in this study were developed in agreement with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The experiments were developed after the approval of the protocol by the Institutional Ethics Committee (CEUA/FURB). All efforts were done in order to minimize animal's suffering and to reduce their number to the minimum necessary to demonstrate solid effects in the experiments.

2.2. Drugs and treatments

This study was carried out in three different treatments. To develop acute and subchronic treatments, mice were divided into 5 groups: control (vehicle – sunflower oil), LUT 0.1, 1, and 10 mg/kg and, fluoxetine 10 mg/kg. The treatments were given orally (*p.o.*) either once or for 7 consecutive days at 10 mL/kg/day, before behavioral tests. The animals were submitted to the tail suspension test (TST) and open-field test (OFT) after 60 min (acute) and after 24 h (subchronic) of the last administration (Zeni et al., 2011; Dalmagro et al., 2017). Fluoxetine (FLU - 10 mg/kg) was purchased from Cadila Healthcare, India and dissolved in saline, whereas LUT (0.1, 1, and 10 mg/kg) purchased from Farma Nostra Distribuidora S.I., Barcelona, and dissolved in sunflower oil. Corticosterone (CORT - 20 mg/kg) was obtained from Sigma Chemical Co., St. Louis, USA and dissolved in distilled water 2% (v/v) of Tween 80 and 0.2% of DMSO. For the chronic treatment mice were divided into six groups: (1) vehicle + vehicle; (2) vehicle + LUT; (3) vehicle + FLU; (4) CORT + vehicle; (5) CORT + LUT, and (6) CORT + FLU. The CORT administered orally (*p.o.*), once a day, for 21 consecutive days to induce depressive-like behavior (Zeni et al., 2017; Camargo et al., 2018) and LUT (10 mg/kg) or FLU (10 mg/kg) administered immediately after the CORT solution during the 21-day treatment. On the 22nd day, 24 h after the last treatment, animals were submitted to the TST, open-field and splash tests (ST). Mice were weighed once a week.

2.3. Behavioral tests

2.3.1. Tail suspension test (TST)

The total immobility time throughout TST was quantified according to Steru et al. (1985) with modifications. The animals, acoustically and visually isolated were suspended by the tail 50 cm above the floor by adhesive tape and immobility time recorded during 6 min (Zeni et al., 2011). Mice were only considered immobile when hung passively and completely motionless.

2.3.2. Open-field test (OFT)

The behavior variables were evaluated as previously described by Lenzi et al. (2015), 10 min after the tail suspension test, using an arena which consists of a wooden box measuring 40 × 60 cm × 50 cm height with the floor divided into 12 equal squares. At the start of each trial, mice were placed in the left corner of the field and allowed to freely explore the arena. Three observational steps were considered: the number of crossings (squares crossed with all paws) as indicative of motor activity, the number of rearings (the animal standing upright on its back legs) as exploratory and the number of fecal boluses as emotionality conditions, registered for 6 min. The arena floor was cleaned between the experiments.

2.3.3. Splash test (ST)

The ST consists of squirting a 10% sucrose solution (w/v) on the dorsal coat of mice placed in clear boxes (9 × 7 × 11 cm) according to Camargo et al. (2018), after the open-field test. Due to its viscosity, the sucrose solution dirties the mice which then initiate a grooming behavior. After applying the sucrose, the time of the start of the first grooming and the total time of grooming were recorded during 5 min, as an index of self-care and motivational behavior, considering any apathetic behavior as symptoms of depression (Willner, 2005). The apparatus was cleaned before each test with a 10% alcoholic solution to remove cues of the previous animal.

2.4. Biochemical analysis

Immediately after behavioral tests, the mice were decapitated and

the plasma, hippocampus and prefrontal cortex dissected. The homogenates were prepared in 20 mM sodium phosphate buffer with 140 mM KCl, pH 7.4, centrifuged at 3000*g* at 4 °C for 15 min to remove cell debris. The supernatant was recovered, saved in aliquots, and stored at −20 °C for the experiments (Lenzi et al., 2015).

2.4.1. Lipid peroxidation assay

Thiobarbituric acid-reactive substances (TBARS) assay was performed according to Ohkawa et al. (1979) measuring malondialdehyde (MDA), a product of lipoperoxidation caused mainly by hydroxyl free radicals, at a wavelength of 535 nm. A calibration curve was built using 1, 1, 3, 3-tetramethoxypropane and TBARS contents were calculated as nmol malondialdehyde per milligram of protein.

2.4.2. Nitrite determination

The production of the nitrites was measured based on Griess (1879). The homogenate was centrifuged at 1800*g* during 10 min, following incubation of the supernatant (50 µl) with 100 µl of Griess reagent at room temperature, for 10 min. The absorbance was measured at 525 nm through a microplate reader and a standard curve prepared with different concentrations of $NaNO_2$ (0–100 µM) was obtained for the nitrite concentration calculations. The results were expressed as nmol of nitrite per mg of protein.

2.4.3. Protein carbonyl (PC) assay

The PC assay was developed according to Reznick and Packer (1994) with modifications. The pellet of the homogenated sample was solubilized in 10 mM 2, 4-dinitro-phenylhydrazine (DNPH) in 2 M HCl and vortexed every 15 min, for 60 min. After protein precipitation, the pellet washed with 1 mL ethanol: ethyl acetate (1:1 v/v) solution dissolved in 6 M guanidine hydrochloride and centrifuged at 14000 rpm for 3 min. The samples' absorbances were read against a blank at 370 nm with 2 M HCl instead of DNPH reagent and the PC quantity was expressed in nmol per mg of protein.

2.4.4. Non-protein thiol groups (NPSH) determination

NPSH level assesses the measure of endogenous defenses against oxidative stress. The method was based on Ellman's reagent (DTNB) reaction with free thiol groups. Briefly, the samples mixed with 0.4 M Tris–HCl buffer, pH 8.9 and 0.01 M DTNB were read at 405 nm and non-protein thiol groups expressed as nmol NPSH per mg of protein (Ellman, 1959).

2.4.5. Protein determination

Protein was quantified applying serum bovine albumin as standard according to Lowry et al. (1951).

2.5. Ex vivo experiments

2.5.1. Treatment of mice with LUT and preparation of slices

LUT (10 mg/kg) and fluoxetine (10 mg/kg) were administered by gavage for 7 days and after 24 h mice were killed by decapitation. The prefrontal cortex and hippocampus were rapidly removed and placed into ice-cold Krebs–Ringer bicarbonate (KRB) buffer (122 mM NaCl, 3 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 0.4 mM KH_2PO_4, 25 mM $NaHCO_3$, and 10 mM D-glucose). The buffer was bubbled with 95% O_2–5% CO_2 up to pH 7.4. Slices (0.4 mm) were quickly prepared in a McIlwain Tissue Chopper, separated in KRB at 4 °C and allowed to recover during 30 min at 37 °C. Hippocampal and prefrontal cortex slices were incubated with glutamate (10 mM or 100 mM, respectively - Sigma Chemical Company, USA) for 1 h in KRB. Subsequently, the medium was withdrawn and replaced by a nutritive medium composed of 50% KRB, 50% Dulbecco's modified Eagle's medium (DMEM, Sigma Chemical Company, USA), 20 mM HEPES, and 100 µg/mL gentamicin, at 37 °C, in a modified CO_2 atmosphere for additional 3 h (Zeni et al., 2011; Dalmagro et al., 2017).

2.5.2. Evaluation of cell viability

The cell viability of hippocampus and prefrontal cortex slices was evaluated by measuring the cells' ability to reduce the reagent 3-(4, 5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT), Sigma Chemical Company, USA (Mosmann, 1983). The slices were incubated with MTT (0.5 mg/mL) in KRB for 30 min, at 37 °C. In this assay, the tetrazolium ring of MTT is cleaved by active dehydrogenases producing a precipitated and colored compound, *i.e.*, formazan, which was further solubilized by adding 200 µL DMSO. The formazan content was measured in a microplate reader at 540 nm.

2.6. Statistical analyses

The results were expressed as means ± S.E.M or S.D.; comparisons between treatments and control groups were performed by one-way or two-way analysis of variance (ANOVA) followed by Tukey's HSD test, when appropriate. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Effects of the acute and subchronic treatments with LUT or fluoxetine in the TST and open-field tests in mice

Acute oral treatment with LUT (0.1 mg/kg and 1 mg/kg) failed to decrease the immobility time in the TST, although LUT at 10 mg/kg significantly decreased it (Fig. 1A, $p < 0.01$) in comparison with the control group, indicating a possible antidepressant-like effect. Remarkably, the same effect was observed in the subchronic treatment by LUT (Fig. 1B) at 10 mg/kg that significantly decreased the immobility time in the TST ($p < 0.001$), as well as fluoxetine ($p < 0.05$), reinforcing an antidepressant-like effect. Noteworthy, as depicted in Table 1 and Suppl. Table 1, LUT or fluoxetine, acute or subchronically administered, did not significantly changed the number of crossings, rearings, and fecal boluses in the open-field test comparing with control ($p > 0.05$). These results indicate that the antidepressant-like effect detected was neither affected by the locomotor or exploratory nor emotionality activities.

