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Effects of α-Humulene and its Nanoparticles in Experimental Model of Alzheimer's Disease: Behavioral Analysis and Anti-Inflammatory Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim was to analyze the effects of α-humulene in experimental models of Alzheimer's disease (AD). A sample composed of 33 rats, divided into the NC group: 3 animals, for analysis of normal tissue and behavior, PC: 10 animals submitted to induction of AD by application of β-amyloid, without treatment, HUM: 10 animals submitted to induction of AD and treated with α-humulene, and NHUM: 10 animals submitted to induction of AD and treated with α-humulene nanoparticles. Treatment was performed once daily for 14 days at a dose of 6.5µg, orally. The animals were submitted to behavioral tests of spatial memory and aversive memory, the cytokines TNF, IL-10, IL-2 and IL-4 were analyzed by flow cytometry. The treated animals obtained superior cognitive performance and expressed lower levels of inflammatory markers, with better results in the group with better results in the NHUM.

Keywords: α-humulene; Alzheimer's; nanoparticles; cytokine; neuroinflammation.

1. INTRODUCTION

Alzheimer's disease (AD) is the most common age-related pathology, affecting approximately 50 million people worldwide [1]. It is defined as a progressive neurodegenerative disorder that causes physical and mental decline gradually, leading the individual to death [2].

Despite the variety of studies related to AD, the etiology remains poorly understood [3]. However, the pathophysiology of AD can be characterized by some factors, such as the abnormal processing of amyloid precursor protein (APP), causing extracellular deposition of β-amyloid peptide (βA), the loss of cholinergic neurons, and the formation of tau protein neurofibrillary tangles [2].

Senile plaques are the first features presented by the brain affected by AD, these are formed by the aggregation of βA, a product of APP metabolism. The deposition of senile plaques and neurofibrillary tangles are responsible for the process of synaptic degeneration in the hippocampus and neocortex. The plaques are produced externally to the neurons, where βA surrounds the neuron and causes its death. Neurofibrillary tangles occur due to hyperphosphorylation of tau protein, thus paired helical filaments are developed within neurons, resulting in disorganization of tubular cytoarchitecture [4,5].

Studies already indicate the relationship of the activation of the immune system triggering inflammation with the progression of neuropathological changes observed in Alzheimer's disease. In this process of inflammation, in order to play a neuroprotective role, microglia release pro-inflammatory agents and other toxic agents, such as reactive oxygen species, nitric oxide, and cytokines [6]. Activation of microglia and astrocytes, which possess transmembrane receptors recognizing pathogen-associated molecules and endogenous substances, induces the production of various cytokines [7].

In addition, exposure to β-amyloid plaques contributes to the intensified production of cytokines, among them interleukins (IL-1β, IL-6, IL-12) and Tumor Necrosis Factor alpha (TNF-α). Thus, the evolution of the disease is also intensified, mainly by the presence of interleukin 1 beta (IL-1β) for example, responsible for stimulating the production of amyloid precursor protein (APP), which results in increased deposition of β-amyloid substance, promote the phosphorylation of tau protein, which results in the formation of more neurofibrillary tangles, and also continue the process of activation of more microglia and astrocytes, producing more cytokines [7].

Cognitive deficits presented by AD patients include impairments in learning, recall of recently learned information, significant difficulties related to visuospatial functions (object agnosia, impaired face recognition, simultanagnosia, and alexia), language functions (word search), and

executive functions (reasoning, judgment, and problem solving) [8].

Regarding treatment, although current drugs can temporarily reduce and delay AD symptoms, they cannot prevent the progression of brain damage; moreover, the presence of the blood-brain barrier hinders the delivery of these drugs. Thus, nanotechnology-oriented drug delivery strategies may be a suitable approach, as the development of different types of drug-loaded nanocarriers result in efficient mechanisms that can reduce the production, aggregation, and deposition of βA, and tau protein neurofibrillary tangles [9].

The α-humulene is a sesquiterpene found in the essential oil of different aromatic plants, such as *Cordia verbenacea* and *Pinus halepensis* [10,11]. This compound stands out for its anti-inflammatory effect [12] but in addition, antioxidant and neuroprotective properties have been attributed to essential oils containing α-humulene in its composition, involving the reduction of the activity of cholinesterases (AChE and BChE) [13].

