



The therapeutic effect of the oily extract of Cannabis sp. in Aluminum Chloride-Induced Alzheimer's Disease in Rats

O efeito terapêutico do extrato oleoso de Cannabis sp. na doença de Alzheimer induzida por cloreto de alumínio em ratos

El efecto terapéutico del extracto oleoso de Cannabis sp. en la enfermedad de Alzheimer inducida por cloruro de aluminio en ratas

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ABSTRACT

Objective: To evaluate the therapeutic potential of Cannabis sp extract in a model of Alzheimer's disease induced by aluminum chloride in rats. **Methods:** Thirty Wistar rats were divided into five groups (n=6) where one group received no treatment, another group received only aluminum chloride and the others, in addition to aluminum chloride, received different doses (50, 100 and 150µl) of oily extract of Cannabis sp. The animals were euthanized one day after treatment, where the brain was collected, fixed, processed and embedded in paraffin. Histological sections of the hippocampus were used for qualitative and quantitative assessment of morphological changes. Blood serum was also obtained to determine indicators of oxidative status. **Results:** Reduction of more than 80% in cell death in the dentate gyrus region of the hippocampus in animals treated with cannabis extract. Furthermore, a reduction in total protein levels by approximately 20% and an increase (15%) in antioxidant enzymes in relation to the positive control were also observed in the serum. **Conclusion:** The use of the oily extract of Cannabis sp. in a 2:1 ratio of delta-9-tetrahydrocannabinol (THC): Cannabidiol (CBD) effectively reduced hippocampal neuron deaths from amyloidosis induced in chronic aluminum chloride intoxication.

Keywords: Alzheimer's disease, Aluminum chloride, Phytocannabinoids, Delta-9-tetrahydrocannabinol (THC): Cannabidiol (CBD).

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RESUMO

Objetivo: Avaliar o potencial terapêutico do extrato de Cannabis sp em modelo de Doença de Alzheimer induzida por cloreto de alumínio em ratos. **Métodos:** Trinta ratos Wistar foram divididos em cinco grupos (n=6) onde um dos grupos não recebeu nenhum tratamento, outro grupo recebeu apenas cloreto de alumínio e os demais, além do cloreto de alumínio, receberam diferentes doses (50, 100 e 150µl) de extrato oleoso de Cannabis sp. Os animais foram submetidos à eutanásia um dia após o tratamento, onde o cérebro foi coletado, fixado, processado e incluído em parafina. Cortes histológicos do hipocampo foram utilizados para avaliação qualitativa e quantitativa das alterações morfológicas. Soro sanguíneo também foi obtido para determinar indicadores do estado oxidativo. **Resultados:** Redução de mais de 80% da morte celular na região do giro denteado do hipocampo dos animais tratados com o extrato de cannabis. Além disso, também foi observada no soro uma redução nos níveis de proteínas totais em aproximadamente 20% e um aumento (15%) nas enzimas antioxidantes em relação ao controle positivo. **Conclusão:** O uso do extrato oleoso de Cannabis sp. em uma proporção de 2:1 de delta-9-tetrahydrocannabinol (THC): Canabidiol (CBD) reduziu efetivamente as mortes de neurônios do hipocampo por amiloidose induzida na intoxicação crônica por cloreto de alumínio.

Palavras-chave: Doença de Alzheimer, Cloreto de alumínio, Fitocannabinóides, Delta-9-tetrahydrocannabinol (THC): Canabidiol (CBD).

