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Behavioral, Neurochemical and Histological Changes in the Use of Low Doses of Naltrexone and Donepezil in the Treatment in Experimental Model of Alzheimer's Disease by Induction of β-Amyloid$_{1-42}$ in Rats

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that promotes the involvement of memory-related functions, characterized by the presence of amyloid plaques formed by the β-amyloid peptide (Aβ), and hyperphosphorylated Tau protein neurofibrillary tangles. Evidence suggests that the use of low doses of Naltrexone, an opioid antagonist, possibly promotes a modulation of the immune system and consequent neuroprotective effect. The present study uses the animal model of induction with β-amyloid$_{1-42}$ (Aβ$_{1-42}$) to verify the behavioral, neurochemical and histological effects of the use of low doses of Naltrexone. Male wistar rats (250-300g) divided into five groups (N = 8) were used: Control, Sham, Aβ$_{1-42}$ subdivided into three groups: treated with water, 05 mg Donepezil and 4.5 mg Naltrexone, orally during the 30-day period. Behavioral tests demonstrated the efficacy of induction to the experimental model with reduced memory of Aβ$_{1-42}$-treated animals as well as reversal of damage in animals treated with Naltrexone. In the structural analysis, observed that the animals induced by Aβ$_{1-42}$ treated with water alone presented alterations in the pyramidal forms of the hippocampal cells and that the animals treated with Naltrexone presented possibly a reversal of the neuronal damages. In conclusion, treatment with Naltrexone promoted a reversal in the memory impairment of rodents induced to the Alzheimer's model with Aβ$_{1-42}$ in the behavioral and histological response.

Keywords: Alzheimer's disease, β-amyloid$_{1-42}$, Naltrexone, Neurochemical, Behaviour, Histological changes.

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Contents

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by extensive neuronal loss, being more evident in the cholinergic system [1, 2] reduction of synaptic connections in the hippocampus and cerebral atrophy [3]. It is characterized by the presence of amyloid plaques, containing beta-amyloid peptide (Aβ) and neurofibrillary tangles formed by the hyperphosphorylated Tau protein [4, 5]. The disease may begin before symptoms such as memory loss occur, making early detection an important tool for preventing, slowing or stopping the course of the disease. Among the most common symptoms, the loss of the ability to remember recent information usually occurs because the death of the first neurons manifests in brain regions involved in memory formation, such as the hippocampus [6]. Because the presence of amyloid plaques is the main structural feature related to AD, the animal model of the induction by intracerebral injection of Aβ1-42 has been widely studied by reproducing the phenotypes of the disease with high fidelity and in a short time [7]. Among the forms of pharmacological treatment, cholinesterase inhibitors (I-ChE) are the main drugs used for treatment. Donepezil is an anticholinesterase that has beneficial effects in patients with this disease. In general, the dose of 5mg / day produces better effects and entails a good tolerance when compared to the dose of 10mg / day of the same substance. However, several side effects are presented, among them: diarrhea, nausea and cramps [8]. Thus, new therapeutic approaches have been proposed for the treatment of this disease, from selective serotonin reuptake inhibitors (SSRIs), antiepileptic drugs, antihypertensives, anti-inflammatories, among other substances, as well as the combination of one or more treatments [9, 10].

Endogenous opioid peptides were discovered by Hughes and colleagues in 1975, and are possibly related to the regulation of growth factors, as well as to neuromodulators and neurotransmitters [11, 12]. Naltrexone is a non-selective opioid antagonist with affinity for mu (μ), delta (δ) and kappa (κ) receptors. The Federal Drug Agency (FDA) approved its use in 1984 for the treatment of chemical dependence disorder [13, 14]. The hypothesis supporting the use of low doses of Naltrexone (LDN) suggests that, when given at doses of 3-4.5 mg / day, this opioid antagonist chronically promotes increased mu (μ), delta (δ) and kappa (κ) as well as enkephalins and beta-endorphins by a compensatory mechanism due to the temporary blockade of these endogenous opioid receptors. This increase in the expression of the opioid receptors and the endogenous opioids possibly is associated with an improvement of the immune system, and consequent neuroprotection [15, 16]. Studies performed with the use of LDN demonstrated significant therapeutic effects in several diseases with unknown etiology. Among the pathologies already studied are Crohn's disease, Multiple sclerosis, Fibromyalgia, Autism, AIDS, Neuroblastoma, and Ovarian cancer [16, 17].

Thus, the hypothesis of the use of LDN suggests that the blockade of the endogenous opioid receptors for a short time (4-6 hours) possibly promotes an increase in the expression, and concomitant action, of the opioid system, and the levels would remain elevated during the the rest of the day (18-20 hours), thus allowing an improvement in the functions related to the modulation of the immune system. Thus, the present study proposes the use of LDN in the treatment of AD, seeking to identify changes related to behavior, possible structural and neurochemical changes.