Studies of this compound in neuroprotection and as an anti-neuroinflammatory agent are scarce in the literature. In this context, the aim of the study was to analyze the effects of α-humulene and its nanoparticles in an experimental model of Alzheimer's disease.

2. PURPOSE

2.1 General

To analyze the effects of α-humulene and its nanoparticles in experimental models of Alzheimer's disease.

2.2 Specifics

Analyze the effects of α-humulene and its nanoparticles on spatial memory and aversive memory in Alzheimer's disease; verify anti-inflammatory actions; analyze and demonstrate the need for new drugs to treat Alzheimer's disease.

3. METHODOLOGY

3.1 Sample

The sample was composed of 33 rats of the Rattus Norvegicus breed, Wistar line, weighing between 300-350 grams, from the UEM's Animal Facility. Four animals were kept per cage, made of acrylic, unbreakable, self-washable. The cages with the animals were arranged on shelves, kept in a room under a 12-hour light/dark cycle at a temperature of 23±1 ºC, controlled by 7000BTU sprint air conditioning. The animals had free access to water and food.

3.2 Experimental Groups

The animals were divided into four groups: Negative Control Group (NC): consisting of 3 animals for testing the predominance of normal tissues; Positive Control Group (PC): composed of 10 animals with lesion in area CA1, without treatment and with collection of material and euthanasia at the end of experimental procedures; α-humulene treated group (HUM): composed of 10 animals with lesion in area CA1 and treated with 6.5μg of α-humulene dissolved by gavage for 14 days and with collection of material and euthanasia at the end of experimental procedures. Group treated with α-humulene nanoparticles (NHUM): composed of 10 animals and treated with 6.5μg of α-humulene by gavage for 14 days and with collection of material and euthanasia at the end of the experimental procedures.

3.3 Surgical Procedure

The animals were anesthetized with 80mg/Kg ketamine hydrochloride to 15mg/Kg xylazine hydrochloride via intraperitoneal. They were soon taken to an extereotaxic apparatus (David Kopf, USA) where their heads were fixed by the petrous temporal bone and upper incisors under coordinates AP= -3.0mm, ML= 1.6mm, -1.6mm and DV= 3.0mm, taking the bregma as reference, receiving 4μl of β-amyloid peptide via Hamilton syringe in the CA1 region of the hippocampus for the process of senile plaque development [14]. After the induction of the neuroinflammation and neurofibrillary process, the animals were rested for a period of 30 days for the inflammatory and neurodegenerative processes of hippocampal neurons to occur.

3.4 Post-surgical Analgesia

For post-surgical analgesia, tramadol hydrochloride was used at a dose of 10 mg/kg every 12 hours orally for 7 days [15]. The animals received the treatment orally (gavage) 6.5μg of a solution of α-humulene (Sigma Aldrich) and 6.5μg of α-humulene nanoparticles according to each group.

3.5 Obtaining the Nanoparticles

The formulation of the nanoparticles was carried out at the Unicentro's Pharmaceutical Nanotechnology Laboratory.

3.6 Behavioral Testing

3.6.1 Spatial memory - morris water maze

This test evaluated the animal's ability to acquire spatial memory, measuring the latency for the animal to locate a platform submerged in a tank of dull water [16]. The animals were trained in an adapted version of the water maze task, where the animals were randomly released at one of the starting points, forcing the animal to orient itself by the spatial relations among the cues to find the platform that remained fixed in the same place throughout the experiment. Each animal was trained 5 times, before the experimental surgery (baseline) and after the treatment period. The acquisition of spatial memory was evaluated after the end of the training.

3.6.2 Aversive memory

The animals were trained on the conditioned fear task. Briefly, this task uses a training chamber (model MED-VFC2- SCT-R, Med Associates Inc., St. Albans, Vermont 05478), which consists of an aluminum box (35 x 35 x 35 cm) with a floor made of parallel stainless steel bars spaced 0.8 mm apart. This training box is located inside a larger, acoustically isolated box to mitigate interference from external sounds. On training days, the animal freely explored the interior of the training chamber for 5 minutes. Next, the animal received the conditioned stimulus, a fast light stimulus. After the conditioned stimulus, the animal received the unconditioned stimulus, a shock for 2 s, 0.5 mA. The triggers of the conditioned and unconditioned stimuli are manually controlled. Long-term memory of acquired conditioning was evaluated 5 days after training by placing the animal again in the training chamber, triggering the conditioned stimulus (light), but this time without the unconditioned stimulus (shock). The rat remained in this chamber for 5 minutes after the end of the conditioned stimulus. The mnemonic performance was measured and expressed as the time the animal remained in a state of paralysis ("freezing"), obtained from the filming by a video camera attached to the top of the larger box. Freezing behavior is associated as an indicator of fear [17].