3.2. Chronic effect of LUT or fluoxetine on the depressive-like behavior induced by CORT

As illustrated in Fig. 2A, B, and C, chronic administration with vehicle + LUT 10, and vehicle + FLU 10 significantly decreased immobility time in the TST, when compared to vehicle-treated control ($p < 0.01$ and $p < 0.001$, respectively). Interestingly, only the treatment vehicle + LUT 10 was significantly able to diminish the latency to grooming ($p < 0.05$) when compared to the control. Moreover, it was noted that CORT (20 mg/kg) chronically administered caused a significant increase in the immobility time in the TST ($p < 0.01$), grooming latency ($p < 0.001$), and decrease the total time of grooming ($p < 0.01$) compared to the control. Two-way ANOVA analysis indicated that CORT + LUT 10 and CORT + FLU 10 treatments were capable of decreasing the immobility time ($p < 0.001$ and $p < 0.05$, respectively) and grooming latency (splash test, $p < 0.001$) compared to the CORT-treated group. Although, just CORT + LUT 10 was able to raise the time spent in grooming ($p < 0.001$). As depicted in Table 1, LUT or fluoxetine treatments did not affect the locomotor, exploratory activities or emotional behavior in the open-field test ($p > 0.05$). Additionally, a complementary description of statistical data is depicted in a Suppl. Table 1. No alterations on mice's body weight were detected during the treatment (data not shown) indicating that the results with CORT cannot be attributed to the weight changes.

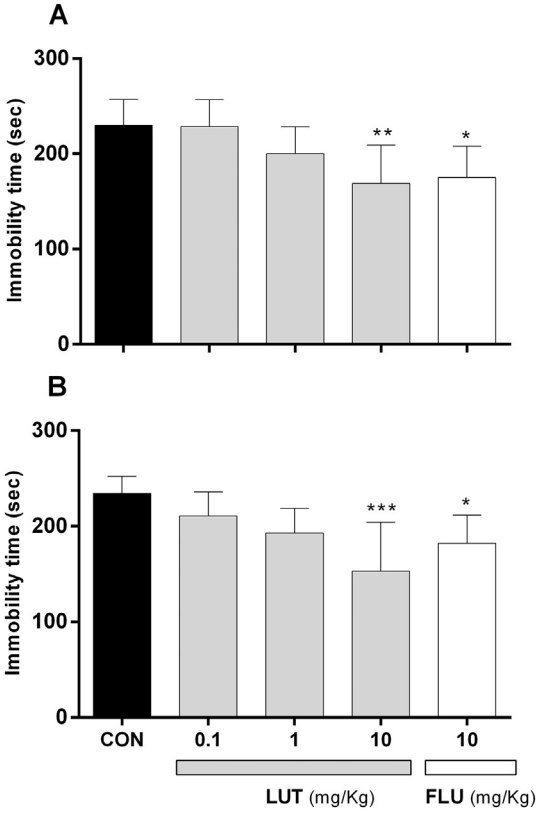

Fig. 1. Effect of the acute (**A**, 60 min after treatment) and subchronic (**B**, 7 days) administration of lutein (LUT 0.1–10 mg/kg, p.o.) or fluoxetine (FLU 10 mg/kg, p.o.) in the TST. Values are expressed as mean ± SEM (n = 7–8). *p < 0.05; **p < 0.01 and ***p < 0.001 as compared with the vehicle-treated control group.

3.3. Subchronic and chronic effects of lutein LUT or fluoxetine administration on the oxidative/nitrosative stress markers in tissues of mice chronically treated or not with CORT

The subchronic treatment with LUT significantly decreased nitrite ($p < 0.05$ and $p < 0.01$) and PC ($p < 0.001$, $p < 0.01$) at doses of 0.1, 1, and 10 mg/kg, respectively. Also, fluoxetine administration reduced the nitrite level ($p < 0.01$) in the mice's hippocampus in comparison with the control (Fig. 3). Moreover, the subchronic treatment with LUT at 10 mg/kg, significantly decreased MDA ($p < 0.05$) and increased NPSH levels ($p < 0.01$) in the prefrontal cortex when compared to control group (Fig. 4). In addition, all doses of LUT in the subchronic treatment diminished nitrite levels ($p < 0.05$) in the prefrontal cortex. However, the subchronic treatment with LUT or fluoxetine did not exhibit any effect on the antioxidant markers in the mice's plasma (Table 2).

The results presented in Fig. 5 show that CORT administration significantly augmented MDA ($p < 0.001$) and PC ($p < 0.001$) contents in the hippocampus, whereas, decreased NPSH level ($p < 0.01$) comparatively to vehicle-treated control. Furthermore, LUT 10 and fluoxetine significantly decreased protein carbonylation ($p < 0.001$) and raised NPSH ($p < 0.001$ and $p < 0.05$, respectively) amounts in respect to the CORT-treated control. Two-way ANOVA analysis revealed that LUT 10 chronically administrated reduced MDA level ($p < 0.001$) when compared to CORT-treated control, despite the fact that chronic administration with fluoxetine increased MDA level in the hippocampus of mice treated or not with CORT ($p < 0.01$ and $p < 0.05$, respectively). Likewise, CORT administration significantly augmented MDA ($p < 0.001$), nitrites ($p < 0.05$) and PC ($p < 0.001$) contents, while decreasing NPSH level ($p < 0.001$) compared to

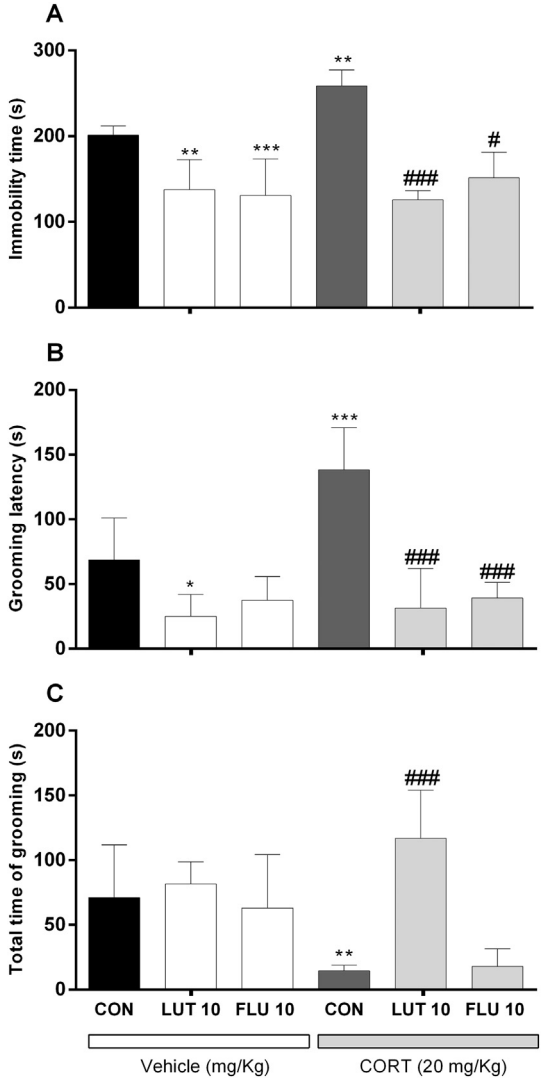

Fig. 2. Effect of lutein (LUT 10 mg/kg, p.o.) or fluoxetine (FLU 10 mg/kg, p.o.) chronic treatment (21 days) in the TST (**A**) and splash-test (**B** and **C**) in mice treated or not with corticosterone (CORT – 20 mg/kg, p.o.). Values are expressed as mean ± SEM (n = 7–8). *p < 0.05; **p < 0.01 and ***p < 0,001 as compared with the vehicle-treated control group; #p < 0.05 and ###p < 0.001 as compared with the CORT-treated control group.

vehicle-treated control in the prefrontal cortex (Fig. 6). However, LUT 10 and fluoxetine significantly diminished nitrites and PC ($p < 0.001$), just as abolished the reduction of NPSH level induced by CORT ($p < 0.001$ and $p < 0.05$, respectively) in comparison to CORT-treated control. Interestingly, LUT chronically administered reduced MDA level ($p < 0.01$), an effect did not promote by fluoxetine. Furthermore, a significant increase in the MDA ($p < 0.01$), nitrite, and PC ($p < 0.001$) amounts caused by CORT administration were detected in the mice's plasma (Table 2). Moreover, an effect prevented by the chronic administration of LUT or fluoxetine ($p < 0.05$). Although, an increase in the nitrite concentration after the fluoxetine administration in the mice's plasma ($p < 0.05$) was found. A complementary description of statistical data is demonstrated in Suppl. Table 1.