2. Materials and Methods

2.1. Animals

Fourty male Wistar rats aged 3-4 months and average weight of 250-300 g were obtained from the Higher Institute of Biomedical Sciences - ISCB of the State University of Ceara - UECE. The animals were divided into polypropylene boxes, separated by homogeneous groups by weight and cognitive profile (Spatial Memory), measured by the Morris Water Maze (MWM) test, preceded by an Open Field test used as initial screening to rule out locomotive disability. In addition, they were kept in a light / dark cycle (12h / 12h), in an environment with controlled temperature between 22 to 25ºC, and with ration and water ad libitum. The infrastructure, as well as all the necessary equipment for the experimental procedures, was made available by the Laboratory of Biochemistry and Gene Expression (LABIEX) and by the Nucleus of Morphology and Image Processing (NEMPI) - UFC. The animals, after using the LAM protocol (considering four days of training and one of the test itself) were divided into five experimental groups (N = 8), described below: Control - C, Sham - S, Aβ1 Aβ1-42 + A; Aβ1-42 treated with Donepezil-Aβ1-42 + D; Aβ1-42 treated with low doses of Naltrexone-Aβ1-42 + LDN. The treatment lasted for 30 days and was administered via oral for all animals with equal volumes and times. The present study was submitted to the Ethics Committee for the Use of Animals - CEUA, of the State University of Ceara, and was approved under the number 5512404/2015.

2.2. Experimental Procedure

For induction to the Alzheimer's model, the Aβ pool was prepared from a freeze-dried powder of $A\beta_{1-42}$ (Sigma-Aldrich, Inc.) in Fetal Bovine Serum (PBS) pH 7.4. The solution was incubated at room temperature for three days under constant stirring to form the Aβ pool and stored at -80ºC [18]. In order to induce the Alzheimer's experimental model, the animals were anesthetized with the combination of Diazepan (10 mg / kg), 10% Ketamine (80 mg / kg) and 2% Xilazine (10 mg / kg) intraperitoneally and fixed in a stereotaxic through earbuds and muzzle fixers (Insight Ltda). Then, the cranial trichotomy, asepsis with iodized alcohol and sagittal incision were performed to expose bregma and lambda in the skull. Once this was accomplished, coordinates to locate the exact points of application of the aggregate were followed by the Anatomical Atlas of Paxinos [19]. The aggregate was aspirated into the Hamilton syringe with a maximum capacity of 10 μL. The needle was lowered into the brain at a

rate of 0.8 mm / min and then held in place for five minutes prior to injection. Then, the Aβ pool was infused in a total of 5ul per hippocampus (1uL / 3min application rate). After injection, the needle was allowed to stand in place for 5 min and then withdrawn at a rate of 0.4 mm / min to ensure adequate diffusion [20]. Afterwards the rats were sutured at the surgery site, housed in individual boxes and observed for seven days. After this phase, to verify the development of the symptoms of AD, the MWM test (Probe Trial only) was applied again, in order to verify if the experimental model worked. After 24 hours of treatment, the animals repeated the MWM test to see the effectiveness of the substances used. Thereafter, the animals were sacrificed and their skulls removed. The area of interest is the hippocampus and, in order to perform all the tests described below, it was standardized that the right hippocampus would be withdrawn integrally for analysis of biochemical tests.

2.3. Histological Tissue Processing for Microscopy

The left hippocampus was used for the histology and placed in cassettes in buffered formaldehyde for 18 hours and in the sequence placed in 70% alcohol for further processing. The slides were stained by Hematoxylin and Eosin (HE) and analyzed by light microscopy.

2.4. Biochemical Assays

Activities of the enzymes Catalase (CAT), Glutathione Peroxidase (GPx) and levels of Malondialdehyde (MDA), Nitrite (NIT) and total protein (BRADFORD) were analyzed. The hippocampal brain region was homogenized with phosphate buffer (pH 7.4) and centrifuged at 12,000 rpm for 15 minutes at 4° C for the production of the supernatant from the samples. To determine the tests, the methods used were Maehly [21]; Ohkawa, et al. [22]; Green and Goldman [23]; Bradford and Thomas [24] respectively.

2.5. Statistical Analysis

To analyze the results, both descriptive and inferential statistics were used. Regarding the descriptive, the values were presented in mean and standard error for better visualization of the group response and for ANOVA analysis was used ANOVA followed by post test of Tuckey for multiple comparisons in the behavioral tests. It was considered a significant difference $p < 0.05$. ANOVA one way with Newman-Keuls post test was used to analyze the neurochemical tests, being considered a significant difference $p < 0.05$.

3. Results and Discussion

3.1. Learning and Memory Spatial

The use of the Open Field test preceded the Morris Aquatic Labyrinth test to rule out locomotor disability. The figure shows the results of the parameters of exploratory locomotor activity (ALE) and vertical exploratory activity (REARING) Figure 1.