The trainings were performed before the experimental surgery (baseline) and post treatment period.

3.7 Euthanasia

The animals were anesthetized with 80 mg/kg ketamine hydrochloride and 15 mg/kg xylazine hydrochloride, after the anesthetic state was verified, 175mg/kg Pentobarbital was injected intraperitoneally [18].

3.8 Histology

After euthanasia, the animals were decapitated and then had their encephalons removed. The encephalons were initially cut near the region of the lesion and placed in 15% formalin. The samples were taken to the pathology laboratory, embedded in paraffin, cut at 2 micrometers and stained with Hematoxylin and Eosin (H.E.).

3.9 Flow Cytometry

Two mL of blood was collected from each animal and subsequently centrifuged at 1500 rpm for 10 min at room temperature. After centrifugation the supernatant (serum) was pipetted and separated for analysis. The kits used for the analysis were BD™ Cytometric Bead Array Mouse Th1/Th2 Cytokine Kit (Becton Dickinson, USA) and the BD™ Cytometric Bead Array Mouse Inflammation Kit (Becton Dickinson, USA), the cytokines analyzed were TNF, IL-10, IL-4 and IL-2.

According to the manufacturer's instructions and analyzed in the BD™ Accuri C6 Flow Cytometer (Becton Dickinson, USA), 10.0 µL of each reagent was added to each sample. After this procedure, 50.0 µL of the cytokine beads, 50.0 µL of the sample (serum), 50.0 µL of detection reagent was placed in a 2.0 mL Eppendorf for each sample; the tubes were placed in the dark for two hours at room temperature. After the two hours, 1.0 mL of the wash buffer was added to each Eppendorf and centrifuged at 200 G, 4°C, for 5 minutes. After centrifugation the supernatant was carefully removed and discarded from each sample, and then 300.0 µL of the wash buffer was added to each tube to re-suspend the samples.

The reading in the cytometer was performed manually by acquiring 10,000 events from each sample. The flow cytometry data were analyzed in FCap 3.0 Array software (Becton Dickinson,

USA) and the results were plotted in graphs of means and standard deviations from the mean.

3.10 Statistical Analysis

The data obtained were arranged in spreadsheets, analyzed by GraphPad Prism 7 software. For Gaussian analysis the Shapiro Wilk test was used. For parametric samples One-Way with Tukey's post-test was used and for non-parametric samples Mann Whitney's U-test and Kruskal-Wallis test with Dunn's post-test was used. All tests adopted $p < 0.05.0$

4. RESULTS AND DISCUSSION

Fig. 1 shows a photomicrograph of the CA1 region of the hippocampus of all groups. It can be seen that senile plaques were present in the groups submitted to induction of AD (PC, HUM and NHUM), except in the NC group, which was used to demonstrate the predominance of normal tissue in the same region.

Fig. 2 shows the means and standard deviation for each group for the test that aimed to assess spatial memory. Significant differences were obtained between the PC and NC (baseline (0.0342), PC (baseline) and NHUM (post-treatment) (0.0375), and NHUM (baseline) and NHUM (post-treatment) (0.0348) groups.

Firstly, it can be observed that the PC group presented a higher mean time than the NC group after induction of AD (only in the PC group), indicating that there was spatial memory impairment in the groups submitted to induction. Then, comparing the treated groups with the PC group, post induction and treatment, it is possible to notice that the HUM group presented a reduced time for learning related to spatial memory, as well as the NHUM group, with an even lower average time. Thus, it is believed that the treatment had a positive influence on this aspect.

Fig. 3 shows the means and standard deviation for each group for the test that evaluated aversive memory. Significant differences were obtained between the groups HUM and NC (post treatment time) (0.0005), and NHUM and NC (post treatment time) (0.0244).

First, it can be observed that the PC group (post-treatment time) had a lower mean than the PC group (baseline), indicating that before the induction of AD, the animals remained longer in freezing due to the memory of the fear condition generated in this test model, which can be confirmed by the higher mean values in the NC group. Regarding the treatment, it was observed that the HUM group showed higher mean time than the PC group after the treatment time, as well as the NHUM group, which showed better results, suggesting that in these cases, despite the memory impairment, the animals performed better than the animals that were not treated.

