<table>
<thead>
<tr>
<th><strong>Journal:</strong></th>
<th><em>Canadian Journal of Physiology and Pharmacology</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manuscript ID:</strong></td>
<td>cjpp-2016-0307.R1</td>
</tr>
<tr>
<td><strong>Manuscript Type:</strong></td>
<td>Article</td>
</tr>
<tr>
<td><strong>Date Submitted by the Author:</strong></td>
<td>05-Nov-2016</td>
</tr>
<tr>
<td><strong>Complete List of Authors:</strong></td>
<td>Araújo, Dayane; Universidade Federal do Rio Grande do Norte, Nursing Camboim, Thaisa; Universidade do Estado do Rio Grande do Norte Silva, Ana; Universidade do Estado do Rio Grande do Norte Fonseca, Caio; Universidade do Estado do Rio Grande do Norte Sousa, Rebeca; Universidade do Estado do Rio Grande do Norte Barbosa, Mabson; Universidade do Estado do Rio Grande do Norte Oliveira, Lucidio; Universidade do Estado do Rio Grande do Norte Cavalcanti, José; Universidade do Estado do Rio Grande do Norte Lucena, Eudes; Universidade do Estado do Rio Grande do Norte Guzen, Fausto; Universidade do Estado do Rio Grande do Norte</td>
</tr>
<tr>
<td><strong>Keyword:</strong></td>
<td>Lipoic acid, Omega 3, Tardive dyskinesia, Oxidative stress, Haloperidol</td>
</tr>
</tbody>
</table>
BEHAVIOURAL AND NEUROCHEMICAL EFFECTS OF ALPHA LIPOIC ACID ASSOCIATED WITH OMEGA 3 IN TARDIVE DYSKINESIA INDUCED BY CHRONIC HALOPERIDOL IN RATS

Dayane Pessoa de Araújo¹*; Thaisa Gracielle Martins Camboim²; Ana Patrícia Magalhães Silva²; Caio da Fonseca Silva²; Rebeca Canuto de Sousa²; Mabson Delânio Alves Barbosa²; Lucidio Clebeson Oliveira¹; José Rodolfo Lopes De Paiva Cavalcanti³; Eudes Euler de Souza Lucena⁴; Fausto Pierdoná Guzen ³

¹ Nursing Department, State University of Rio Grande do Norte – UERN/FAEN
² Students of Medicine, Department of Medicine, College of Health Sciences, State University of Rio Grande do Norte - UERN/FACS.
³ Department of Medicine, College of Health Sciences, State University of Rio Grande do Norte - UERN/FACS.
⁴ Department of Dentistry, State University of Rio Grande do Norte - UERN.

Conflict of interest

This work presents no conflict of interest
Arte.saber@yahoo.com.br

Addresses for correspondence to Dayane Pessoa de Araújo, Nursing Department, State University of Rio Grande do Norte -UERN/FAEN. Des. Dionísio Filgueira Street, 383, Centro, CEP 59610-090, – Mossoró-RN, Brazil. E-mail: dayanepessoa@uern.br
ABSTRACT

Tardive dyskinesia (TD) is characterized by involuntary movements of the lower portion of the face being related to typical antipsychotic therapy. TD is associated with the oxidative imbalance in the basal ganglia. Lipoic acid (LA) and omega 3 (ω-3) are antioxidants acting as enzyme cofactors, regenerating antioxidant enzymes. This study aimed to investigate behavioral and neurochemical effects of supplementation with of LA (100mg/Kg) and ω-3 (1g/Kg) in the treatment of TD-induced by chronic use haloperidol (HAL) (1mg/Kg) in rats. Wistar male rats were used, weighing between 180-200g. The animals were treated chronically (31 days) with LA alone or associated with HAL or ω-3. Motor behavior was assessed by open-field test, the catalepsy test and evaluation of orofacial dyskinesia. Oxidative stress was accessed by determination of lipid peroxidation and concentration of nitrite. LA and ω-3 alone or associated caused an improvement in motor performance by increasing locomotor activity in the open-field test and decreased the permanence time in the bar in the catalepsy test and decreased the orofacial dyskinesia. LA and ω-3 showed antioxidant effects, decreasing lipid peroxidation and nitrite levels. Thus, the use of LA associated with ω-3 reduced the extrapyramidal effects produced by chronic use of the HAL.