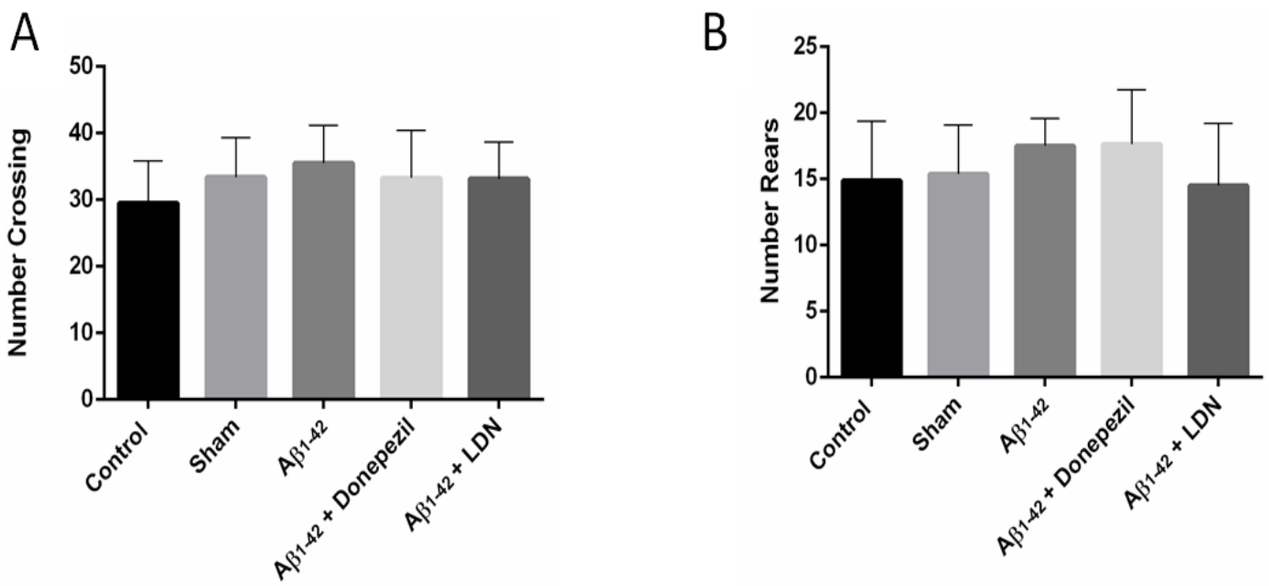

Figure-1. Open Field test data. (A) The values represent the mean and standard deviation of the groups in relation to the number of crossing in the 5-minute period. (B) The values represent the mean and standard deviation of the groups in relation to the number of rears in the 5-minute period $P < 0.05$.

The results presented demonstrate that the animals were distributed homogeneously before the LAM test, in order not to direct any subsequent response. The data regarding the amount of exploratory locomotor activity were: Control (C): 29.50 ± 2.22, Sham (S): 33.38 ± 2.09, Alzheimer (A): 35.50 ± 1.99, Alzheimer + Donepezil (AD): 33.25 ± 2, 53; Alzheimer + Low Dose Naltrexone (ALDN): 33.13 +/- 1.95); The data in relation to the amount of vertical exploratory activity were: C: 14.88 ± 1.59; S: 15.38 ± 1.30; A: 17.50 ± 0.73; AD: 17.63 ± 1, 46; ALDN: 14.50 ± 1.66).

In our study, the use of the open field test was used to determine the locomotor disability of the animals before they were submitted to the LAM test, where memory and learning are evaluated as parameters indicative of the efficacy of induction to the Alzheimer model by of the stereotactic surgery, performed before surgery, seven days after surgery and thirty days after the treatment.

The Morris Aquatic Labyrinth tests were used to measure spatial memory parameters. The figure presents the results before any surgical procedure in order to standardize the groups with each other. After surgery the animals

remained separated in individual boxes for 07 days and repeated the LAM test. Thus, with data obtained after surgery, the efficacy of the surgical procedure was verified, since the three groups that received the aggregate were statistically different from both control and sham. After treatment for 30 consecutive days, the results showed that there were losses in the group induced to the Alzheimer model. It is also observed that a reversal of cognitive impairment occurred in the Donepezil group Figure 2.