3.4. Effect of LUT or fluoxetine pretreatment against glutamate-induced toxicity in the mice's hippocampal and prefrontal cortex slices

As shown in Fig. 7 and Suppl. Table 1, mice's hippocampal (A) and prefrontal cortex (B) slices incubated with glutamate (10 or 100 mM, respectively) for 1 h showed a significant diminished cell viability when

Table 1
Acute, subchronic or chronic effect of lutein (LUT) or fluoxetine (FLU) administration in the open-field test in mice treated or not with corticosterone (CORT).

Group/dose mg/kg/day	Treatment	Parameters		
		Crossing	Rearing	Fecal bolus
CON	Acute	64.5 ± 21.92	4.2 ± 0.94	1.2 ± 0.62
	Subchronic	94.2 ± 23.03	56.2 ± 22.72	0.7 ± 0.75
	Chronic	54.2 ± 11.84	10.2 ± 2.87	2.5 ± 0.50
LUT 0.1	Acute	60.2 ± 10.81	11.5 ± 3.96	1.2 ± 0.94
	Subchronic	90.7 ± 19.57	25.7 ± 6.72	2.2 ± 0.85
LUT 1	Acute	66.5 ± 18.78	6.5 ± 2.39	1.0 ± 0.70
	Subchronic	85.0 ± 22.20	32.5 ± 10.14	1.0 ± 0.57
LUT 10	Acute	83.0 ± 19.44	14.5 ± 2.06	2.5 ± 1.05
	Subchronic	99.0 ± 22.14	25.2 ± 8.29	1.7 ± 0.62
	Chronic	80.2 ± 6.30	23.7 ± 2.40	3.0 ± 0.92
FLU 10	Acute	90.0 ± 12.26	15.7 ± 6.56	0.7 ± 0.48
	Subchronic	99.7 ± 8.59	19.5 ± 5.21	1.7 ± 0.75
	Chronic	55.2 ± 1.34	7.7 ± 4.29	2.5 ± 1.04
CORT	Chronic	42.2 ± 9.42	9.7 ± 2.75	2.0 ± 1.41
CORT + LUT 10	Chronic	74.7 ± 7.20	12.0 ± 5.60	3.2 ± 1.25
CORT + FLU 10	Chronic	47.5 ± 5.72	9.5 ± 4.18	2.0 ± 0.58

Mean ± SEM (n = 7–8), $p > 0.05$ significant difference as compared to vehicle-treated or CORT-treated control.

compared to the control ($p < 0.01$). The glutamate-induced damage was not observed in the hippocampal slices from mice previously treated during seven days with LUT or fluoxetine ($p < 0.05$). Likewise, none cell viability reduction ($p > 0.05$) was detected in the mice's hippocampal and prefrontal cortex slices previously treated with LUT or fluoxetine and both treatments were able to prevent the slices from glutamatergic excitotoxicity ($p < 0.05$). Interestingly, fluoxetine was able to augment the cell viability in the prefrontal cortex compared to control group ($p < 0.05$).

4. Discussion

In this study, we have provided evidence that the acute, subchronic and chronic administration of LUT, a well-known antioxidant, has an antidepressant-like effect without changes in the motor performance in mice. Furthermore, we also noted that treatment with LUT modulates the antioxidant system, reducing oxidative stress in the mice's hippocampus, prefrontal cortex, and plasma, as well as protecting hippocampus and prefrontal cortex against glutamatergic excitotoxicity.

LUT is a dietary xanthophyll that has been recommended for intake (Sasaki et al., 2012) since it is widely known by its biological properties and low toxicity (Sommerburg et al., 1998; Gao et al., 2011). This carotenoid is predominantly present in the macular region and acts as an efficient pigment for absorbing high-energy blue light. In fact, the noticeable role of LUT in the nervous system has been demonstrated (Li et al., 2012; Woo et al., 2013; Sun et al., 2014; Silva et al., 2017) and its ability to cross blood-retina barrier accumulating in the macula lutea, the central region responsible for sharp central vision (Bone et al., 1988). Recently, a study showed that LUT is the major carotenoid present in the primate's brain regions, such as occipital cortex, prefrontal cortex and the cerebellum which had almost 10–20 times more than its isomer zeaxanthin (Vishwanathan et al., 2013). These data sustain the role that this fat-soluble molecule crosses the blood-brain barrier by straight passing through the lipids in the brain's capillary wall (Nataraj et al., 2016).

Noteworthy, LUT has been associated with memory function and depression in the young human population (Johnson, 2014). In this study, LUT acute or subchronically administered decreased the immobility time in the TST at 10 mg/kg. As expected, fluoxetine (10 mg/kg), a conventional antidepressant drug, also demonstrated antidepressant-like property. Recently, Badgujar and Saraf (2015) showed that LUT chronically administered at 40 mg/kg in rats presented an antinociceptive-antidepressant-like effect, modulating reactive oxygen species (ROS) and monoamine levels in the brain. In addition, Sharma et al. (2017) showed evidence that acute and chronic treatment with lutein-zeaxanthin association (5, 10, and 20 mg/kg) produced an antidepressant-like effect in the TST with the involvement of serotonergic, noradrenergic and opioid systems when administered to female mice. Noteworthy, to the best of our knowledge, this is the first reporting LUT effect in a mice depression model since LUT exhibited antidepressant-like effect reinforced in a chronic model of depression induced by CORT that mimics depression symptoms in humans.

The CORT chronic model has been widely performed in rodents resulting in marked depressive-like behavior and neurological

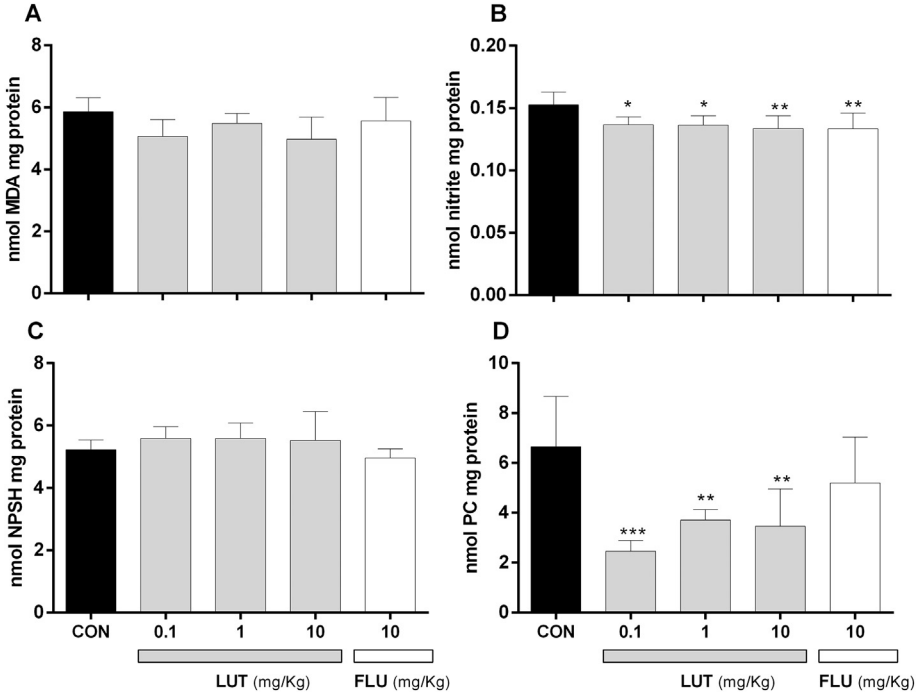

Fig. 3. Effect of lutein (LUT 0.1–10 mg/kg, p.o.) or fluoxetine (FLU 10 mg/kg, p.o.) subchronic treatment (7 days) on the oxidative stress (MDA – **A**, nitrite – **B**, NPSH – **C** and PC – **D**) in the mice's hippocampus. Values are expressed as mean ± SD (n = 7–8). *p < 0.05; **p < 0.01 and ***p < 0.001 as compared with the vehicle-treated control.