Keyword: Lipoic acid; Omega 3; Tardive dyskinesia; Haloperidol; Oxidative stress.
INTRODUCTION

Antipsychotics are a group of psychoactive drugs used to treat schizophrenia. The first drugs generation described as typical antipsychotics, such as chlorpromazine, haloperidol (HAL) and sulpiride present main mechanism of action blocking the dopamine D2 receptor. This blockage produces the desired therapeutic effects and side effects, the tardive dyskinesia and catalepsy main drawbacks of this class of drugs (Menegatti et al. 2004).

Tardive dyskinesia (TD) is characterized by repetitive, involuntary movements. The most serious aspect of TD is that it may persist for months or years after the drug withdrawal and in some patients are irreversible (Glazer et al. 1990). Thus, TD has been considered an important clinical problem in the treatment of schizophrenia.

The catalepsy is a typical side effect produced by chronic treatment with neuroleptic. It is characterized by loss of voluntary movement, impaired postural stability, inability to actively initiate movements and persistent abnormal postures. This effect is due to the blockade of D2 receptors at the central level, especially in striatal areas. The catalepsy development occurs after thirty days of treatment with HAL and this effect correlates with reduced locomotor activity. These effects disappeared after three days of suspension of the drug. After seven days of this suspension, it was observed up-regulation of D2 receptors in the striatum. These phenomena are due to blockade of D2 receptors, though other mechanisms may also be involved (Vasconcelos et al. 2003). The catalepsy test it is used for an important model for predicting extrapyramidal effects induced by neuroleptics (Chittiprol et al. 2010).
Evidence suggests that oxidative stress plays an important role in the pathophysiology of TD and catalepsy, a fact proved by studies published about this topic (Macêdo et al. 2011; Oliveira et al. 2013). Some of these studies have reported problems with the antioxidant defense and increased lipid peroxidation in animals chronically treated with haloperidol (Aguiar et al. 2010; Lister et al. 2014; Macêdo et al. 2011; Oliveira et al. 2013).

Based on the presence of oxidative stress in the pathophysiological process of TD, studies have been investigating the use of antioxidant therapies in an attempt to minimize extrapyramidal effects produced by chronic use of these typical antipsychotics (Daya et al. 2011; Macêdo et al. 2011; Peroza et al. 2013). Among these antioxidant therapies, we can mention the alpha lipoic acid (LA) (Thaakur and Himabindhu 2009) and omega-3 (ω-3) as potent antioxidant (Barcelos et al. 2010).

The LA is an antioxidant naturally synthesized in the human body and has been used to treat various diseases when provided as an oral supplement. Besides acting against free radicals, it promotes the reduction of lipid peroxidation, acts as a cofactor in many enzyme complexes and regenerates damaged tissues (Araújo et al. 2011; Ferreira et al. 2009). LA has the ability to combat reactive oxygen species (ROS) both in the lipophilic and hydrophilic environment (Packer et al. 1995). Due to its powerful antioxidant effect, LA would also be able to prevent neuronal damage caused by ROS produced during neurodegenerative diseases (Araújo et al. 2013). In addition, dihydrolipoic acid, the reduced form of the LA, is able to regenerate other antioxidants of low molecular weight, such as glutathione, coenzyme Q10, and vitamins A and C (Packer et al. 1995). It is also attributed to this substance anti-
inflammatory activity, and therefore the effect of short- and long-term reduction in oxidative processes related to neurodegenerative diseases. Furthermore, it works as a metal chelator, reducing ROS production (Araújo et al. 2013; Silva et al. 2013).

The ω-3 is an essential polyunsaturated fatty acid (PUFA-n3) being acquired from external sources through diet or supplementation (Cardoso 2009).