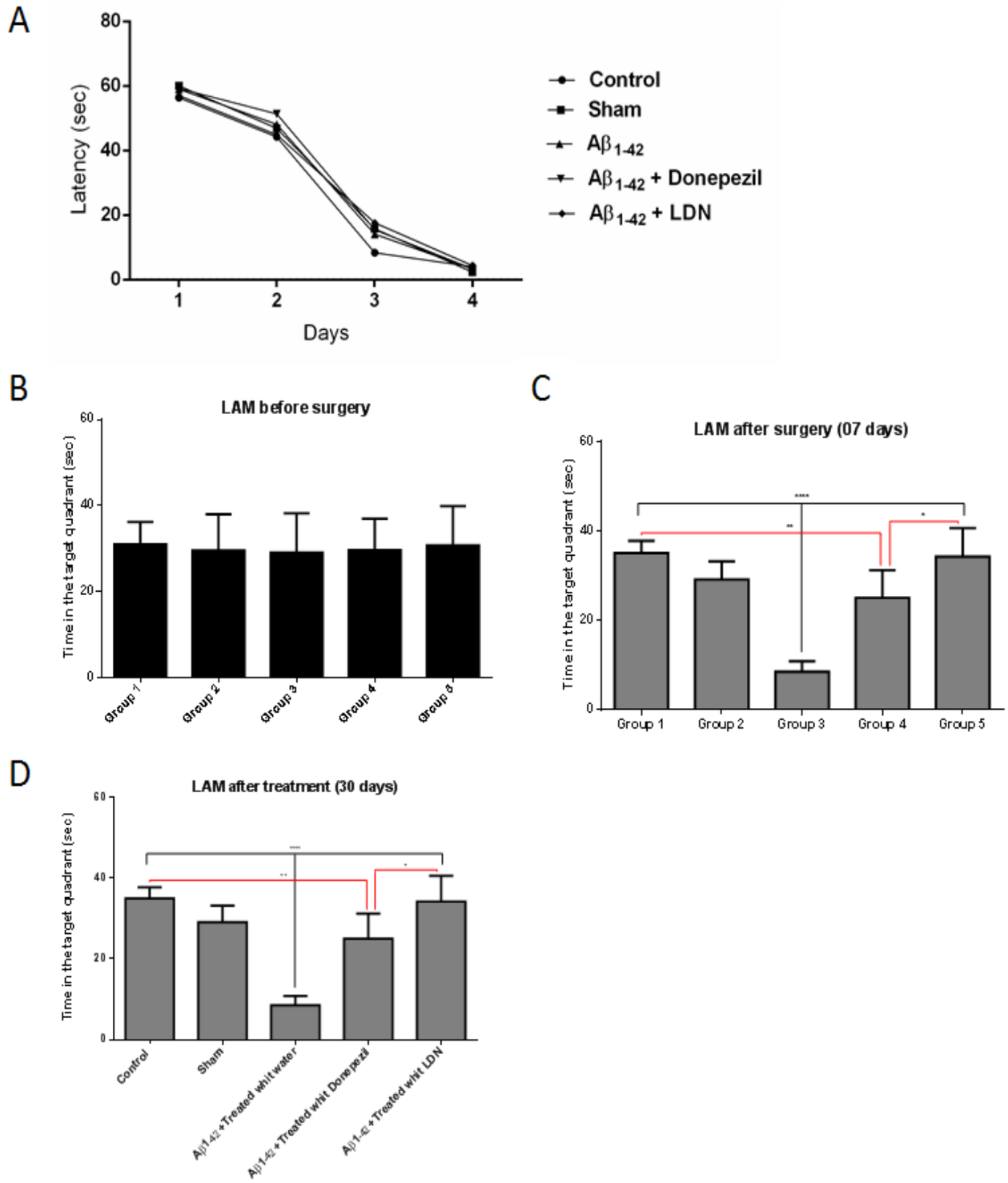

Figure-2. (A) Data related to the results of the comparison between the groups through the Morris Water Maze (MWM) test during the training period. Result of the mean time in seconds of all groups over the four training days in relation to the time to find the platform in the target quadrant. There was no significant difference between the groups when compared to each other on the same days. (B) Results of the comparison between groups using the Morris Water Maze (MWM) test prior to surgery. (C) Results of the comparison between groups using the MWM test 07 days after surgery. (D) Results of the comparison between the groups using MWM test after treatment. It was considered $p < 0.05$ (95%).

The results presented (B) show that the animals were homogeneously distributed before the surgical procedure in order not to direct any subsequent response (C: 30.97 ± 1.83 s; S: 29.59 ± 2.97 s; A: 29.09 ± 3.21 s, AD: 29.66 ± 2.57 s, ALDN: 30.72 ± 3.23 s). To measure the data, the mean time the animals remained in the target quadrant of the LAM was observed, after the animal was released in the four quadrants. Thus, with the homogeneous groups regarding memory, we could determine the groups that would undergo surgery and the application of $A\beta_{1-42}$.

After surgery the animals remained separated in individual boxes for 07 days and repeated the LAM test. Thus, with data obtained after surgery, the efficacy of the surgical procedure was verified, since the three groups that received the aggregate were statistically different from both control and sham. It was thus realized that the surgery was effective in causing damage to the animals' memory, serving as a basis for the use of conventional and experimental treatment. The Sham group (25.47 ± 2.57 s) presented a significant difference ($p < 0.05$) when

compared to the control group (33.88 ± 1.85 s), probably due to the stress caused by the surgery itself, and not by the PBS, since the posttreatment values did not generate differences between this group and the control (C).

The groups that received Aβ1-42 had a significant difference ($p < 0.0001$) when compared to the control group (A: 9.88 ± 1.21 s; AD: 11.91 ± 1.03 s; ALDN: 8.88 ± 1.05 s) and sham ($p < 0.001$ and $p < 0.0001$). This fact may possibly occur due to damage caused by Aβ1-42 in the hippocampus region. Thus, once the experimental model proved to be effective, it was appropriate to start the treatment to visualize the possibility of new substances with the intention of treating the experimental model. For this, two groups were created, where one used the base drug used in the clinic for the treatment of this condition: Donepezil, and the other drug is of interest in the present study: LDN. After treatment for 30 consecutive days, the results presented above, show that Alzheimer's losses (8.50 ± 0.82 s) remained with a significant difference when compared to the other groups, mainly to the control group (35.00 ± 0.97 s) and LDN (34.22 ± 2.25 s). In this way, the cognitive damages promoted by the experimental model by induction by $A\beta_{1-42}$ remain during the period of 30 days. In addition, this period was sufficient to reverse the losses presented in the Sham group (29.09 ± 1.44 s) when compared to the test 7 days after surgery (D). In this way, it can be noticed that although losses occur with the surgical procedure, they reduce with the passage of time.