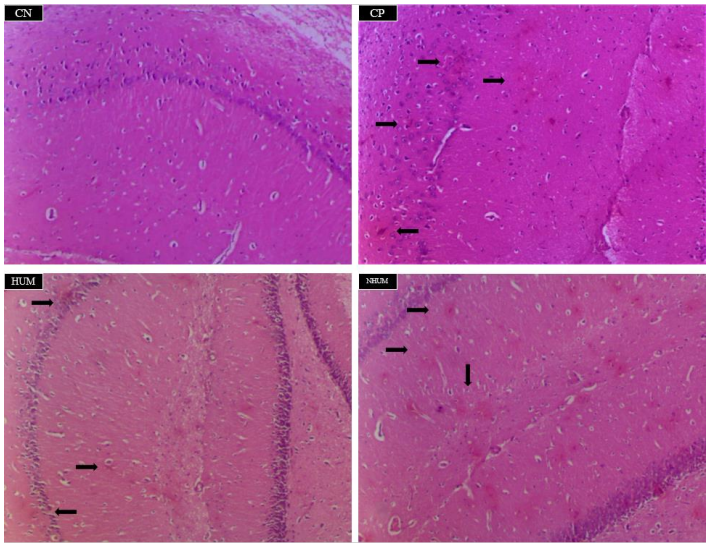

Fig. 1. Photomicrograph of the CA1 region of the hippocampus from each group (NC, PC, HUM, NHUM). Panoramic view, light microscopy, 40x magnification. Hematoxylin Eosin staining

Note: arrows indicate presence of senile plaques in the groups submitted to induction of AD

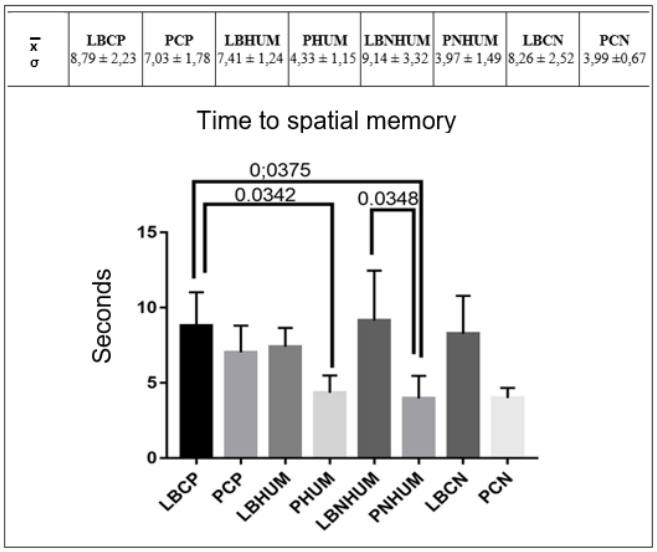

Fig. 2. Representation of the means and standard deviation of the groups in the spatial memory test before Alzheimer's induction (except NC group) and post treatment

LBCP= baseline PC group, PCP= post treatment time, PC group, LBHUM= baseline HUM group, PHUM= post treatment HUM group, LBNHUM= baseline NHUM group, PNHUM= post treatment NHUM group, LBCN= baseline NC group, PCN= post treatment time NC group

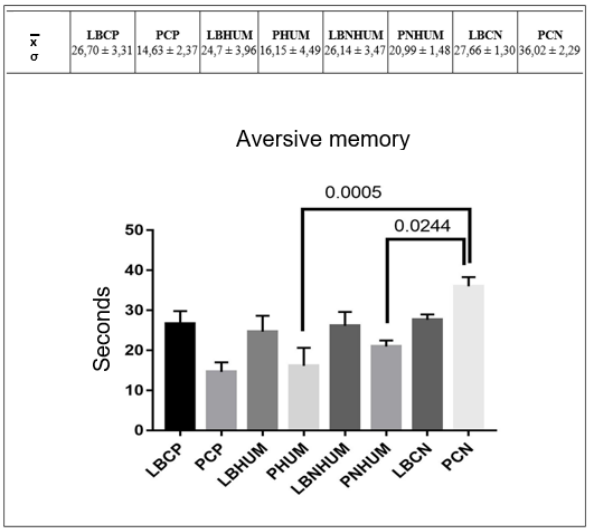

Fig. 3. Representation of the means and standard deviation of the groups in the aversive memory test before Alzheimer's induction (except NC group) and post treatment