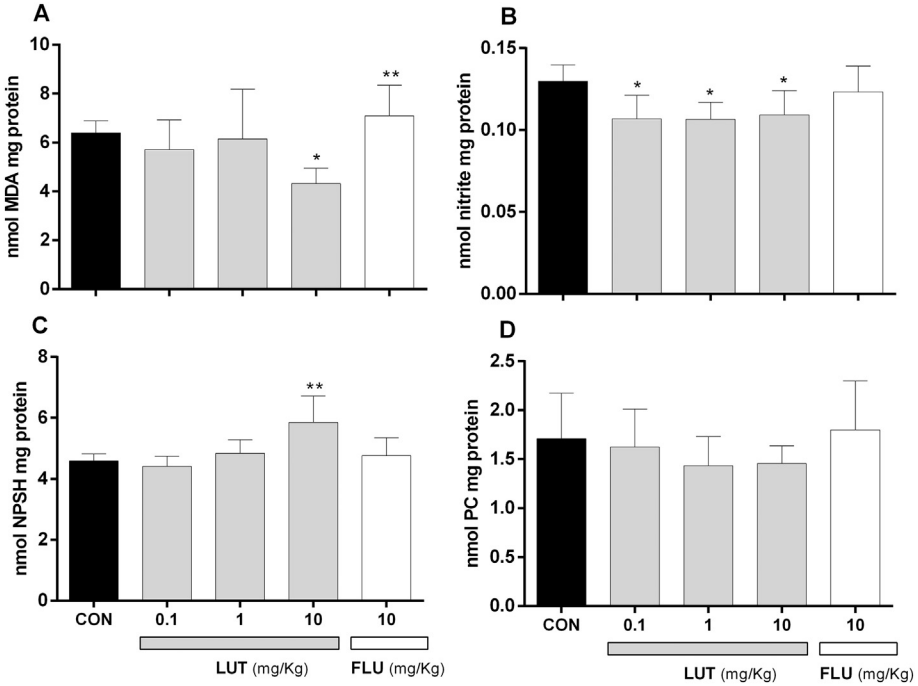

Fig. 4. Effect of lutein (LUT 0.1–10 mg/kg, p.o.) or fluoxetine (FLU 10 mg/kg, p.o.) subchronic treatment **(7 days)** on the oxidative stress (MDA – **A**, nitrite – **B**, NPSH – **C** and PC – **D**) in the mice's prefrontal cortex. Values are expressed as mean ± SD (n = 7–8). *p < 0.05 and **p < 0.01 as compared to the vehicle-treated control.

alterations (Ali et al., 2015; Demuyser et al., 2016; Freitas et al., 2016; Pazini et al., 2016a, 2016b; Weng et al., 2016; Yan et al., 2016; Camargo et al., 2018). Throughout this paper, the results demonstrated that CORT administration for 21 days was effective in inducing depressive-like behavior in mice, since it increased immobility time, grooming latency and decreased total time of grooming, findings in line with recent studies (Freitas et al., 2016; Zeni et al., 2017; Camargo et al., 2018). The augmented glucocorticoid contents have been implicated in the pathophysiology of stress-related disorders, such as depression, due to a feedback mechanism of HPA axis dysfunction (Nguyen et al., 2017).

Indeed, it has been shown that CORT treatment reduced BDNF amounts and decreased maturation and neuronal plasticity (Arnal et al., 2010; Pazini et al., 2016a; Weng et al., 2016). Furthermore, CORT reduced cell proliferation and neuronal differentiation, as well as induced alteration on astrocytes and microglia morphologies in the hippocampus region (Freitas et al., 2016; Pazini et al., 2016b). These events may lead to a dysregulation of HPA axis causing neurobehavioral alterations since under normal conditions the hippocampus controls the negative feedback (Demuyser et al., 2016). Conversely, LUT and fluoxetine both at 10 mg/kg were able to reverse the increase of immobility time and grooming latency in CORT-treated animals, previously demonstrated with fluoxetine (Zeni et al., 2017). However, only lutein treatment was effective to prevent the reduction in the time spent in grooming. On the other hand, Pazini et al. (2016b) demonstrated the ability of fluoxetine to prevent the reduction in sucrose consumption, other behavioral paradigm used to assess anhedonic-like behavior.

Recently, a number of studies have pointed out that high level of glucocorticoids present in the physiological stress induces reactive oxygen and nitrogen species overproduction leading to oxidative damage (Zafir et al., 2009; Spiers et al., 2015; Stanić et al., 2016). Possibly due to an exacerbated stimulation of mitochondrial respiration (Spiers et al., 2015) and glutamate release (Freitas et al., 2016) which have been implicated in the depression pathophysiology (Maes et al., 2011; Maurya et al., 2016). In particular, some studies have been demonstrated the increase of MDA (Palta et al., 2014), nitrites (Savas et al.,

Table 2
Subchronic or chronic effect of lutein (LUT) or fluoxetine (FLU) treatment on the oxidative stress in the plasma of mice treated or not with corticosterone (CORT).

Group/dose mg/kg	Treatment	Parameters (nmol mg/protein)			
		MDA	Nitrite	PC	NPSH
CON	Subchronic	5.87 ± 5.83	0.057 ± 0.01	3.83 ± 0.92	2.16 ± 0.56
	Chronic	7.74 ± 4.03	0.055 ± 0.02	4.08 ± 0.75	4.34 ± 0.89
LUT 0.1	Subchronic	5.99 ± 4.15	0.054 ± 0.04	4.57 ± 0.61	1.59 ± 0.26
LUT 1	Subchronic	5.04 ± 3.12	0.049 ± 0.05	3.56 ± 0.83	2.24 ± 0.81
LUT 10	Subchronic	9.77 ± 5.13	0.049 ± 0.06	3.65 ± 0.76	2.01 ± 0.52
	Chronic	4.80 ± 2.19	0.061 ± 0.05	2.88 ± 0.73	3.96 ± 0.55
FLU 10	Subchronic	10.15 ± 6.65	0.049 ± 0.04	3.85 ± 0.85	2.10 ± 0.57
	Chronic	3.49 ± 2.55	0.064 ± 0.06*	4.37 ± 1.38	5.37 ± 2.27
CORT	Chronic	14.03 ± 3.61**	0.074 ± 0.05***	11.98 ± 4.87***	3.75 ± 1.10
CORT + LUT 10	Chronic	2.25 ± 0.44###	0.060 ± 0.06###	2.65 ± 1.17###	5.95 ± 1.89
CORT + FLU 10	Chronic	2.79 ± 0.28###	0.065 ± 0.03#*	7.42 ± 1.78##	5.51 ± 0.89

Mean ± SD (n = 7–8), **p < 0.01; ***p < 0.001 significant difference as compared to vehicle-treated and #p < 0.05; #p < 0.01; ###p < 0.001 significant difference as compared CORT-treated control.

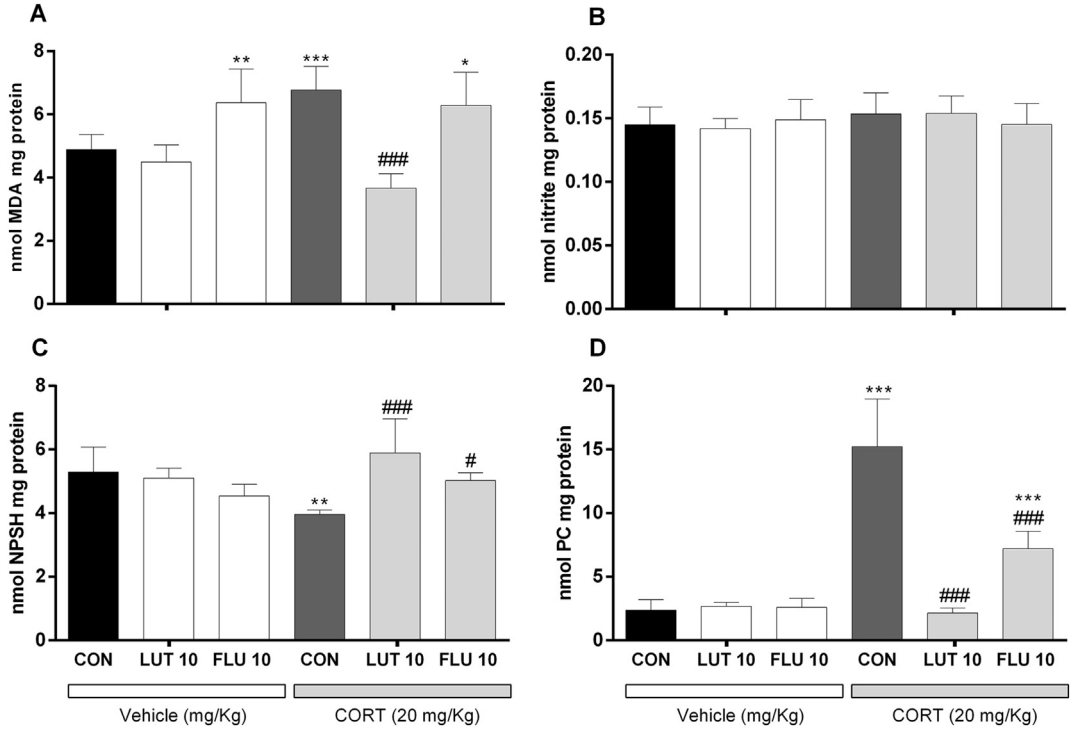

Fig. 5. Effect of lutein (LUT 10 mg/kg, p.o.) or fluoxetine (FLU 10 mg/kg, p.o.) chronic treatment (21 days) on the oxidative stress (MDA – **A**, nitrite – **B**, NPSH – **C** and PC – **D**) in mice's hippocampus treated or not with corticosterone (CORT – 20 mg/kg, p.o.). Values are expressed as mean ± SD (n = 7–8). *p < 0.05; **p < 0.01 and ***p < 0.001 as compared with the vehicle-treated control; #p < 0.05 and ###p < 0.001 as compared with the CORT-treated control.