RESUMEN

Objetivo: Evaluar el potencial terapéutico del extracto de Cannabis sp en un modelo de enfermedad de Alzheimer inducida por cloruro de aluminio en ratas. **Métodos:** Treinta ratas Wistar fueron divididas en cinco grupos (n=6) donde uno de los grupos no recibió tratamiento, otro grupo recibió solo cloruro de aluminio y los demás, además de cloruro de aluminio, recibieron diferentes dosis (50, 100 y 150µl) de extracto oleoso de Cannabis sp. Los animales fueron sometidos a eutanasia un día después del tratamiento, donde se recolectó el cerebro, se fijó, procesó e incrustó en parafina. Se utilizaron secciones histológicas del hipocampo para la evaluación cualitativa y cuantitativa de los cambios morfológicos. También se obtuvo suero sanguíneo que se usó para determinar indicadores del estado oxidativo. **Resultados:** Reducción de más del 80% de la muerte celular en la región del giro dentado del hipocampo de los animales tratados con el extracto de cannabis. Además, también se observó en el suero una reducción de los niveles de proteínas totales en aproximadamente un 20% y un aumento (15%) de las enzimas antioxidantes en comparación con el control positivo. **Conclusión:** Que utilizando el extracto oleoso de Cannabis sp. en una proporción de 2:1 de delta-9-tetrahydrocannabinol (THC): Cannabidiol (CBD) redujo efectivamente las muertes de neuronas del hipocampo por amiloidosis inducida en la intoxicación crónica por cloruro de aluminio.

Palabras-clave: Enfermedad de Alzheimer, Cloruro de aluminio, Fitocannabinoides, Delta-9-tetrahydrocannabinol (THC): Cannabidiol (CBD).

INTRODUCTION

Studies have shown that individuals who ingested foods with high aluminum (Al) contents were twice as likely to develop Alzheimer's disease (AD) (ROGERS M, 1999). This is because Al salt causes selective neuronal loss and loss of cholinergic function and the β -amyloid accumulation acts as a toxin for neurons and affects neuron-neuron communication (AHANGARPOUR A, et al., 2015) similar to Alzheimer's disease (AL-OKBI SY, et al., 2017; YIN Z, 2020).

Alzheimer's disease (AD) is a pathology that causes the development of dementia, especially in the elderly, and has become very prevalent, affecting millions of people worldwide (GHUMATKAR PJ, et al., 2015). AD involves cognitive dysfunction related to memory, behavior, judgment, orientation, and reasoning

(TANZI RE e BERTRAM L, 2005). Currently, the conventional treatments for Alzheimer's patients are anticholinergics, which have only palliative and not curative properties.

In the last decades, there has been a growth for molecules that can be used as targets to improve cognition, preventing AD (LOERA-VALENCIA R, et al., 2019). Among these possible treatments demonstrated, Cannabis has been widely reported due to its antioxidant properties. Cannabis (*Cannabis sativa* L.) plants, from the family Cannabidaceae, and there are three known subspecies: *Cannabis sativa* ssp. *sativa* L., *Cannabis sativa* ssp. *ruderalis* Janisch and *Cannabis sativa* ssp. *indica* Lam., although, in some studies, these plants are classified as distinct species (KOPUSTINSKIENE DM, et al., 2022). Cannabis is a plant of over 1,000 chemical constituents, of these 177 are phytocannabinoids (PCBN) (HANUŠ LO and HOD Y, 2020).

The phytocannabinoids Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and cannabidiol (CBD) are the most abundant and exert different pharmacological effects (POLLASTRO F, et al., 2017). The biological properties of these compounds are associated with their various interactions with the endocannabinoid system in humans (PISANTI S, et al., 2017). THC is a product strictly controlled in great part of the world due to its psych activity (HAO F and FENG Y, 2021) on the other side cannabidiol (CBD) is a non-psychoactive phytocannabinoid known for its beneficial effects, including antioxidant and anti-inflammatory properties (SILVESTRO S, et al., 2020).

The combination of CBD and THC may have specific pharmacological effects because CBD can enhance the potential therapeutic benefits of THC through pharmacokinetic interactions (DRYBURGH LM, et al., 2018). In this context, it has been suggested that co-administration of CBD may alter the pharmacological profile of THC by attenuating some undesirable effects of this cannabinoid. Hence, the present study aims to evaluate the therapeutic potential of Cannabis sp extract in the aluminum chloride chronicle intoxication, an Alzheimer's disease model assay.

METHODS

Animals

Thirty male Wistar rats were used so that there was no interference of the estrous cycle, with 90 days of age, young adults, so that there was no interference of degenerative lesions by age. The animals were kept on a light/dark cycle (12/12), with free access to water and food. The protocol adopted is by the Brazilian guide for Animal Experimentation (COBEA), filed and approved by the Committee on Ethics in the Use of Animals (CEUA) under number 3001170720. The experiment was carried out in the vivarium of the Department of Animal Morphology and Physiology of the University Federal Rural de Pernambuco (DMFA-UFRPE).