The ω-3 shows a fundamental role in maintaining neuronal integrity to promote brain development and synaptic plasticity. Besides ω-3 present antioxidant effect by promoting the removal or interfere with the production of ROS (Barcelos et al. 2010). The EPA and DHA can alter production of catecholamines like dopamine and serotonin, are fundamental for the maintenance of motor function controlled by the dopaminergic system in the corpus striatum (Delattre et al. 2010).

Considering that oxidative stress is involved in the pathophysiology of dyskinesias induced by chronic use of haloperidol, and the ω-3 (Barcelos et al. 2010) and LA (Araújo et al. 2013) have antioxidant effects proven in the literature, we hypothesized that the LA and/or ω-3 co-administration could prevent the development of these dyskinesias. Therefore, the aim of this study was to evaluate the behavioral and neurochemical effects of LA and/or ω-3 in HAL-induced TD in rodents.
MATERIALS AND METHODS

ANIMALS

Male Wistar rats (weighing 180–200 g), 8 per group from the animal colony of UERN. The animals were housed at an average temperature of 24 ± 2°C in light / dark cycle of 12 hours, receiving food and water ad libitum. The study was approved by the Ethics Committee on Animal Research UERN under the protocol 001/12.

DRUGS

Lipoic acid and HAL were purchased from Sigma (USA). ω-3 fish oil EPA-DHA 180/120mg (1000mg) were purchased from TopTherm (Brazil).

EXPERIMENTAL PROTOCOL

The animals were treated with saline (control), LA (100 mg/kg) by gavage, or HAL (1mg/kg) intraperitoneally, or ω-3 fish oil EPA-DHA 180/120mg (1g/kg) by gavage alone or with associations of the (LA + ω-3), or (ω-3 + LA+ HAL), or (LA + HAL), or (ω-3 + HAL). The drugs were administered chronically (31 days).

The open field tests and catalepsy were performed after 31 days. The orofacial dyskinesia test was performed on 11, 21 and 31 days of treatment. All behavioral tests were performed 1 hour after drug administration. After behavioral tests, the animals will be sacrificed by guillotine, the brain of the animals will be removed and the brain areas of interest (hippocampus - HC; prefrontal cortex - PFC; striatum-S) dissected on ice and stored at -70°C to perform the neurochemical tests (Supplementary Figure S1).
ANALYSIS OF MOTOR BEHAVIOR

OPEN-FIELD TEST (OF)

The OF area was made of timber (50 cm × 50 cm × 50 cm) divided into four squares of equal area. The OF was used to evaluate the exploratory activity of the rats. Each rat was placed in the center of the arena and the number of squares crossed, with the four paws (locomotor activity) was recorded for 5 min after a minute of habituation (1 minute). Before introducing each animal, the arena was cleaned with 10% alcohol to eliminate the possible bias due to the odor that could be left by previous animals (Archer 1973).

CATALEPSY TEST

In this test, front legs of the animals were placed on a rigid bar 2cm thick and 15cm in height. Each rat was placed with its forepaws near the edge of the bar and the amount of time spent in this atypical position was recorded for three times: 60 minutes, 90 minutes and 120 minutes after drug administration. All the rats treated were individually placed on the inclined grid and observed for the 60s (Sanberg et al. 1988).

EVALUATION OF DYSKINESIA OROFACIAL

The animals were placed on a scale similar to the open field arena, with mirrors at the base and sides. Thus, the observer had higher viewing angles. The animals were individually assessed by evaluating the number of vacuous chewing movement and protrusions of the tongue.

In this study, vacuous chewing movement (VCM) is referred to as openings in the vertical plane, not facing physical material. The protrusion of the tongue (PT) is referred to as the stereotypical behavior of the tongue with protrusions. If the protrusion of the tongue or VCM occurs over a period of
preparation, they will not be taken into account. The counting is stopped whenever the animal start grooming. The VCM and PT were measured continuously for 6 min after a period of adaptation (6min) (Naidu et al. 2003).