LBCP= baseline PC group, PCP= post treatment time, PC group, LBHUM= baseline HUM group, PHUM= post treatment HUM group, LBNHUM= baseline NHUM group, PNHUM= post treatment NHUM group, LBCN= baseline NC group, PCN= post treatment time NC group

Fig. 4 shows the means and standard deviation for each group regarding the expression of tumor necrosis factor that was used to evaluate neuroinflammation. Non-significant differences were obtained, numerically, between the HUM and NC groups (0.0001), and the same difference was obtained between the NHUM and NC groups (0.0001).

According to the analysis, the PC group had a higher mean expression of the pro-inflammatory cytokine than the other groups, treated or not treated with neuroinflammation induction. This indicates that the treated groups, both the HUM group, which had a slightly higher average than the NHUM, and the NHUM group itself had positive results with the treatment, since the

inflammation marker TnF had a greater action in the PC groups, while in the HUM and NHUM treated groups the inflammatory cascade was less intense.

Fig. 5 shows the means and standard deviation for each group regarding the expression of interleukin 10, which also aimed to evaluate neuroinflammation. Non-significant differences were obtained, numerically, between the HUM and NC groups (0.0001), as well as the difference between the NHUM and NC groups (0.0008).

According to the analysis, the PC group presented a higher mean anti-inflammatory cytokine than the other groups, treated or untreated, that did not undergo neuroinflammation induction. This indicates a greater action of IL-10 in untreated animals, with the objective of regulating the immune response, and thus reducing potentially damaging inflammatory responses. Cytokine levels were lower in the treated groups, the HUM group, which had a slightly higher mean than the NHUM, and also the NHUM group itself, due to a good treatment result, not requiring such an intense IL-10 action in the anti-inflammatory process.

Fig. 6 shows the means and standard deviation for each group, referring to the expression of interleukin 2, which also had the purpose of evaluating neuroinflammation. Significant differences were obtained between groups PC (5.60) and NC (2.97), HUM (3.72) and NHUM (3.20), post-treatment.

The PC group showed higher mean pro-inflammatory cytokine expression than the other groups, treated or untreated, that did not undergo neuroinflammation induction. This expression is indicative of a greater action of IL-2 in untreated animals, aiming to regulate immune response by increasing inflammatory responses. Cytokine levels were lower in the treated groups, with the HUM group having a higher mean than the NHUM group.

The low expression of IL-2 in the treated groups may be due to the pharmacological anti-inflammatory reaction, since the inflammatory markers need to be present, but at controlled levels. The difference of the treated groups to the PC group is indicative of treatment efficacy.

Fig. 7 shows the averages and standard deviation for each group regarding the expression of interleukin 4 in order to evaluate neuroinflammation. The difference between groups PC (6.94) and NC (3.80), HUM (4.84) and NHUM (6.15), post-treatment, is remarkable.

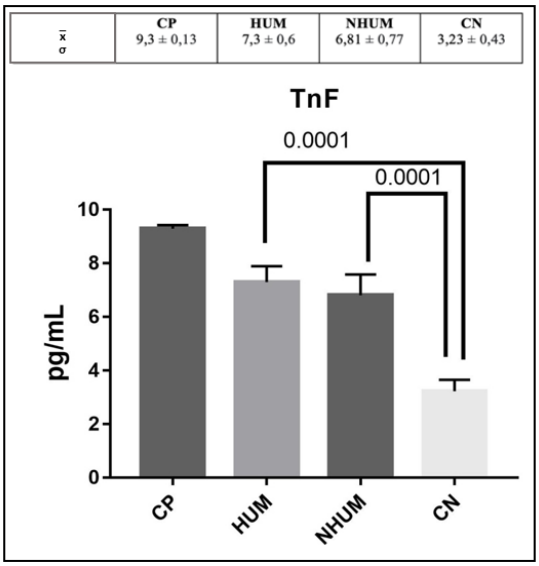

Fig. 4. Representation of the means and standard deviation of the groups in tumor necrosis factor (TnF) expression in the post-treatment of CNS neuroinflammation induced by beta-amyloid1-42 (except NC group)

PC = positive control group, HUM = α-humulene treated group, NHUM = α-humulene nanoparticle treated group, NC = negative control group