It is also observed that a reversal of cognitive impairment occurred in the group treated with Donepezil (25.00 ± 2.19 s). Because it is the standard drug for pharmacological treatment in AD, the results demonstrated that this drug, probably through the inhibition of acetylcholinesterase, is capable of reversing cognitive alterations in the initial period of the disease, where the cholinergic system apparently is most affected by pathology. However, the use of LDN for a period of 30 days showed a greater reversion of the memory-related impairment than the standard drug ($p < 0.05$), observing a similarity in the means of the ALDN and Control groups.

The results of the behavioral tests performed after the training period corresponding to four days, where the acrylic platform transparent and invisible to the animals, remained placed in the quadrant with the signal corresponding to the ball being considered the target quadrant are presented as satisfactory and without significant difference between groups Figure 2A. The use of open field is an evaluation parameter of motor activity and may indicate alteration in motor regulation systems in several areas of the central nervous system. In general, the use of the open field is related to observation of the effects of substances, where the initial test is compared with the test after the duration of a given treatment. In a study conducted with rats to verify the effects of treatment of Efarizenz, a substance commonly used in the treatment of HIV carriers, an increase in the ALE parameter was observed after the 34-day treatment [25]. Other studies conducted to verify the efficacy of pharmacological treatments in animal models with CNS disorders such as anxiety, depression and schizophrenia also used the open field test as a form of evaluation [26, 27].

In a recent study in mice to verify the effects of Alpinia oxyphylla extract (used in Chinese medicine) in a model similar to that used in the present study, LAM was used to verify the cognitive impairment related to the surgical procedure. Thus, a significant difference was observed between the animals submitted to surgery when compared to the control group ($p < 0.001$) [28]. These findings confirm the efficacy of the surgical procedure used in our experiments, where similar results were found. In a study conducted to verify the effects of Lycopene in rats submitted to the surgical procedure of induction by $A\beta_{1-42}$, found results similar to ours in relation to the damages caused by the surgery. The $A\beta_{1-42}$ induction model promoted damage related to memory loss from seven days after surgery in the aforementioned study, as well as in our work [29].

Similar results were observed in a study using Chitosan in the $A\beta_{1-42}$ induction model [30]. Another study corroborated the results found in our research, where the effects of the use of a soybean isoflavone, were tested in an $A\beta_{1-42}$ model that presented similar memory impairment to those obtained in our study [31]. The use of Tamoxifen, a selective modulator of estrogen receptors is intended for the treatment of cancer. Possibly this substance has anti-inflammatory properties acting on the microglia [32]. In a recent study using tamoxifen in Alzheimer-induced animals for $A\beta_{1-42}$, it was observed through behavioral tests, a response similar to that presented in our study, regarding the memory impairment due to the surgical procedure with the $A\beta_{1-42}$ peptide [33]. In a recent study, low levels of testosterone with amyloid plaques were associated as a risk factor for AD. The application of testosterone was used as treatment in animal model induced to DA with $A\beta_{1-42}$. The study demonstrated cognitive losses in animals receiving only the $A\beta_{1-42}$ peptide dilution vehicle compared to the control group receiving only anesthesia, similar to the results obtained in our study. Similar cognitive losses were observed in all groups induced by $A\beta_{1-42}$, and reported as a model causing greater damage in the induction of AD animals [34]. In our study, reversal of cognitive impairment in the LDN-treated group through behavioral testing may occur due to the hypothesis that modulation of the immune system responds to treatment with Naltrexone by means of a compensation mechanism, where endogenous opioid receptors and opioids themselves such as enkephalin and opioid growth factors (OGF) increase their expression when used at low doses, such as 3-4.5mg / day [15, 16].

3.2. Biochemical Assays

Under pathological conditions there may be an imbalance between the production of reactive oxygen and nitrogen species and the antioxidant defenses, promoting the occurrence of oxidative stress. The results of MDA demonstrated that the administration of Donepezil was able to reduce levels of lipid peroxidation when compared to the Alzheimer group and it is also possible to observe that the use of LDN was not able to reduce the levels of MDA, possibly by not acting through mechanisms of antioxidant action, although the behavioral results demonstrate that there was a reversal of cognitive impairment caused by the surgical procedure, as well as a similarity with the control group in the histological slides of all hippocampal regions.