2006; Klinedinst and Regenold, 2015) and PC (Gibson et al., 2012) levels, as well as a decrease of NPSH content (Klinedinst and Regenold, 2015; Maurya et al., 2016) in depressive patients. In addition, damage to the DNA, lipids, and proteins (Niki, 2012; Pandya et al., 2013) leads to neurodegeneration implicated in depressive-like behavior (Maes et al., 2011). Finally, according to evidence that antidepressants may reverse oxidative stress (Ng et al., 2008), the present study concurs, at least in part, that LUT could exert the antidepressant-like effect by the

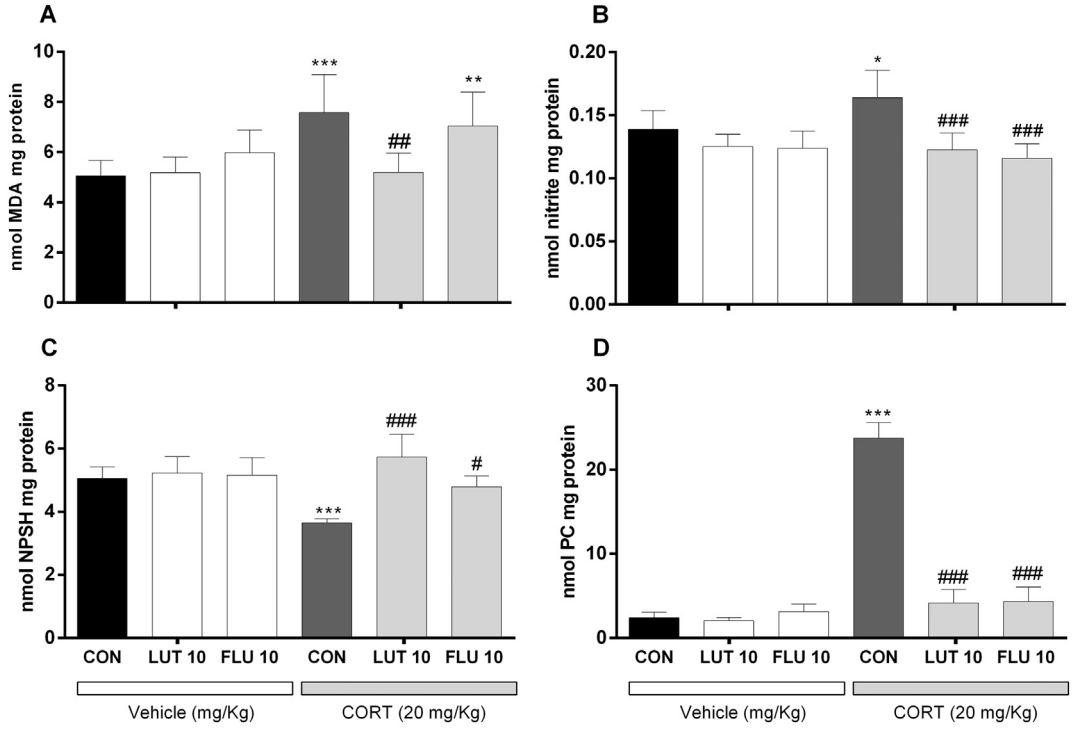

Fig. 6. Effect of lutein (LUT 10 mg/kg, p.o.) or fluoxetine (FLU 10 mg/kg, p.o.) chronic treatment (21 days) on the oxidative stress (MDA – **A**, nitrite – **B**, NPSH – **C** and PC – **D**) in mice's prefrontal cortex treated or not with corticosterone (CORT – 20 mg/kg, p.o.). Values are expressed as mean ± SD (n = 7–8). *p < 0.05; **p < 0.01 and ***p < 0.001 as compared with the vehicle-treated control; #p < 0.05; ##p < 0.01 and ###p < 0.001 as compared with the CORT-treated control.

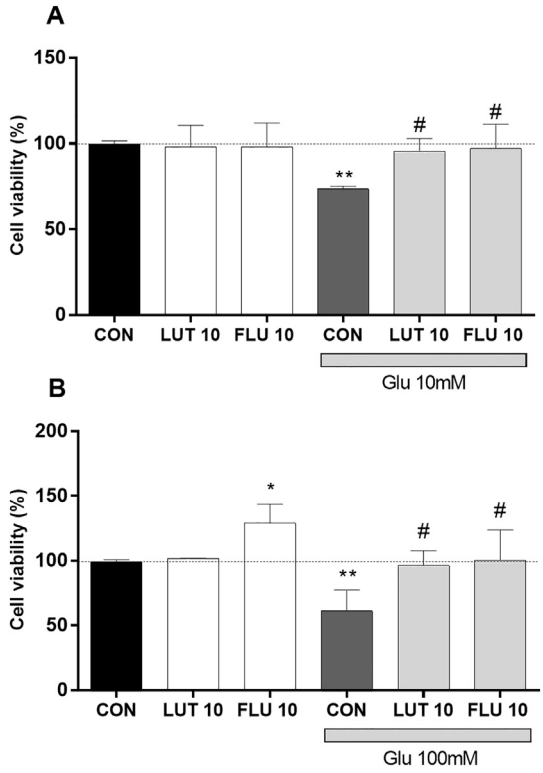

Fig. 7. *Ex vivo* cell viability analysis in hippocampal (**A**) and prefrontal cortex (**B**) slices incubated with glutamate (GLU 10 and 100 mM, respectively) for 1 h. When present, lutein (LUT 0.1–10 mg/kg, p.o.) or fluoxetine (FLU 10 mg/kg, p.o.) was pre-administered in mice by gavage (7 days). After this period, incubation media was withdrawn and replaced by fresh culture medium without GLU and maintained for additional 3 h. The control group (first black bar) was considered as 100% and represents cell viability of slices incubated only in the culture medium. The values represent mean ± SD of at least 5 experiments carried out in triplicates. *p < 0.05 and **p < 0.01 significantly different from control group (100%); #p < 0.05 and ## p < 0.01 significantly different from GLU group.

modulation of the nitro-oxidative stress response. In this study, acute or subchronic treatment with LUT was able to decrease nitrite and protein carbonyl (PC) levels in the hippocampus, also lessening MDA and nitrites, as well as elevating non-protein thiols in the prefrontal cortex of mice. Furthermore, CORT administration augmented the MDA, PC, and nitrite levels, while reduced NPSH in mice's hippocampus, prefrontal cortex, and plasma. Previous reports demonstrated CORT ability to increase these markers in mice's brain (Zafir and Banu, 2009; Gupta et al., 2015; Zeni et al., 2017). In this study, both LUT and fluoxetine were effective to reverse the elevation of nitrite, NPSH, and PC amounts in the hippocampus, prefrontal cortex, and plasma of mice.

In this study, LUT subchronic treatment did not counteract TBARS elevation in the hippocampus but decreased PC levels, while in the prefrontal cortex it decreased TBARS and did not PC level. In this sense, an experiment showed that repeated administration of ascorbic acid prevented depressive-like behavior accompanied by decreased TBARS levels only in the hippocampus and treatment with fluoxetine restored the lipid peroxidation in stressed mice only in the cerebral cortex (Moretti et al., 2012), thereby these effects demonstrated a region-specific nature of the redox balance. Fluoxetine counteracted the oxidative stress induced by corticosterone administration probably inhibiting glutamate release in the hippocampus and cerebral cortex (Freitas et al., 2016; Bonanno et al., 2005). Moreover, according to Weckmann et al. (2017), the reduction of PC level can be occurred by the capture of ROS, which includes antioxidant molecules and enzymes. Also, the increase in NPSH levels suggests that glutathione (GSH) concentration was able to change the effects of nitric oxide (NO) since GSH forms adducts with NO: S-nitrosoglutathione, a reservoir of NO (Seddon et al., 1994). LUT acts including trapping chain-carrying peroxyl radical, singlet oxygen and decelerating the chain propagation reaction retarding the cumulative effects even at high oxygen tension (Wang et al., 2013).

However, the involvement of NMDA receptor cannot rule out since it is oxidative stress sensible and LUT could exert antioxidant effect decreasing the excitatory events including oxidative stress induced by NMDA (Zhang et al., 2016). Therefore, these findings are not a surprise, since LUT has a long carbon chain with alternating single and double carbon-carbon bonds and a hydroxyl group attached to each extremity of the molecule, which confers certain hydrophilic property. The lipophilic polyene chain inside the lipid bilayer with its polar hydrophilic hydroxyls close to the hydrophilic head groups of the phospholipids, optimizes contact with the cell membrane lipids and enhances the its ability to react with ROS than other carotenoids (Ribaya-Mercado and Blumberg, 2004; Kijlstra et al., 2012; Wang et al., 2013). Notwithstanding, it is well known the remarkable antioxidant role of LUT evidenced by reducing lipid peroxidation, nitrite, and PC production, and also raising glutathione level in the brain of mice subjected to transient cerebral ischemia (Binawade and Jagtap, 2013; Sun et al., 2014; Nataraj et al., 2016). Further, Muriach et al. (2006) pointed out that LUT was able to restore MDA and glutathione levels in the hippocampus of diabetic mice.