NEUROCHEMICAL STUDY

EVALUATION OF LIPID PEROXIDATION

Brain areas, the PFC, HC and S from all groups were dissected to prepare 10% homogenates (w/v, in 1.15% KCl). The formation of lipid peroxides during lipid peroxidation was followed by measuring the thiobarbituric acid reactive substances (TBARS), as previously described by Draper and Hadley. Briefly, samples were mixed with 1mL of 10% trichloroacetic acid and 1mL of 0.6% thiobarbituric acid. The reaction media was heated in a boiling water bath for 15 min, and n-butanol (2:1 v/v) was added to the media. After centrifugation (800 ×g, 5min), TBARS contents were determined at 535 nm. The results were expressed as micromoles of malondialdehyde (MDA) per mg protein (Draper and Hadley 1990).

NITRITE DETERMINATION

Tissue samples from PFC, HC or S were used to prepare 10% homogenates (w/v). After centrifugation (800 ×g, 10 min), supernatants were collected and the NO production was determined by the Griess reaction. Briefly, 100 μL of the supernatant were incubated with 100 μL of the Griess reagent [1% sulfanilamide in 1% H₃PO₄/0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride/ 1% H₂PO₄/distilled water (1:1:1:1)] at room temperature for 10 min. The absorbance was measured at 550 nm microplate reader. Nitrite concentration (μM) was determined from a standard NaNO₂ curve (Green 1981).
STATISTICAL ANALYSIS

All tests were analyzed by One-way ANOVA using Prism 5.0 software. For meaningful results, multiple comparisons were made by the Tukey as the post hoc tests. Results were considered significant at $P < 0.05$ and presented as mean ± SEM.

RESULTS

BEHAVIORAL TESTS

OPEN FIELD TEST

In the open field test, HAL group (0.0±0.0) showed a significant decrease in locomotor activity compared to the control group (41.4±3.9) ($p<0.0001$). In LA group alone (20.7±2.7) as well as in the ω-3 group alone (25.7±1.6) was observed a decrease in the number of intersections of the quadrants when compared to the control group and increased when compared to the HAL group ($p<0.0001$)(Figure 1A).

In the groups of associations with lipoic acid and haloperidol (HAL+ LA: 1.6±1.6) or ω-3 and haloperidol (ω-3+HAL: 8.7±2.0) or lipoic acid, ω-3 and haloperidol group (ω-3+LA+HAL: 11.0±2.9) all showed reduced locomotor activity when compared to control group. However, the latter two groups showed an increase in locomotor activity when compared with the haloperidol group (Figure 1A).

In the evaluation of vertical exploratory activity (rearing) the results showed that LA group (5.7±0.7) or the ω-3 alone group (6.2±1.4) increased the frequency of rearing compared to the HAL group (0.0±0.0). The associations of ω-3 and HAL (2.4±0.5) or LA, ω-3 and HAL group (5.6±0.2) ($p<0.009$) showed increasing the number of rearing compared to the HAL group (Figure 1B).
CATALEPSY TEST

In the catalepsy test HAL group (60min: 150,3±29,3; 90min: 186,8±25,5; 120min: 186,4±26,0) remained more time in the bar when compared to the control group (60min: 0,5±0,5; 90min: 4,1±4,1; 120min: 5,8±5,8). The groups treated with LA alone (60min: 0,6±0,6; 90min: 3,0±3,0; 12 min: 5,8±5,8) or ω-3 alone (60min: 0,6±0,6; 90min:4,0±4,0; 120min: 6,0±6,0) or associations, HAL and LA (60min: 93,0±19,0; 90min: 76,2±9,0; 120min: 71,6±7,1) or ω-3 and HAL (60min: 8,6±4,1; 90min: 43,4±23,9; 120min: 50,0±15,3) or LA, ω-3 and HAL (60min: 1,6±1,4; 90min: 5,1±2,4; 120min: 22,3±12,8) were able to decrease the time spent in the bar when compared to the HAL group (Figure 2).