The presence of amyloid plaques may induce oxidative stress and consequently promote mitochondrial dysfunction and lipid peroxidation. Lipid peroxidation is capable of causing destruction to the structural integrity of cell membranes, and is generally presented in brains of patients with neurodegenerative diseases when compared to healthy individuals. Lipid peroxidation can be revealed using several markers, such as thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA) [35]. In a study in AD-induced rats where the antioxidant

effects of vitamins A, e C were observed, it was observed that in animals induced by $A\beta_{1-42}$ the levels of TBARS were found to be elevated when compared to the control group, similar to the results obtained in our study. Vitamin E demonstrated a greater efficacy in reducing MDA levels after the treatment period, data similar to the results found in the Donepezil group in our study [36].

Decreases in GP-x activity result in a decrease in the antioxidant system, evidenced in AD as presented in one study [37]. These data differ from a human study, where serum levels were measured through enzymatic activities. It was observed that although there is a reduction in the activity of GP-x throughout the aging process, patients who presented reduction in the specific memory tests presented elevation of GP-x levels [38]. In a study that utilized Resveratrol as a possible antioxidant agent in the treatment of AD, there were no changes related to EROS decrease as well as changes in the activity levels of antioxidant enzymes such as Catalase, results similar to those obtained in the present study in relation to activity of the catalase enzyme. However, a capacity of reduction of inflammatory mediators, specifically TNF-α, was observed, demonstrating a possible anti-inflammatory activity of this substance [39].