Drugs and herbal medicines

The drugs used in this experimental model were aluminum chloride ($AlCl_3$), the oily extract of Cannabis was obtained from the Associação Brasileira de Apoio Cannabis Esperança – ABRACE.

Experimental induction of Alzheimer's disease

For the induction of Alzheimer's disease, the animals were treated for 21 consecutive days with 100 mg/kg of aluminum chloride ($AlCl_3$) via gavage (LAKSHMI BVS, et al., 2015).

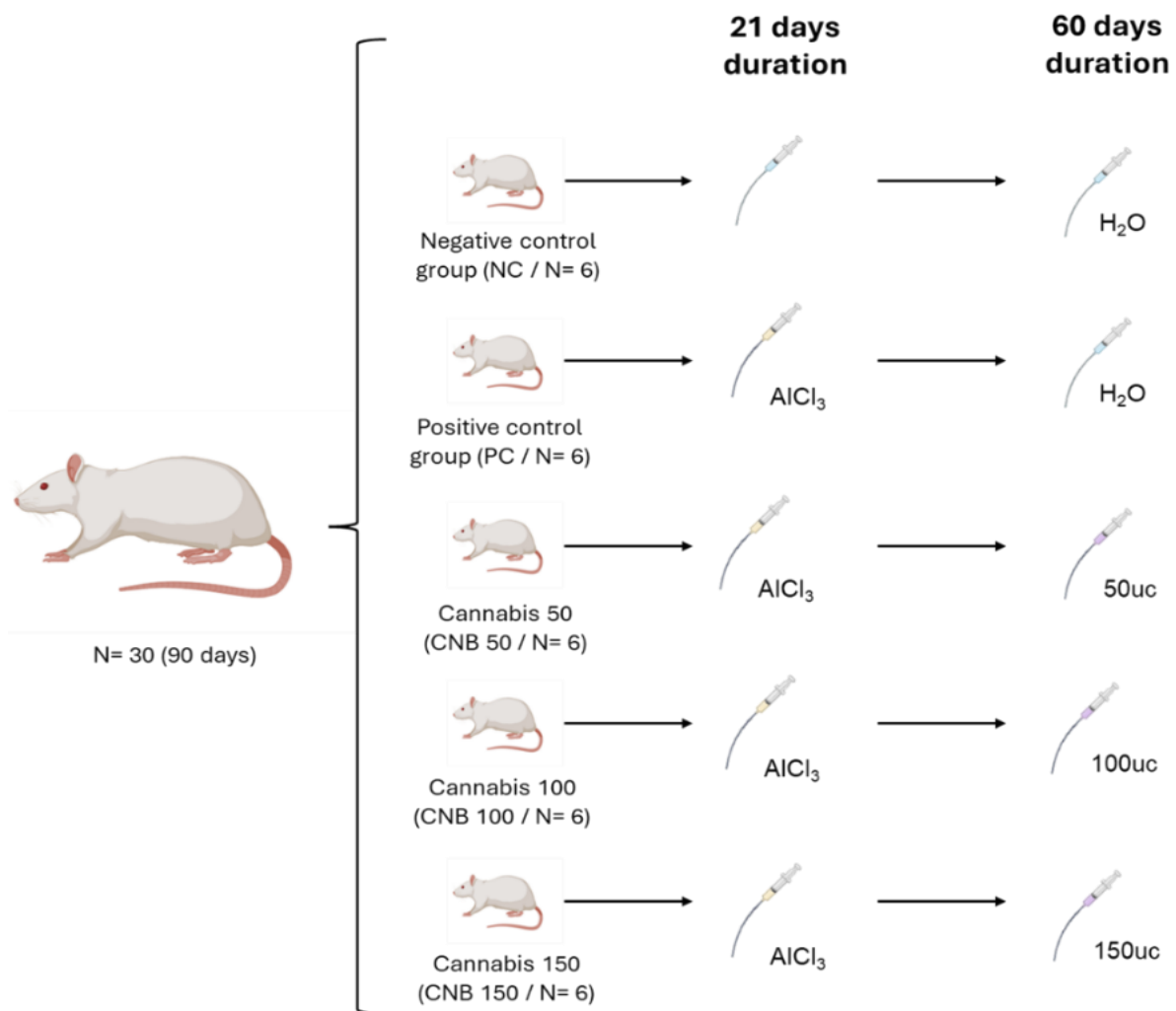
Experimental groups

The animals were divided into five groups (n=6, 270 g). The negative control group (NC) did not receive any treatment, and it just went through the containment and gavage procedure to produce the same stress as the other groups; The positive control group (PC) had AD induction (21 days receiving 100mg/kg of $AlCl_3$) but received no treatment; The Cannabis 50 (CNB 50) group had AD induced (21 days receiving 100mg/kg of $AlCl_3$) and then treated with cannabis extract 50 μ L/animal for 60 days; The Cannabis 100 (CNB 100) group had AD induced (21 days receiving 100mg/kg of $AlCl_3$) and then treated with cannabis extract 100 μ L/animal

for 60 days; The Cannabis 150 (CNB 150) group had AD induced (21 days receiving 100mg/kg of $AlCl_3$) and then treated with cannabis extract 150 μ L/animal for 60 days. Being the administered dose of an oily extract of Cannabis sp. at a ratio of approximately 2:1 (THC: CBD) twice a day, at 6:00 am and 6:00 pm via gavage (Figure 1).

One day after the experimental period, the animals were weighed submitted to blood collection and the procedures for performing euthanasia. Initially, propofol (8mg/kg) was administered intraperitoneally for induction, and soon after, an association of ketamine (20mg/kg) and xylazine (4mg/kg) intraperitoneally, to deepen until the confirmation of death (CFMV - Federal Council of Veterinary Medicine). After verification of death, the blood was removed via cardiac puncture, placed in 2mL tubes with separator gel, centrifuged in a refrigerated centrifuge, and stored at -80°C until the time of analysis. An autopsy of the animals was performed to collect the brain. The organ was fixed in 10% buffered formalin.