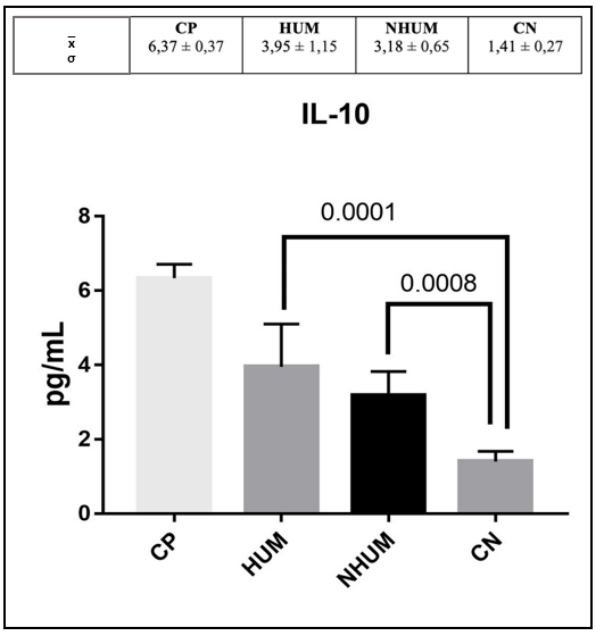

Fig. 5. Representation of the means and standard deviation of groups on interleukin 10 (IL-10) expression in the post-treatment of beta-amyloid1-42-induced CNS neuroinflammation (except NC group)

PC= positive control group, HUM= α-humulene treated group, NHUM= α-humulene nanoparticle treated group, NC= negative control group

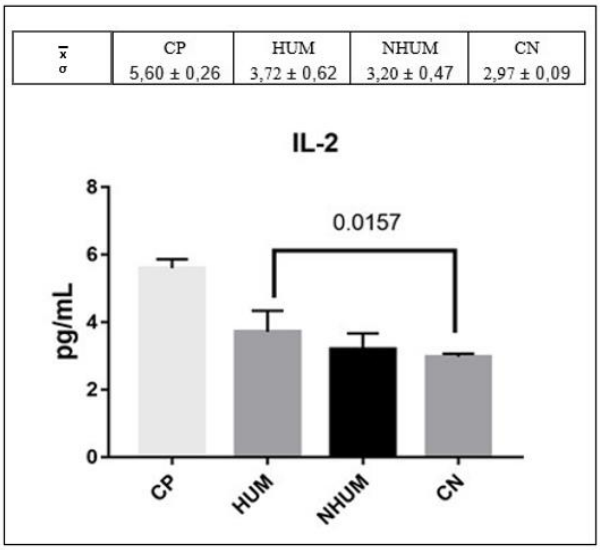

Fig. 6. Mean and standard deviation representation of groups on interleukin 2 (IL-2) expression in the post-treatment of Central Nervous System neuroinflammation, induced by beta-amyloid1-42 (except NC group)

PC= positive control group, HUM= α-humulene treated group, NHUM= α-humulene nanoparticle treated group, NC= negative control group

The PC group showed higher mean expression of the anti-inflammatory cytokine than the other groups, treated or untreated. This expression is indicative of a greater action of IL-4 in the animals that did not receive treatment, due to the permanence of the inflammatory process in this untreated group. The mean of the treated groups is lower, with the NHUM group higher than HUM and NC group with the lowest expression.

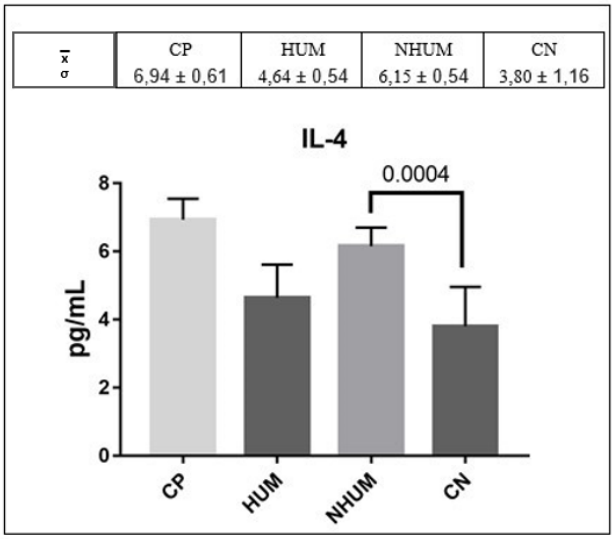

Fig. 7. Mean and standard deviation representation of groups on interleukin 4 (IL-4) expression in the post-treatment of Central Nervous System neuroinflammation, induced by beta-amyloid1-42 (except NC group)