EVALUATION OF OROFACIAL DYSKINESIA

In the orofacial dyskinesia test HAL group (11th day: 13±1,8; 21th day: 17,1±3,3; 31th day: 10,3±1,3) showed an increase in the VCM compared to the control group (11th day: 0,2±0,2; 21th day: 0,2±0,2; 31th day: 0,2±0,2). The LA group (11th day: 0,2±0,2; 21th day: 0,2±0,2; 31th day: 0,2±0,2) or ω-3 group (11th day: 0,2±0,2; 21th day: 0,4±0,2; 31th day: 2,4±1,5) or the group treated with LA and HAL (11th day: 2,6±0,4; 21th day: 4,0±0,9; 31th day 3,0±0,7) they were able to decrease the number of VCM when compared to the group HAL (p<0.0001) (Figure 3).

The association group ω-3 and HAL on the eleventh day (5,2±1,4) showed an increase in the number of VCM compared to the control group (0,2±0,2) or ω-3 alone group (0,2±0,2). Also in relation to the association group with ω-3 and HAL, this decreased the number of ECM compared to the HAL group in the three-time analyzed. The association group with LA, ω-3 and HAL (11th day: 4,5±1,0; 21th day: 3,9±1,0; 31th day: 3,5±0,8) exhibited on the
eleventh day, increase in VCM compared to the control group (0.2±0.2) or the LA group (0.2±0.2) alone. However, when compared to the HAL group with association LA, ω-3 and HAL decreased the number of VCM in the three times studied ($p<0.0001$) (Figure 3).

Also in the orofacial dyskinesia test the HAL group (11\textsuperscript{th} day: 5.6±1.1; 21\textsuperscript{th} day: 4.5±0.8; 31\textsuperscript{th} day: 5.2±1.2) showed an increase in the number of protrusions tongue when compared to the control group (11\textsuperscript{th} day: 0.0±0.0; 21\textsuperscript{th} day: 0.0±0.0; 31\textsuperscript{th} day: 0.0±0.0) (Figure 4).

The groups treated of LA alone (11\textsuperscript{th} day: 0.0±0.0; 21\textsuperscript{th} day: 0.0±0.0; 31\textsuperscript{th} day: 0.0±0.0), ω-3 alone (11\textsuperscript{th} day: 0.0±0.0; 21\textsuperscript{th} day: 0.0±0.0; 31\textsuperscript{th} day: 0.0±0.0), and associations groups of LA and HAL (11\textsuperscript{th} day: 0.8±0.4; 21\textsuperscript{th} day: 0.6±0.4; 31\textsuperscript{th} day: 0.2±0.2), ω-3 and HAL (11\textsuperscript{th} day: 0.6±0.5; 21\textsuperscript{th} day: 2.1±0.6; 31\textsuperscript{th} day: 0.4±0.2) or LA, ω-3 and HAL (11\textsuperscript{th} day: 0.4±0.2; 21\textsuperscript{th} day: 0.3±0.2; 31\textsuperscript{th} day: 1.7±0.5) showed a decrease in the number of protrusions tongue compared to the HAL group in the three time periods analyzed (Figure 4).

**NEUROCHEMICAL STUDY**

**CONCENTRATION OF LIPID PEROXIDATION (TBARS)**

The results showed that chronic exposure of the cells of the PFC, HC and S to HAL (1480±46.7; 1525±69.9; 1522±53.3, respectively) caused an increase in MDA ($\mu$mol MDA/g of tissue) content when compared to control group (100.6±22.7, 94.6±20.4, 80.4±7.9, respectively) (Figure 5A,B,C).

In the three areas studied the groups treated with LA (PFC: 1041±37.0; HC: 980.6±45.0; S: 1052±59.0) or ω-3 (PFC: 1085±42.5, HC: 1066±53.1, S: 1054±57.6) alone showed a significant reduction in the levels of TBARS compared to the HAL group ($p<0.0001$). Comparing the associations groups
with the group of HAL alone was possible to observe a significant reduction in lipid peroxidation in the TBARS assay in the three brain areas (ω-3+HAL PFC: 834.6±55.6, HC: 780.1±43.7, S: 882.8±77.3; LA+HAL PFC: 1091±41.6, HC: 1067±37.3, S: 1051±57.7; HAL PFC: 1480±46.7; HC: 1525±69.9; S: 1522±53.3) (p<0.0001) (Figure 5A,B,C).