Figure 1 - Experimental design showing the steps for inducing Alzheimer's disease with $AlCl_3$ gavage for 21 days and treatment with different doses of the CBD:THC combination for 60 days, twice a day.



Source: Silva RN, et al., 2024. Figure created with Biorender.

Histological processing

The material intended for histology was dehydrated in an increasing series of ethanol and embedded in paraffin. Semi-serial sections of 4 μ m were obtained using a rotating microtome (RM 2255, Leica Biosystems, Nussloch, Germany), respecting an interval of at least 40 μ m between sections. The histological preparations

were stained with hematoxylin and eosin for histopathological analysis and Congo red for identifying the amyloid substance in a polarized light microscope performed at Instituto Aggeu Magalhães. Photomicrographs obtained from an image capture system coupled to a Leica model DM500E microscope were used for the morphometric analyses. All images were analyzed using Image J® software (National Institute of Health, USA).

Confirmation of induction of Alzheimer's Disease

Confirmation of AD was done by quantifying the amount of beta-amyloid, and this quantification was done in two ways:

Quantification of Serum β Amyloid

The β A content was evaluated in the serum of the animals using the commercial Rat β A42 ELISA Kit (Elabscience) as described in the kit insert.

Quantification of β Amyloid in nervous system

The quantification of areas marked by Congo red was performed in photomicrographs at 40x magnification. These areas were quantified by pixel count. The stained areas were selected using the color distribution as a discriminant parameter. After defining the color range by trial and error, a mask was applied to derive the area and percentage of beta-amyloid. A computerized histophotometry technique was used (OBERHOLZER M, et al., 1996). A total area of $30 \times 104 \mu\text{m}^2$ was quantified for each group.

Congo red color

Staining was performed according to Stoopler (STOOPLER ET, et al., 2003) for observation of amyloid substance, observed through a microscope with polarized light model Leica/DMi8 carried out at Instituto Aggeu Magalhães (FIOCRUZ – PE). One hundred photomicrographs were taken at 10x and 40x magnification for qualitative analysis.