PC= positive control group, HUM= α-humulene treated group, NHUM= α-humulene nanoparticle treated group, NC= negative control group

The lower levels of IL-4 expression in the treated groups indicate that the anti-inflammatory process has already occurred, and that the groups are now in a more advanced stage of resolution of the neuroinflammation, given the difference in markers at this particular moment of the analysis. The group without treatment has a later resolution of the inflammatory process in relation to the treated groups, therefore concluding that the treatment was efficient in accelerating the anti-inflammatory process.

According to Arya [19], poor cholinergic transmission at the synapse is one of the main factors for AD progression, and molecules such as sesquiterpenes, due to their great structural diversity, may be useful in inhibiting acetylcholine (ACh) degradation. In this context, the compound α-humulene was used as a treatment in order to verify whether its isolated activity may be responsible for the already reported neuroprotective effects of essential oils.

Postu et al. [11] reported that essential oils may be a natural treatment option for AD. The authors identified the effects of *Pinus halepensis* essential oil, 1% and 3%, administered for three weeks, in an experimental model with acute toxicity caused by βA 1-42. The animals underwent behavioral tests and biochemical analyses of brain homogenates for acetylcholinesterase (AChE) and oxidative stress biomarkers were also performed. The essential oil was able to reverse βA 1-42-induced cognitive deficits, restore oxidant-antioxidant balance, and reverse AChE action in the hippocampus.

In the study by Arruda et al. [20] the AChE inhibition and the antioxidant and cytotoxic activities of the essential oils from the leaves of *Hedychium gardnerianum*, a plant that also has α-humulene, were analyzed. AChE inhibition was reported and this action was attributed to the sesquiterpenes present in more than 60% of the oils' composition. Such outcomes support the application of essential oils in the treatment of diseases that trigger cognitive impairment, such as AD, due to their ability to increase acetylcholine levels and also to combat oxidation responsible for the degeneration of neurons.

As observed in Postu's study [11], the present study also showed positive results regarding the reduction of cognitive deficits, since it obtained improvements in both spatial memory and aversive memory tests, suggesting the involvement of α-humulene, since this compound is also found in the essential oil of *Pinus halapensis*. Furthermore, it corroborates with Arruda's study, since α-humulene is also a constituent of *Hedychium gardnerianum*, indicating that the cognitive improvement seen in behavioral tests may be related to increased ACh and less neuronal degeneration.

Da Silva et al. [21] identified that in the essential oil of *Piper hispidum* the α-humulene is among the main constituents, this species was reported to be one hundred times more active than physostigmine in inhibiting AChE, the first drug used in the treatment of AD, [22].

This class is used due to the degeneration of cholinergic neurons in the limbic and cerebral cortex, and presents positive effects in relation to cognitive disorders, being able to delay the progression of the disease, providing a better quality of life for patients [23,24]. In addition, these drugs can reduce βA deposition [7].

Regarding the inflammatory process, the deposition of β-amyloid, characteristic of Alzheimer's disease neuropathology, results in the activation of microglia, which alongside astrocytes, is responsible for releasing inflammatory mediators, such as cytokines and chemokines, that are important in the inflammatory cascade and also in the recruitment process of important cells acting in the immune defense, such as lymphocytes, monocytes, and neutrophils [25,26]. The production of these inflammatory mediators is essential for tissue repair, and also serves as a neuroprotective factor, but it is important that there is a balance between the pro-inflammatory and repair processes, because a prolonged inflammatory situation can generate a negative feedback loop in which the continuous activation of inflammatory factors may cause neuronal damage [27].

The first neuroinflammatory signals coordinate nuclear factor kappa B (NF-κB), a transcription factor responsible for the expression of genes that encode pro-inflammatory cytokines, such as tumor necrosis factor (TNF). In a positive feedback loop, TNF ends up amplifying and perpetuating inflammation by activating NF-κB, indirectly participating in the induction of other inflammatory cytokines, and interacting with receptors that promote apoptosis [28]. Following the principle of the need for balance in the release of inflammatory mediators, some studies have already demonstrated the neurotoxic capacity of TNF by potentiating the neurodegenerative process of excitotoxicity, causing neuronal death, through the intense activation of the excitatory neurotransmitter glutamate [29].