Also with respect to the associations, the group of ω-3 and HAL presented a greater reduction in MDA levels when compared to the combination of LA and HAL group in the PFC and HC (Fig. 5A,B). In contrast, the association group with LA, ω-3 and HAL showed better response in the reduction of lipid peroxidation induced by chronic use of HAL presenting results close to those obtained in the control group in the three areas studied (ω-3+LA+HAL PFC: 211.1±44.0, HC: 101.1±38.9, S: 121.9±43.2) (Figure 5A,B,C).

NITRITE DETERMINATION

The results showed that the concentrations of nitrite/nitrate (µmol/g of tissue) in the HAL group (2.8±0.0) increased in the PFC group compared to the control group (1.0±0.0). The group of LA alone (1.9±0.1) or ω-3 alone (1.8±0.0) showed an increase in the concentrations of nitrite/nitrate compared to the control group in the PFC and a decrease when compared to the HAL group (Figure 6A).

All associations, LA and HAL (1.0±0.2), ω-3 and HAL (1.6±0.0) or ω-3 and LA and HAL (1.2±0.1) showed increased concentration of nitrite/nitrate compared to the control group in the PFC and a decrease when compared to the HAL group (p<0.0001) (Figure 6A).

Also in relation to concentrations of nitrite/nitrate, the group treated with HAL (1.7±0.0) showed an increase when compared to the control group
(0,9±0,0) in the HC. The group of LA alone (0,4±0,0) showed a decrease compared to the HAL group (Figure 6B).

The group of ω-3 alone (1,6±0,0) as well as the association’s groups with LA and HAL (1,6±0,0) or ω-3 and HAL (1,6±0,1) showed increased in the concentrations of nitrite-nitrate compared to the control group in HC. The group of the association with LA, ω-3 and HAL (1,0±0,0) showed a decrease in the concentrations of nitrite-nitrate compared to HAL group in HC (Figure 6B).

In the S, the concentrations of nitrite/nitrate in the HAL group (2,5±0,2) increased compared with the control group (1,0±0,0). The groups treated with LA alone (1,6±0,0) or ω-3 alone (1,7±0,1) or association group treated with LA and HAL (1,7±0,1) showed increased concentrations of nitrite / nitrate compared the control group and decreased when compared to the HAL group in S (Figure 6C).

The associations with ω-3 and HAL (44,0±0,0) or LA, ω-3 and HAL (1,2±0,1) exhibited decreased of concentrations of nitrite/nitrate compared to the HAL group (Figure 6C).

DISCUSSION

The open field test aims to study the action of the dopamine system, serotonergic and noradrenergic emotional and exploratory behavior through the horizontal scanning (mobility), vertical (rearing) and self-cleaning (grooming). It is widely used as an evaluation tool of the substances action that may act on these neurotransmitter systems promoting motor abnormalities (bradykinesia or hyperlocomotion) and emotional (anxiety) (Schallert 2000).

The results showed that LA and ω-3 promoted improvement in motor performance in the open field test. This improves motor due to the antioxidant
effect of these substances, reflecting on monoamines levels. The LA has the capacity to alter the levels of monoamines and their metabolites by stimulating the synthesis, release in the synaptic cleft and reduced metabolism of these neurotransmitters thereby increasing its availability in the CNS (Chang et al. 2009; Santos et al. 2010).

The LA and ω-3 they were able to reduce the time spent in the bar in the catalepsy test, indicating a possible neuroprotective effect. This effect is related to the ability of these substances has to control the state oxidant / antioxidant, stabilizing the membranes of brain tissue structures and acting as antiapoptotic (Barcelos et al. 2010; Thaakur et al. 2009).

The typical neuroleptics promote blocking dopamine D2 receptors such block results in up-regulation of these receptors and dopamine-rich areas catecholamines, such as the basal ganglia. The increase in catecholamine levels those results in the overproduction of ROS. The HAL reduces gene expression of superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GHP), thus reducing the complex antioxidant defense (Thaakur et al. 2009).