Determination of indicators of oxidative stress

Moments before euthanasia, blood was collected by cardiac puncture, 2mL from each animal, placed in tubes with clot activator, centrifuged in a cooled centrifuge, and, after separation of the clot from the serum, the serum was frozen at -80° until the analysis day.

Samples were homogenized in potassium phosphate buffer (pH 7.4, 0.2 M), containing 1 M EDTA, using a homogenizer (OMNI) and centrifuged ($13,800 \times g$ at four $^\circ\text{C}$ for 10 min). NO production was quantified indirectly through nitrite/nitrate by the standard Griess reaction (RICART-JANÉ D, et al., 2002). SOD activity was determined according to Siddiqui IA, et al. (2005).

Total protein concentration was quantified using bovine serum albumin as the standard curve (LOWRY OH, et al., 1951). The absolute levels of MDA in each sample were determined using a standard curve from known concentrations of 1,1,3,3-tetra methoxy propane (TMPO) (WALLIN B, et al., 1993). Analyzes were performed in duplicate. All enzyme activities were determined using an ELISA reader (ThermoScientific, Waltham, MA, USA).

Histopathological and Histomorphometric Evaluation

The hippocampus regions (C1, C2, C3, C4, and dentate gyrus) were photomicrographed using an optical microscope (DM500-Leica) coupled to a digital video camera (ICC50E-Leica). Photomicrographs were evaluated using image analysis software (LASEZ 4.11 and Image J). The ratio of live/necrotic neurons was determined (MC V, et al., 2005).

A qualitative evaluation of the regions mentioned above was also performed to determine the intensity and the most frequent injuries in the different experimental groups. In the histomorphometric evaluation, dead or degenerated neurons were counted in four individuals from each group.

The raw values were transformed into lesion intensity or “scores” such that up to 100 cells (0) no change, up to 500 cells (+) mild, up to 1000 (+) moderate cells, above 1000 cells (+) ++ intense.

Statistic

The results were evaluated for normality using the Shapiro-Wilk test, followed by an analysis of variance (ANOVA) and the Student Newman-Keus test. STATISTICA for WINDOWS 3.11 software was used. Pearson's correlation was used to assess the relationship between two variables. Differences were significant when $P < 0.05$. All results were expressed as mean and standard deviation. In addition, to assess the diversity between treatments and define the variables that most contributed to the separation of groups, principal component analysis (PCA) was performed.

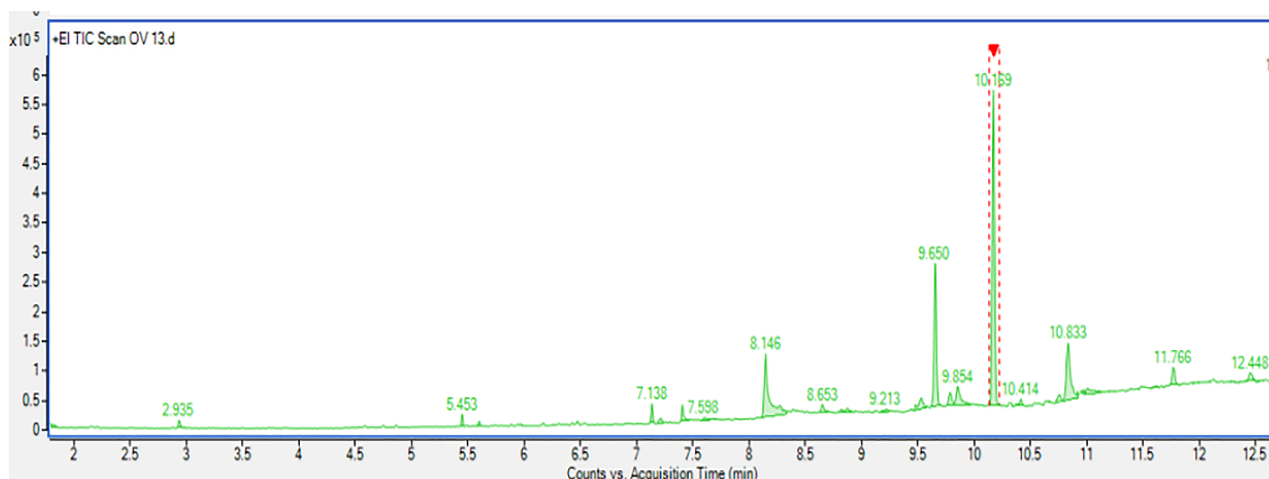
The variables were centered and scaled before the examination. To explore the possible relationships between the variables, exploratory linear correlations (Pearson). Principal component analysis was performed using R Software (RStudio version 4.0.2, 2020).