Subsequently, LUT's ability to protect hippocampal and prefrontal cortex slices against glutamatergic toxicity was tested, since the glucocorticoids excess, particularly corticosterone, simultaneously also heighten extracellular glutamate concentrations, leading to glutamatergic excitotoxicity and neural damage (Lau and Tymianski, 2010; Sałat et al., 2015; Freitas et al., 2016). Glutamate excess involvement in depression pathophysiology has been suggested in some studies (Sanacora et al., 2012; Freitas et al., 2016). Herein, it was found that fluoxetine in agreement with Dalmagro et al. (2017) and LUT were able to protect the slices against glutamate-induced excitotoxicity and cell death by apoptosis (Zeni et al., 2011), reinforcing the possible involvement of glutamate receptors. Furthermore, LUT demonstrated neuroprotective effects on NMDA-induced retinal ganglion cell injury in rats by protein kinase B (Akt) activation and inhibition of p38 mitogen-activated protein kinases (MAPK) and c-Jun protein phosphorylation, which resulted in increased of B-cell lymphoma 2 (Bcl-2) protein (Bcl-2) levels and reduced levels of Bcl-2-associated X protein (Bax), caspase-3, and cytochrome *c* (Zhang et al., 2016). This carotenoid also protected against MPTP and ischemic stroke by modulating oxidative and inflammatory pathways shown by Li et al. (2012) and Nataraj et al. (2016). According all aforementioned and since high amounts of glucocorticoids, glutamate and oxidative species play critical role in the depression physiopathology cascade (Maes et al., 2011; Popoli et al., 2011) is conceivable the antidepressant-like and neuroprotective effects exerted by LUT could have the implication by both, antioxidant and glutamatergic systems modulation.

5. Conclusion

Taken together, the evidence could suggest the participation of antioxidant and protective properties against cell death induced by glutamatergic excitotoxicity, in the antidepressant-like effect of LUT. Further research will be necessary to clarify in more details the exact mechanism by which lutein induces the antidepressant-like effect. Apart from the wide usage of this xanthophyll as an ocular health enhancer the evidence encourages investigations of LUT as a promising agent with clinical implications in the management of depression associated with stress.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2019.02.004.

Acknowledgments

This work was supported by grants from Universidade Regional de Blumenau (FURB), PIBIC-FURB and PIBIC-CNPq scholarships.

Conflict of interest

The authors have no conflict of interest to declare.

References

Ali, S.H., Madhana, R.M., Athira, K.V., Kasala, E.R., Bodduluru, L.N., Pitta, S., Mahareddy, J.R., Lahkar, M., 2015. Resveratrol ameliorates depressive-like behavior in repeated corticosterone-induced depression in mice. Steroids 101, 37–42. https://doi.org/10.1016/j.steroids.2015.05.010.

Arnal, E., Miranda, M., Barcia, J., Bosch-Morell, F., Romero, F.J., 2010. Lutein and docosahexaenoic acid prevent cortex lipid peroxidation in streptozotocin-induced diabetic rat cerebral cortex. Neuroscience 166, 271–278. https://doi.org/10.1016/j.neuroscience.2009.12.028.

Badgujar, L., Saraf, M., 2015. Behavioral, biochemical and molecular modulation in brain and spinal cord: a potential antinociceptive-antidepressant mechanism of xanthophylls. FASEB J. 29, LB502.

Binawade, Y., Jagtap, A., 2013. Neuroprotective effect of lutein against 3-nitropropionic acid-induced Huntington's disease-like symptoms: possible behavioral, biochemical, and cellular alterations. J. Med. Food 16, 934–943. https://doi.org/10.1089/jmf.2012.2698.

Bonanno, G., Giambelli, R., Raiteri, L., Tiraboschi, E., Zappettini, S., Musazzi, L., Raiteri, M., Racagni, G., Popoli, M., 2005. Chronic antidepressants reduce depolarization-evoked glutamate release and protein interactions favoring formation of SNARE complex in hippocampus. J. Neurosci. 25, 3270–3279. https://doi.org/10.1523/JNEUROSCI.5033-04.2005.

Bone, R.A., Landrum, J.T., 2010. Dose-dependent response of serum lutein and macular pigment optical density to supplementation with lutein esters. Arch. Biochem. Biophys. 504, 50–55. https://doi.org/10.1016/j.abb.2010.06.019.

Bone, R., Landrum, J., Fernandez, L., Tarsis, S.L., 1988. Analysis of the macular pigment by HPLC: retinal distribution and age study. Invest. Ophthalmol. Vis. Sci. 29, 843–849.

Camargo, A., Dalmagro, A.P., Rikel, L., Silva, E.B., Silva, K.A.B.S., Zeni, A.L.B., 2018. Cholecalciferol counteracts depressive-like behavior and oxidative stress induced by repeated corticosterone treatment in mice. Eur. J. Pharmacol. 833, 451–461. https://doi.org/10.1016/j.ejphar.2018.07.002.

Choi, J.S., Kim, D., Hong, Y.M., Mizuno, S., Joo, C.K., 2006. Inhibition of nNOS and COX-2 expression by lutein in acute retinal ischemia. Nutrition 22, 668–671. https://doi.org/10.1016/j.nut.2005.08.011.

Dalmagro, A.P., Camargo, A., Zeni, A.L.B., 2017. *Morus nigra* and its major phenolic, syringic acid, have antidepressant-like and neuroprotective effects in mice. Metab. Brain Dis. 28, 1–11. https://doi.org/10.1007/s11011-017-0089-y.

Dean, O., Giorlando, F., Berk, M., 2011. N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. J. Psychiatry Neurosci. 36, 78–86. https://doi.org/10.1503/jpn.100057.

Deepmala, S.J., Kumar, N., Delhey, L., Berk, M., Dean, O., Spielholz, C., Frye, R., 2015. Clinical trials of N-acetylcysteine in psychiatry and neurology: a systematic review. Neurosci. Biobehav. Rev. 55, 294–321. https://doi.org/10.1016/j.neubiorev.2015.04.015.

Demuyser, T., Bentea, E., Deneyer, L., Albertini, G., Massie, A., Smolders, I., 2016. Disruption of the HPA-axis through corticosterone-release pellets induces robust depressive-like behavior and reduced BDNF levels in mice. Neurosci. Lett. 626, 119–125. https://doi.org/10.1016/j.neulet.2016.05.026.

Ellman, G.L., 1959. Tissue sulfydryl groups. Arch. Biochem. Biophys. 82, 70–77.

Freitas, A.E., Egea, J., Buendía, I., Navarro, E., Rada, P., Cuadrado, A., Rodrigues, A.L.S., López, M.G., 2015. Agmatine induces Nrf2 and protects against corticosterone effects in hippocampal neuronal cell line. Mol. Neurobiol. 51, 1504–1519. https://doi.org/10.1007/s12035-014-8827-1.

Freitas, A.E., Egea, J., Buendia, I., Gómez-Rangel, V., Parada, E., Navarro, E., Casas, A.I., Wojnicz, A., Ortiz, J.A., Cuadrado, A., Ruiz-Nuño, A., Rodrigues, A.L.S., Lopez, M.G., 2016. Agmatine, by improving neuroplasticity markers and inducing Nrf2, prevents corticosterone-induced depressive-like behavior in mice. Mol. Neurobiol. 53, 3030–3045. https://doi.org/10.1007/s12035-015-9182-6.

Gao, S., Qin, T., Liu, Z., Caceres, M.A., Ronchi, C.F., Chen, C.-Y.O., Yeum, K.-J., Taylor, A., Blumberg, J.B., Liu, Y., Shang, F., 2011. Lutein and zeaxanthin supplementation reduces H_2O_2-induced oxidative damage in human lens epithelial cells. Mol. Vis. 17, 3180–3190.

Ghasemi, M., Phillips, C., Trillo, L., De Miguel, Z., Das, D., Salehi, A., 2014. The role of NMDA receptors in the pathophysiology and treatment of mood disorders. Neurosci. Biobehav. Rev. 47, 336–358. https://doi.org/10.1016/j.neubiorev.2014.08.017.

Gibson, S.A., Korade, Ž., Shelton, R.C., 2012. Oxidative stress and glutathione response in tissue cultures from persons with major depression. J. Psychiatr. Res. 46, 1326–1332. https://doi.org/10.1016/j.jpsychires.2012.06.008.

Griess, P., 1879. Bemerkungen zu der Abhandlung der HH. Weselsky und Benedikt Ueber einige Azoverbindungen. Ber. Dtsch. Chem. Ges. 12, 426–428. https://doi.org/10.1002/cber.187901201117.

Gupta, D., Radhakrishnan, M., Kurhe, Y., 2015. Effect of a novel 5-HT 3 receptor antagonist 4i, in corticosterone-induced depression-like behavior and oxidative stress in mice. Steroids 96, 95–102. https://doi.org/10.1016/j.steroids.2015.01.021.

Jiang, X., Zhu, K., Xu, Q., Wang, G., Zhang, J., Cao, R., Ye, J., Yu, X., 2017. The antidepressant-like effect of trans-astaxanthin involves the serotonergic system. Oncotarget 8, 25552–25563. https://doi.org/10.18632/oncotarget.16069.