The LA and ω-3 promote a reduction in the number of VCM and protrusion of the tongue induced HAL, indicating a possible neuroprotection. This effect is due to the reduction in ROS levels as well as increased content of SOD, CAT, and GHP. Studies have shown that the D2 receptor blockade is related to the increase in glutamate release in the striatum, it produces such an increase resulting in a chronic neurotoxicity extrapyramidal events resulting from chronic use of such antipsychotics (Bošković et al. 2013; Naidu et al. 2003; Thaakur et al. 2009).
The nervous system is more sensitive to the damaging action of free radicals than other tissues of the organism once the metabolism of the brain is extremely high which favors the continual formation of reactive oxygen species and nitrogen, addition of the antioxidant defense system not be as effective in removing these agents, thus favoring neurodegeneration (Ferreira et al. 2016).

Evidence suggests that oxidative stress plays an important role in the pathophysiology of TD, a fact proven by studies published about this topic (Aguiar et al. 2010; Oliveira et al. 2013).

In this research, the HAL increased the concentration of nitrite/nitrate in the striatum. Another interesting result is that oxidative damage was not restricted to this area of the brain, but also mostly affected the prefrontal cortex and hippocampus. This demonstrates that the changes produced by chronic use of HAL are not limited only to the neurons of the striatum.

As an antioxidant LA and ω-3 work removing the hydroxyl radicals, hydrogen peroxide in its free form, superoxide and peroxynitrite (Araújo et al 2011; Bošković et al. 2016). Because of this potent effect LA and ω-3, would also be able to prevent neuronal damage caused by reactive species derived from oxygen and nitrogen (Bošković et al. 2016; Maczurek et al. 2008; Thaakur et al. 2009).

The omega-3 plays a fundamental role in maintaining neuronal integrity to promote brain development and synaptic plasticity. Besides ω-3 present a possible antioxidant effect by promoting the removal or interfere with the production of ROS. The EPA and DHA can alter production of catecholamines like dopamine and serotonin, are fundamental for the maintenance of motor function controlled by the dopaminergic system in the striatum (Cardoso 2009).
Lipid peroxidation is the broader process of oxidative damage by promoting the breakdown of the cell membrane lipids and the formation of the peroxyl radical. Once started the event, this spreads inducing cell destruction chain (Ferreira et al. 2016).

LA and ω-3 showed a possible neuroprotective effect since it was able to significantly reduce the lipid peroxidation in the TBARS assay in the striatum and prefrontal cortex and hippocampus.

The EPA and DHA to be metabolized by cyclooxygenase-2 (COX-2) resolvin produce the E and D series, respectively. The DHA can be converted to protectine D1 and D1 neuroprotectine by the action of lipoxygenase (LOX). The neuroprotectine D1 is produced in response to oxidative damage in the brain, serving as antiinflammatory and antioxidant (Cardoso 2009).

CONCLUSION

The LA and the ω-3 were able to promote an improvement of motor function observed through the open field test and reduced the time spent in the bar in the catalepsy test and the number of empty movements of chewing and tongue protrusion in test orofacial dyskinesia. They also were able to promote a reduction in the concentrations of nitrite/nitrate as well as haloperidol-induced lipid peroxidation in three brain areas investigated. Such a response would be related to the antioxidant and anti-inflammatory effect of these substances, suggesting a neuroprotective action against extrapyramidal injury induced by chronic use of haloperidol.

DECLARATION OF INTEREST

No conflict of interest.
REFERENCES


https://mc06.manuscriptcentral.com/cjpp-pubs
Cardoso, P.M.F. 2009. Efeitos da suplementação com ácidos graxos Ω-3 nos distúrbios motores e cognitivos de pacientes psiquiátricos tratados com antipsicóticos. (Dissertação de mestrado) Universidade Federal Santa Maria: RS, Brazil.