RESULTS

Extract phytochemistry

The oily extract of Cannabis sp. contained cannabidiol CBD (2.66 mg/mL) and tetrahydrocannabinol (5.34 mg/mL), confirming the 2:1 ratio of THC:CBD. This information was obtained from the gas chromatography method coupled to mass spectrometry – an in-house method (**Figure 2**). Analysis carried out in the laboratory of the federal police in Recife - PE.

Figure 2 - HPLC result of the main compounds of the oil extract of Cannabis sp (CBD and THC) used in the present study. Analysis carried out in the laboratory of the federal police in Recife - PE.

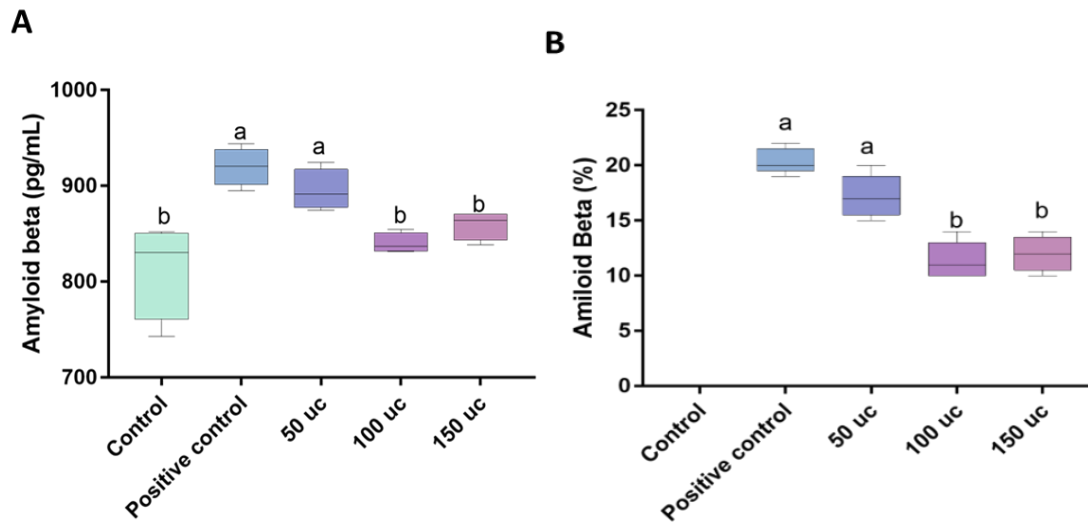


Source: Silva RN, et al., 2024.

Induction of Alzheimer's Disease

In the serum dosage of β -amyloid, an increase of 13% of the peptide was observed in the positive control group, concerning the negative control, and an increase of 3% in the positive control concerning the 50 μ l group, 9% concerning the 100 μ l group, and 7 % concerning the 150 μ l group (**Figure 3A**). The quantification of tissue amyloid beta, through Congo red staining, maintained the same response between the groups, the positive control group had an increase in the marked area while the treated groups reduced it to the positive control group (**Figure 3B**).