Meanwhile, interleukin 10 (IL-10), an anti-inflammatory cytokine, is considered a control mechanism between neuroprotection and neurodegeneration. One of its suppressive actions is related to the inhibition of the production of pro-inflammatory cytokines, among them TNF [30,29]. In CNS pathologies, it is common for IL-10 levels to increase, acting on the inflammatory response and protecting neurons from excitotoxicity [26]. Interleukin-2 is pro-inflammatory, produced mainly by activated type 1 T lymphocytes (Th0 and Th1), neuroinflammatory signals are stimuli for its production (IFN-a and IL-1). Interleukin 4, an anti-inflammatory cytokine, is considered a control mechanism between neuroprotection and neurodegeneration. IL-4 is the much studied suppressor molecule in its regulatory function of the inflammatory cascade. It is synthesized by T-lymphocytes-CD4, mast cells, eosinophils and basophils [31].

The IL-2 expressed at lower levels in the treated groups possibly represents the pharmacological anti-inflammatory reaction, considering the controlled presence of inflammatory markers, this difference in the result of the groups that received treatment and the treated group is suggestive and a possible effectiveness in the treatment. In contrast, IL-4, also expressed at lower levels in the treated groups, possibly indicates a more resolutive and advanced stage of the anti-inflammatory process, also aiming at the difference of markers at this particular moment that the analysis occurred.

The same occurred with TNF expression, which indicates the action of a pro-inflammatory marker in the inflammatory cascade of the neuroinflammation induction process, and the group without treatment was the one with the highest expression of this cytokine, which may indicate the efficacy of the anti-inflammatory action of α-humulene. As a consequence of the increase in TNF levels, an increase in IL-10 levels was observed in the groups that presented β-amyloid-induced neuroinflammation. Regarding IL-10 levels, they were also higher in the animals that did not receive treatment, regulating the action of TNF, in order to control potentially damaging inflammatory responses, and the treated groups, probably due to a good treatment outcome, did not require such intense action of the anti-inflammatory cytokine IL-10.

In a general analysis, the treated groups expressed the inflammatory mediators, IL-2, IL-4, TNF, and IL-10, in lower intensity, indicating that the degree of induced neuroinflammation was

lower compared with the PC group, the untreated group. In addition, the groups treated with α-humulene nanoparticles showed lower expression of cytokines compared to the other group treated with α-humulene, which may indicate a more effective action with the use of nanoparticles, since these are developed in order to provide advantages, either by physical-chemical properties that can influence the absorption capacity, or by mechanisms that interfere with the duration of action, and other pharmacokinetic properties [32].

However, despite advances in pharmacology and understanding AD at the systemic and molecular level, novel therapies that can slow pathological progression are not yet available and existing treatment has extremely limited effect and high liver toxicity [33,7].

For this reason, many studies have reported the promising effects of phytocompounds and medicinal plant extracts, which can stimulate and/or recover cognitive functions because of antioxidant and anti-inflammatory properties. These compounds can inhibit βA aggregation, TAU protein hyperphosphorylation, and cytokine production [33],7]. Moreover, they have the advantage of causing fewer adverse effects and the possibility of having their efficacy and safety improved through structural modifications and formulation studies [33]. Therefore, associating therapeutics with nanotechnology becomes a possible solution to obstacles when it comes to the use of drugs in the treatment of neurodegenerative diseases. This is because nanoparticles are able to provide greater efficacy with lower dose, while improving the bioavailability and kinetic profile of these drugs [34,35].

5. CONCLUSION

It was concluded that treatment with α-humulene provided cognitive improvements related to spatial and aversive memory possibly by indirectly increasing acetylcholine through inhibition of the enzyme acetylcholinesterase. Regarding the anti-inflammatory potential the results showed promising effects due to lower expression of the pro-inflammatory cytokines TNF and IL-2 and controlled expression of IL-4 and IL-10 (anti-inflammatory cytokines). As for treatment with nanoparticles, a superior effect was observed, thus, studies are suggested to analyze the effects through different mechanisms, at different doses, exploring both the therapeutic effects of α-humulene and also of its nanoparticle form.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experiments were conducted in accordance with the approval by the ethics committee on the use of animals, protocol number: 009/2021.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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