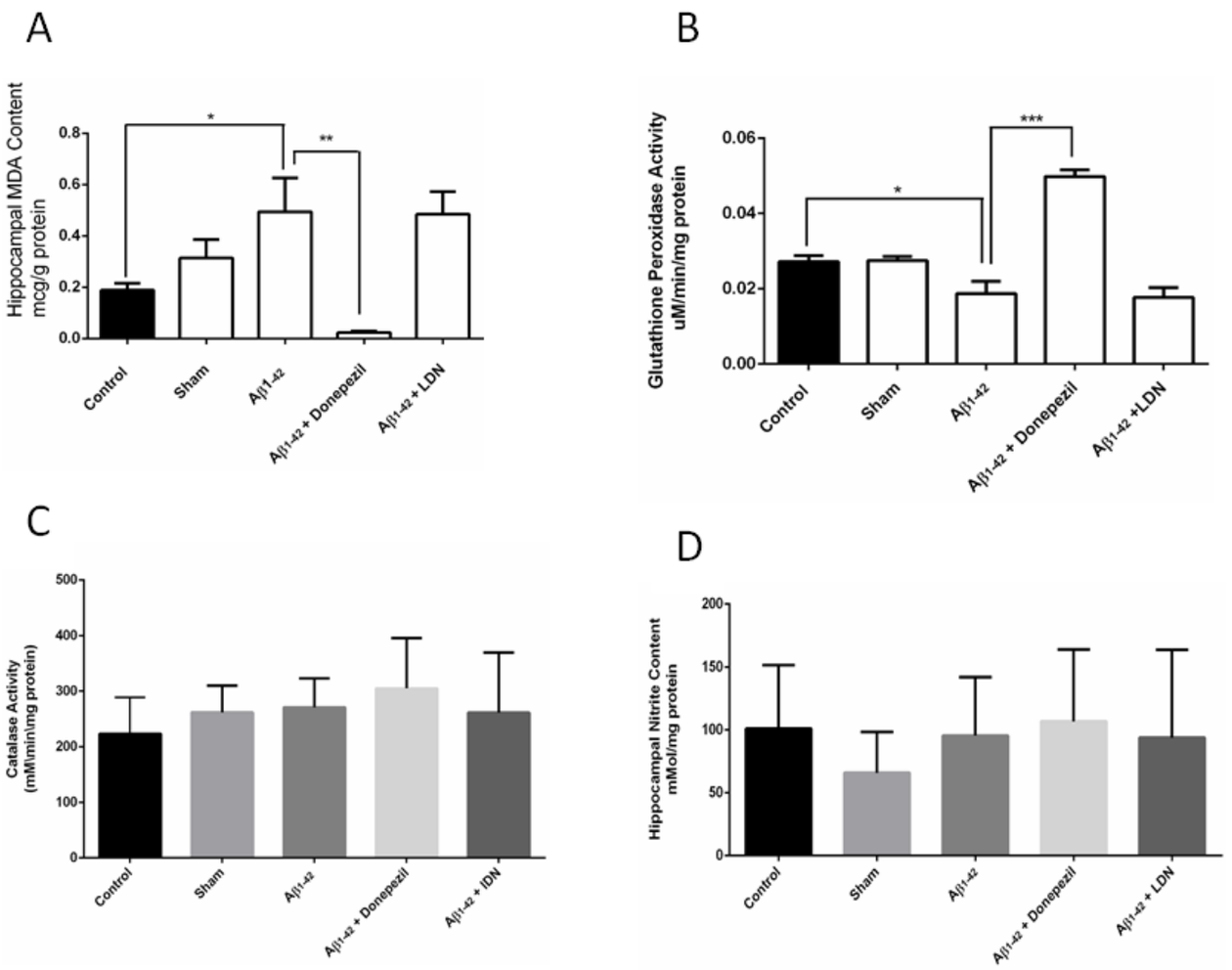

Figure-3. (A) Data related to the results of lipid peroxidation levels (TBARS) in the hippocampus region of animals of all groups. Results presented in MDA levels (malondialdehyde). (B) Data related to the results of enzymatic activity levels glutathione peroxidase (GP-x) in the hippocampus region of animals of all groups. (C) Result of the mean of the enzymatic catalase activity of all animals in the groups. The activity of the catalase enzyme was determined by spectrophotometry. There was no significant difference between the groups when compared to each other on the same days. (D) Data related to the determination of nitrite levels in the hippocampus region of animals of all groups. Nitrite levels were determined by a microplate reader. There was no significant difference between the groups when compared to each other on the same days. It was considered * = $p < 0.05$ (95%) ** = $p < 0.01$ (99%); **** = $p < 0.001$ (99.99%).

Orientin (ORI) is a flavonoid component found in abundance in the shell of passion fruit and bamboo leaves, presenting a long history in Asian medicine for possibly exerting antioxidant properties [40]. In a study with animals treated for a period of 15 days intraperitoneally, there was improvement in memory parameters when tested by LAM, as well as reduction in MDA levels [19]. These results are similar to the results found in the Donepezil group and demonstrate that LDN treatment does not promote the same results as an acetylcholinesterase inhibitor such as Donepezil promotes, suggesting that its action is not through antioxidant activity.

In a study with mice induced to the AD model, by surgery similar to that carried out in our study, a reduction in nitrite levels was observed when treated with synaptic acid, the most significant component extracted from the canola seed, possibly having antioxidant effects and anti-inflammatory drugs [41]. In contrast to the aforementioned study, it was not possible to verify a significant difference in nitrite levels when comparing the groups in our study. It is possible to verify a reduction in the NIT mean of the animals treated with LDN compared to those that were submitted to treatment with Donepezil, but did not express significant differences.