Johnson, E.J., 2014. Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan. Nutr. Rev. 72, 605–612. https://doi.org/10.1111/nure.12133.

Johnson, E., McDonald, K., Caldarella, S., Chung, H.-Y., Troen, A., Snodderly, D., 2008. Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women. Nutr. Neurosci. 11, 75–83. https://doi.org/10.1179/147683008X301450.

Kijlstra, A., Tian, Y., Kelly, E.R., Berendschot, T.T.J.M., 2012. Lutein: more than just a filter for blue light. Prog. Retin. Eye Res. 31, 303–315. https://doi.org/10.1016/j.preteyeres.2012.03.002.

Klinedinst, N.J., Regenold, W.T., 2015. A mitochondrial bioenergetic basis of depression. J. Bioenerg. Biomembr. 47, 155–171. https://doi.org/10.1007/s10863-014-9584-6.

Lau, A., Tymianski, M., 2010. Glutamate receptors, neurotoxicity and neurodegeneration. Pflugers Arch. - Eur. J. Physiol. 460, 525–542. https://doi.org/10.1007/s00424-010-0809-1.

Lee, A.L., Ogle, W.O., Sapolsky, R.M., 2002. Stress and depression: possible links to neuron death in the hippocampus. Bipolar Disord. 4, 117–128. https://doi.org/10.1034/j.1399-5618.2002.01144.x.

Lenzi, J., Rodrigues, A.F., Rós, A. de S., de Castro, B.B., de Lima, D.D., Magro, D.D.D., Zeni, A.L.B., 2015. Ferulic acid chronic treatment exerts antidepressant-like effect: role of antioxidant defense system. Metab. Brain Dis. 30, 1453–1463. https://doi.org/10.1007/s11011-015-9725-6.

Li, S.-Y., Fu, Z.-J., Ma, H., Jang, W.-C., So, K.-F., Wong, D., Lo, A.C.Y., 2009. Effect of lutein on retinal neurons and oxidative stress in a model of acute retinal ischemia/reperfusion. Invest. Ophthalmol. Vis. Sci. 50, 836–843. https://doi.org/10.1167/iovs.08-2310.

Li, S.-Y., Yang, D., Fu, Z.J., Woo, T., Wong, D., Lo, A.C.Y., 2012. Lutein enhances survival and reduces neuronal damage in a mouse model of ischemic stroke. Neurobiol. Dis. 45, 624–632. https://doi.org/10.1016/j.nbd.2011.10.008.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, J.R., 1951. Protein measurement with the Folin phenol reagent. Anal. Biochem. 217, 220–230. https://doi.org/10.1016/0304-3894(92)87011-4.

Maes, M., Galecki, P., Chang, Y.S., Berk, M., 2011. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Prog. Neuro-Psychopharmacology Biol. Psychiatry 35, 676–692. https://doi.org/10.1016/j.pnpbp.2010.05.004.

Malinowski, A.S., Veselka, L., Atkinson, B.E., 2017. An investigation of vulnerability factors for depression. Pers. Individ. Differ. 107, 126–130. https://doi.org/10.1016/j.paid.2016.11.049.

Maurya, P.K., Noto, C., Rizzo, L.B., Rios, A.C., Nunes, S.O.V., Barbosa, D.S., Sethi, S., Zeni, M., Mansur, R.B., Maes, M., Brietzke, E., 2016. The role of oxidative and nitrosative stress in accelerated aging and major depressive disorder. Prog. Neuro-Psychopharmacology Biol. Psychiatry 65, 134–144. https://doi.org/10.1016/j.pnpbp.2015.08.016.

Milaneschi, Y., Bandinelli, S., Penninx, B.W., Corsi, A.M., Lauretani, F., Vazzana, R., Semba, R.D., Guralnik, J.M., Ferrucci, L., 2012. The relationship between plasma carotenoids and depressive symptoms in older persons. World J. Biol. Psychiatry 13, 588–598. https://doi.org/10.3109/15622975.2011.597876.

Moretti, M., Colla, A., De Oliveira Balen, G., Dos Santos, D.B., Budni, J., De Freitas, A.E., Farina, M., Severo Rodrigues, A.L., 2012. Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. J. Psychiatr. Res. 46, 331–340. https://doi.org/10.1016/j.jpsychires.2011.11.009.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63. https://doi.org/10.1016/0022-1759(83)90303-4.

Muriach, M., Bosch-Morell, F., Alexander, G., Blomhoff, R., Barcia, J., Arnal, E., Almansa, I., Romero, F.J., Miranda, M., 2006. Lutein effect on retina and hippocampus of diabetic mice. Free Radic. Biol. Med. 41, 979–984. https://doi.org/10.1016/j.freeradbiomed.2006.06.023.

Myers, B., McKlveen, J.M., Herman, J.P., 2014. Glucocorticoid actions on synapses, circuits, and behavior: implications for the energetics of stress. Front. Neuroendocrinol. 35, 180–196. https://doi.org/10.1016/j.yfrne.2013.12.003.

Nataraj, J., Manivasagam, T., Justin Thenmozhi, A., Essa, M.M., 2016. Lutein protects dopaminergic neurons against MPTP-induced apoptotic death and motor dysfunction by ameliorating mitochondrial disruption and oxidative stress. Nutr. Neurosci. 19, 237–246. https://doi.org/10.1179/1476830515Y.0000000010.

Nemeroff, C.B., 2007. The burden of severe depression: a review of diagnostic challenges and treatment alternatives. J. Psychiatr. Res. 41, 189–206. https://doi.org/10.1016/j.jpsychires.2006.05.008.

Ng, F., Berk, M., Dean, O., Bush, A.I., 2008. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int. J. Neuropsychopharmacol. 11, 851–876. https://doi.org/10.1017/s1461145707008401.

Nguyen, E.T., Streicher, J., Berman, S., Jody, L., Ghisays, V., Estrada, C.M., Aynara, C., Solomon, M.B., 2017. A mixed glucocorticoid/mineralocorticoid receptor modulator dampens endocrine and hippocampal stress responsivity in male rats. Physiol. Behav. https://doi.org/10.1016/j.physbeh.2017.01.020.

Niki, E., 2012. Do antioxidants impair signaling by reactive oxygen species and lipid oxidation products? FEBS Lett. 586, 3767–3770. https://doi.org/10.1016/j.febslet.2012.09.025.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Palta, P., Samuel, L.J., Miller, E.R., Szanton, S.L., 2014. Depression and oxidative stress: results from a meta-analysis of observational studies. Psychosom. Med. 76, 12–19. https://doi.org/10.1097/PSY.0000000000000009.

Pandya, C.D., Howell, K.R., Pillai, A., 2013. Antioxidants as potential therapeutics for neuropsychiatric disorders. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 46, 214–223. https://doi.org/10.1016/j.pnpbp.2012.10.017.

Pazini, F.L., Cunha, M.P., Rosa, J.M., Colla, A.R.S., Lieberknecht, V., Oliveira, A., Rodrigues, A.L.S., 2016a. Creatine, similar to Ketamine, counteracts depressive-like behavior induced by corticosterone via PI3K/Akt/mTOR pathway. Mol. Neurobiol. 53, 6818–6834. https://doi.org/10.1007/s12035-015-9580-9.

Pazini, F.L., Cunha, M.P., Azevedo, D., Rosa, J.M., Colla, A., de Oliveira, J., Ramos-Hryb, A.B., Brocardo, P.S., Gil-Mohapel, J., Rodrigues, A.L.S., 2016b. Creatine prevents corticosterone-induced reduction in hippocampal proliferation and differentiation: possible implication for its antidepressant effect. Mol. Neurobiol. 54, 6245–6260. https://doi.org/10.1007/s12035-016-0148-0.

Perrone, S., Longini, M., Marzocchi, B., Picardi, A., Bellieni, C.V., Proietti, F., Rodriguez, A., Turrisi, G., Buonocore, G., 2009. Effects of lutein on oxidative stress in the term newborn: a pilot study. Neonatology 97, 36–40. https://doi.org/10.1159/000227291.

Perrone, S., Tei, M., Longini, M., Santacroce, A., Turrisi, G., Proietti, F., Felici, C., Picardi, A., Bazzini, F., Vasarri, P., Buonocore, G., 2014. Lipid and protein oxidation in newborn infants after lutein administration. Oxidative Med. Cell. Longev. 2014, 1–7. https://doi.org/10.1155/2014/781454.

Popoli, M., Yan, Z., McEwen, B.S., Sanacora, G., 2011. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat. Rev. Neurosci. 13, 22–37. https://doi.org/10.1038/nrn3138.

Pytka, K., Dziubina, A., Młyniec, K., Dziedziczak, A., Zmudzka, E., Furgała, A., Olczyk, A., Sapa, J., Filipek, B., 2016a. The role of glutamatergic, GABA-ergic, and cholinergic receptors in depression and antidepressant-like effect. Pharmacol. Rep. 68, 443–450. https://doi.org/10.1016/j.pharep.2015.10.006.