Silva, M.C., de Sousa, C.N., Sampaio, L.R., Ximenes, N.C., Araújo, P.V., da Silva, J.C., et al. 2013. Augmentation therapy with alpha-lipoic acid and


Figure captions

FIGURE 1 - Determination of the effect of lipoic acid and/or omega 3 alone or associated with haloperidol on the number of crossings in the quadrants and rearing in the open field test. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey test as post hoc test were used. a vs the control, b vs HAL, c vs ω-3, d vs ω-3+HAL with \( p < 0.0001 \).

FIGURE 2 - Determination of the effect of lipoic acid and/or omega 3 alone or associated with haloperidol in time spent in the bar in the catalepsy test. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, c vs ω-3, with \( p < 0.0001 \).

FIGURE 3 - Determination of the effect of lipoic acid and/or omega-3 alone or associated with haloperidol on the number of chewing motion in vacuo at orofacial dyskinesia test. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, c vs LA, d vs ω-3 with \( p < 0.0001 \).

FIGURE 4 - Determination of the effect of lipoic acid and/or omega-3 alone or associated with haloperidol on the number of tongue protrusion in the test orofacial dyskinesia. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, c vs LA, d vs ω-3, e vs LA+HAL, f vs ω-3+LA+HAL, with \( p < 0.0001 \).

FIGURE 5 - Effect of α-lipoic acid and/or omega 3 on lipid peroxidation in the prefrontal cortex, hippocampus and striatum of rats subjected to chronic treatment with haloperidol. PFC: prefrontal cortex, HC: hippocampus, S: striatum. Values represent mean ± SEM of TBARS quantities expressed in µmol
MDA/g of tissue. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, e vs AL+HAL, f vs O3+HAL with p<0.0001.

FIGURE 6 - Effect of α-lipoic acid and/or omega 3 on the concentration of nitrite/nitrate in the prefrontal cortex, hippocampus and striatum of rats subjected to chronic treatment with haloperidol. PFC: prefrontal cortex, HC: hippocampus, S: striatum. Values represent mean ± SEM of the amounts of nitrite/nitrate expressed in µmol/g of tissue. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, with p<0.0001.
Figure 1 - Determination of the effect of lipoic acid and/or omega 3 alone or associated with haloperidol on the number of crossings in the quadrants and rearing in the open field test. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey test as post hoc test were used. a vs the control, b vs HAL, c vs ω-3, d vs ω-3+HAL with p <0.0001.
Figure 2- Determination of the effect of lipoic acid and/or omega 3 alone or associated with haloperidol in time spent in the bar in the catalepsy test. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, c vs ω-3, with p<0.0001.
FIGURE 3 - Determination of the effect of lipoic acid and/or omega-3 alone or associated with haloperidol on the number of chewing motion in vacuo at orofacial dyskinesia test. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, c vs LA, d vs ω-3 with p<0.0001.
FIGURE 4 - Determination of the effect of lipoic acid and/or omega-3 alone or associated with haloperidol on the number of tongue protrusion in the test orofacial dyskinesia. Values are expressed as mean ± SEM of the number of observations. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, c vs LA, d vs ω-3, e vs LA+HAL, f vs ω-3+LA+HAL, with p<0.0001.
FIGURE 5 - Effect of α-lipoic acid and/or omega 3 on lipid peroxidation in the prefrontal cortex, hippocampus and striatum of rats subjected to chronic treatment with haloperidol. PFC: prefrontal cortex, HC: hippocampus, S: striatum. Values represent mean ± SEM of TBARS quantities expressed in µmol MDA/g of tissue. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, e vs AL+HAL, f vs O3+HAL with p<0.0001.

80x223mm (300 x 300 DPI)
FIGURE 6 - Effect of α-lipoic acid and/or omega 3 on the concentration of nitrite/nitrate in the prefrontal cortex, hippocampus and striatum of rats subjected to chronic treatment with haloperidol. PFC: prefrontal cortex, HC: hippocampus, S: striatum. Values represent mean ± SEM of the amounts of nitrite/nitrate expressed in µmol/g of tissue. ANOVA and Tukey test as post hoc test were used. a vs control, b vs HAL, with p<0.0001.