3.3. Histopatological Changes

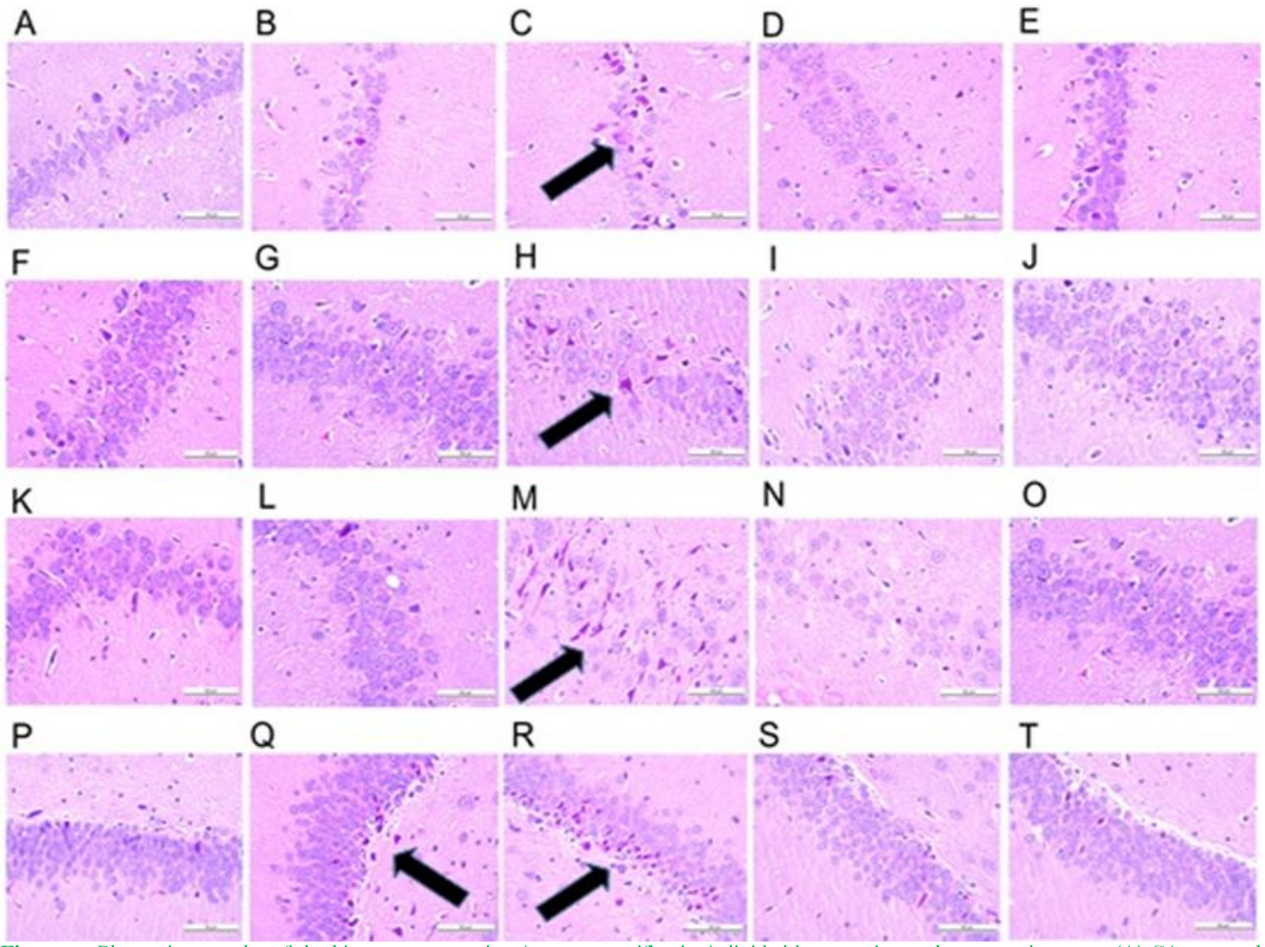

Figure-4. Photomicrography of the hippocampus region (x 400 magnification) divided by experimental areas and groups. (A) CA1 control group. (B) CA1 Sham group. (C) CA1 water-treated Aβ1-42 group. (D) CA2 group Aβ1-42 treated with Donepezil (E) CA1 group Aβ1-42 treated with LDN (F) CA2 control group (G) CA2 Sham group (H) CA2 group Aβ1-42 treated with water. (I) CA2 group Aβ1-42 treated Donepezil (J) CA2 group Aβ1-42 treated LDN (K) CA3 control group (L) CA3 Sham group (M) CA3 group $A\beta_{1-42}$ treated water (N) CA3 group $A\beta_{1-42}$ treated with Donepezil (O) CA3 LDN-treated group $A\beta_{1-42}$ (P) Gyrus control group (Q) gyrus group Sham (R) gyrus group Aβ1-42 treated with water. (S) dendritic group Aβ1-42 treated with Donepezil. (T) LDN treated group $A\beta_{1-42}$ gyrus. The black arrows indicate degeneration in the pyramidal neurons.

The definitive diagnosis for AD is performed using the structural analysis after the death of the patient. Due to the specific alterations of the pathology, the presence of amyloid plaques and neurofibrillary tangles of phosphorylated Tau protein, the structural study is necessary for the alterations to be confirmed. Through HE staining it is also possible to observe changes in the neuronal forms of specific regions of the hippocampus, a region related to memory and learning. Morphological changes are one of the main characteristics related to cellular apoptosis, being the main form of cell death [42]. When neurons in the hippocampus region change, they are possibly followed by dysfunctions and consequent pathologies such as dementias, including AD [6]. In our study, it is possible to verify changes in the pyramidal form, characteristic of the neurons of the hippocampus region. These changes suggest the possibility of neuronal death, a characteristic that configures AD.

In a study that used vitamin P to verify possible neuroprotective effects in a rat model induced by AD, it was possible to verify structural changes similar to what was found in our study, neuronal loss and changes in the pyramidal form of neurons of the hippocampus region by through the observation of photomicrographs [20]. To corroborate the above, scientific evidence demonstrates the association of memory-related cognitive impairments with neuronal damage possibly caused by the $A\beta_{1-42}$ accumulation of animals also submitted to the surgical procedure similar to the one performed in our study, through the verification of neuronal losses and structural alterations through histological analysis when Ibuprofen was used as a treatment for AD-induced by $A\beta_{1-42}$ [18].

Changes in pyramidal forms of neurons in the hippocampus region become more evident in the AD-induced group by $A\beta_{1-42}$ and treated only with water. Thus, in our study, treatment with Donepezil and LDN demonstrated a similarity observed in the histological comparison with the control group. The mechanisms by which these treatments possibly reduced or reversed the morphological changes may follow hypotheses related to antioxidant action and modulation of the immune system, respectively. In a study on the use of LDN, a blockade in the production of TNF-α in a murine cell model was reported, suggesting the possibility of LDN therapy to affect different endogenous opioids such as endorphins, although not measured in the study [18].

4. Conclusion

In conclusion, with regard to the treatment substances used, both Donepezil and LDN were able to reverse the memory deficit in the treated groups, but in a very interesting way, the LDN presented a better effect than the drug base of Donepezil treatment in the obtained results in the behavioral test of Morris Aquatic Labyrinth. Regarding the results obtained through the neurochemical tests, it was possible to observe that Donepezil

promoted reduction in MDA levels and that the use of low doses of Naltrexone was not able to promote changes in relation to oxidative stress in the hippocampus region. Regarding the structural analyzes in the hippocampus region, in the different groups through observation of histological slides by staining of Hematoxylin and Eosin, it was possible to conclude that the alterations in relation to the structural conformation of the pyramidal neurons occurred in the Alzheimer group and that although the groups induced for Aβ1-42 but treated with Donepezil and low doses of Naltrexone did not present such changes, resembling the Control group.

It is known that although the results presented in the behavioral and histological tests of the use of low doses of Naltrexone are satisfactory, they are not sufficient to consider adequate for the treatment of Alzheimer's Disease. Thus, other experimental research is needed to prove its efficacy and by what mechanisms of action this substance acts so that possibly in the near future is considered a possible therapy in Alzheimer's Disease.

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