Pytka, K., Podkowa, K., Rapacz, A., Podkowa, A., Zmudzka, E., Olczyk, A., Sapa, J., Filipek, B., 2016b. The role of serotonergic, adrenergic and dopaminergic receptors in antidepressant-like effect. Pharmacol. Rep. 68, 263–274. https://doi.org/10.1016/j.pharep.2015.08.007.

Reznick, A.Z., Packer, L., 1994. Oxygen radicals in biological systems part C. Methods Enzymol. 233, 357–363. https://doi.org/10.1016/S0076-6879(94)33041-7.

Ribaya-Mercado, J.D., Blumberg, J.B., 2004. Lutein and zeaxanthin and their potential roles in disease prevention. J. Am. Coll. Nutr. 23, 567S–587S.

Sałat, K., Siwek, A., Starowicz, G., Librowski, T., Nowak, G., Drabik, U., Gajdosz, R., Popik, P., 2015. Antidepressant-like effects of ketamine, norketamine and dehydronorketamine in forced swim test: role of activity at NMDA receptor. Neuropharmacology 99, 301–307. https://doi.org/10.1016/j.neuropharm.2015.07.037.

Sanacora, G., Treccani, G., Popoli, M., 2012. Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology 62, 63–77. https://doi.org/10.1016/j.neuropharm.2011.07.036.

Sasaki, M., Yuki, K., Kurihara, T., Miyake, S., Noda, K., Kobayashi, S., Ishida, S., Tsubota, K., Ozawa, Y., 2012. Biological role of lutein in the light-induced retinal degeneration. J. Nutr. Biochem. 23, 423–429. https://doi.org/10.1016/j.jnutbio.2011.01.006.

Savas, H.A., Gergerlioglu, H.S., Armutcu, F., Herken, H., Yilmaz, H.R., Kocoglu, E., Selek, S., Tutkun, H., Zoroglu, S.S., Akyol, O., 2006. Elevated serum nitric oxide and superoxide dismutase in euthymic bipolar patients: impact of past episodes. World J. Biol. Psychiatry 7, 51–55. https://doi.org/10.1080/15622970510029993.

Seddon, J.M., Ajani, U.A., Sperduto, R.D., Hiller, R., Blair, N., Burton, T.C., Farber, M.D., Gragoudas, E.S., Haller, J., Miller, D.T., 1994. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA 272, 1413–1420.

Sharma, A., Oommen, S., Kumar, A., 2017. Antidepressant-like activity of lutein-zeaxanthin in mice: evidence for involvement of monoaminergic and opioid systems. Int. J. Pharm. Technol. 9, 28551–28566.

Sheline, Y.I., Gado, M., Kraemer, H.C., 2003. Untreated depression and hippocampal volume loss. Am. J. Psychiatry 161, 1516–1518.

Silva, J.T., Geiss, J.M.T., Oliveira, S.M., Brum, E. da S., Sagae, S.C., Becker, D., Leimann, F.V., Ineu, R.P., Guerra, G.P., Gonçalves, O.H., 2017. Nanoencapsulation of lutein and its effect on mice's declarative memory. Mater. Sci. Eng. C 76, 1005–1011. https://doi.org/10.1016/j.msec.2017.03.212.

Sommerburg, O., Keunen, J.E., Bird, C., van Kuijk, F.J., 1998. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. Br. J. Ophthalmol. 82, 907–910. https://doi.org/10.1136/bjo.82.8.907.

Spiers, J.G., Chen, H.J.C., Sernia, C., Lavidis, N.A., 2015. Activation of the hypothalamic-pituitary-adrenal stress axis induces cellular oxidative stress. Front. Neurosci. 9, 1–6. https://doi.org/10.3389/fnins.2014.00456.

Stanić, D., Plećaš-Solarović, B., Petrović, J., Bogavac-Stanojević, N., Sopić, M., Kotur-Stevuljević, J., Ignjatović, S., Pešić, V., 2016. Hydrogen peroxide-induced oxidative damage in peripheral blood lymphocytes from rats chronically treated with corticosterone: the protective effect of oxytocin treatment. Chem. Biol. Interact. 256, 134–141. https://doi.org/10.1016/j.cbi.2016.07.006.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370. https://doi.org/10.1007/BF00428203.

Stringham, N.T., Holmes, P.V., Stringham, J.M., 2016. Lutein supplementation increases serum brain-derived neurotrophic factor (BDNF) in humans. FASEB J. 30, 689–6893.

Sun, Y., Liu, T., Dai, X., Zheng, Q., 2014. Treatment with lutein provides neuroprotection in mice subjected to transient cerebral ischemia. J. Asian Nat. Prod. Res. 16, 1084–1093. https://doi.org/10.1080/10286020.2014.939584.

Vishwanathan, R., Neuringer, M., Snodderly, D.M., Schalch, W., Johnson, E.J., 2013. Macular lutein and zeaxanthin are related to brain lutein and zeaxanthin in primates. Nutr. Neurosci. 16, 21–29. https://doi.org/10.1179/1476830512Y.0000000024.

Wang, W., Shinto, L., Connor, W.E., Quinn, J.F., 2008. Nutritional biomarkers in Alzheimer's disease: the association between carotenoids, n-3 fatty acids, and dementia severity. J. Alzheimers Dis. 13, 31–38.

Wang, M., Jiao, J., Li, Z., Liu, R., Shi, Q., Ma, L., 2013. Lutein supplementation reduces plasma lipid peroxidation and C-reactive protein in healthy nonsmokers. Atherosclerosis. 227, 380–385. https://doi.org/10.1016/j.atherosclerosis.2013.01.021.

Wąsik, A., Antkiewicz-Michaluk, L., 2017. The mechanism of neuroprotective action of natural compounds. Pharmacol. Rep. 69, 851–860. https://doi.org/10.1016/j.pharep.2017.03.018.

Weckmann, K., Deery, M.J., Howard, J.A., Feret, R., Asara, J.M., Dethloff, F., Filiou, M.D., Iannance, J., Labermaier, C., Maccarone, G., Webhofer, C., Teplytska, L., Lilley, K., Muller, M.B., Turck, C.W., 2017. Ketamine's antidepressant effect is mediated by energy metabolism and antioxidant defense system. Sci. Rep. 7, 1–11. https://doi.org/10.1038/s41598-017-16183-x.

Weng, L., Guo, X., Li, Y., Yang, X., Han, Y., 2016. Apigenin reverses depression-like behavior induced by chronic corticosterone treatment in mice. Eur. J. Pharmacol. 774, 50–54. https://doi.org/10.1016/j.ejphar.2016.01.015.

WHO - The World Health Organization, 2017. Depression and Other Common Mental Disorders: Global Health Estimates. Document Production Services, Geneva, Switzerland.

Willner, P., 2005. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52, 90–110. https://doi.org/10.1159/000087097.

Woo, T.T.Y., Li, S.Y., Lai, W.W.K., Wong, D., Lo, A.C.Y., 2013. Neuroprotective effects of lutein in a rat model of retinal detachment. Graefes Arch. Clin. Exp. Ophthalmol. 251, 41–51. https://doi.org/10.1007/s00417-012-2128-z.

Yan, T., Xu, M., Wan, S., Wang, M., Wu, B., Xiao, F., Bi, K., Jia, Y., 2016. *Schisandra chinensis* produces the antidepressant-like effects in repeated corticosterone-induced mice via the BDNF/TrkB/CREB signaling pathway. Psychiatry Res. 243, 135–142. https://doi.org/10.1016/j.psychres.2016.06.037.

Zafir, A., Banu, N., 2009. Modulation of in vivo oxidative status by exogenous corticosterone and restraint stress in rats. Stress 12, 167–177. https://doi.org/10.1080/10253890802234168.

Zafir, A., Ara, A., Banu, N., 2009. *In vivo* antioxidant status: a putative target of antidepressant action. Prog. Neuro-Psychopharmacology Biol. Psychiatry 33, 220–228. https://doi.org/10.1016/j.pnpbp.2008.11.010.

Zeni, A.L.B., Zomkowski, A.D.E., Dal-Cim, T., Maraschin, M., Rodrigues, A.L.S., Tasca, C.I., 2011. Antidepressant-like and neuroprotective effects of *Aloysia gratissima*: investigation of involvement of l-arginine-nitric oxide-cyclic guanosine monophosphate pathway. J. Ethnopharmacol. 137, 864–874. https://doi.org/10.1016/j.jep.2011.07.009.

Zeni, A.L.B., Camargo, A., Dalmagro, A.P., 2017. Ferulic acid reverses depression-like behavior and oxidative stress induced by chronic corticosterone treatment in mice. Steroids 125, 131–136. https://doi.org/10.1016/j.steroids.2017.07.006.

Zhang, C., Wang, Z., Zhao, J., Li, Q., Huang, C., Zhu, L., Lu, D., 2016. Neuroprotective effect of lutein on NMDA-induced retinal ganglion cell injury in rat retina. Cell. Mol. Neurobiol. 36, 531–540. https://doi.org/10.1007/s10571-015-0231-5.