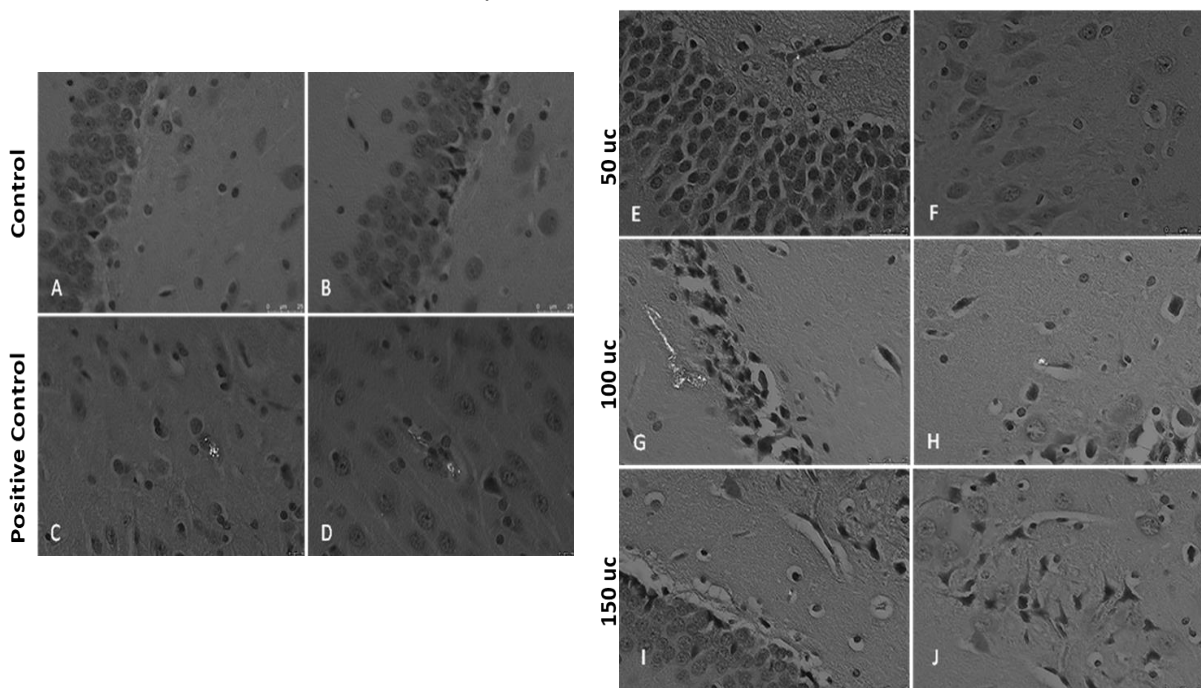
Figure 3 - Serum β -amyloid levels of mice experimentally induced for Alzheimer's and treated with Cannabis sp extract. in a 2:1 THC: CBD ratio.



Source: Silva RN, et al., 2024. Values are mean \pm SD. Different letters (a, b) = $P < 0.05$ (ANOVA and Student Newman Keuls Test).

In evaluating samples stained in Congo red, the lesions caused by the deposition of β -Amyloid protein were confirmed through the fluorescence produced by polarized light microscopy (**Figure 4**). The frequency at which the lesions were observed followed the same pattern as the other analyses: no lesions were observed in the negative control; in the positive control, there was a marked presence of fluorescent structures; in the others, some lesions were observed, however, at a reduced intensity concerning positive control.

Figure 4 - Histological photomicrographs stained in Congo red of rats experimentally induced for Alzheimer's and treated with Cannabis sp extract. in a 2:1 THC: CBD ratio.

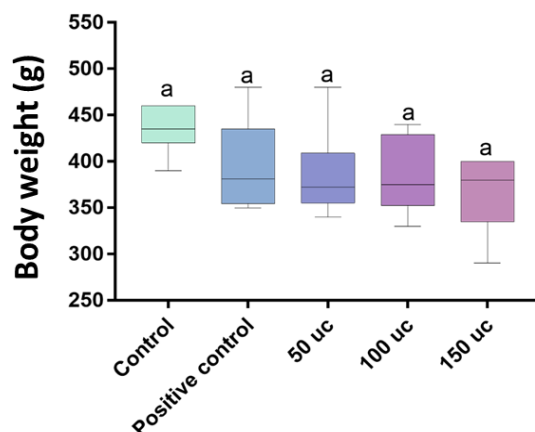


Source: Silva RN, et al., 2024.

Body biometrics

There was no change in the animal body weight in any experimental group (**Figure 5**).

Figure 5 - Graph representing the average weight of the animals on the day of euthanasia in animals induced with Alzheimer's disease and treated with cannabis.

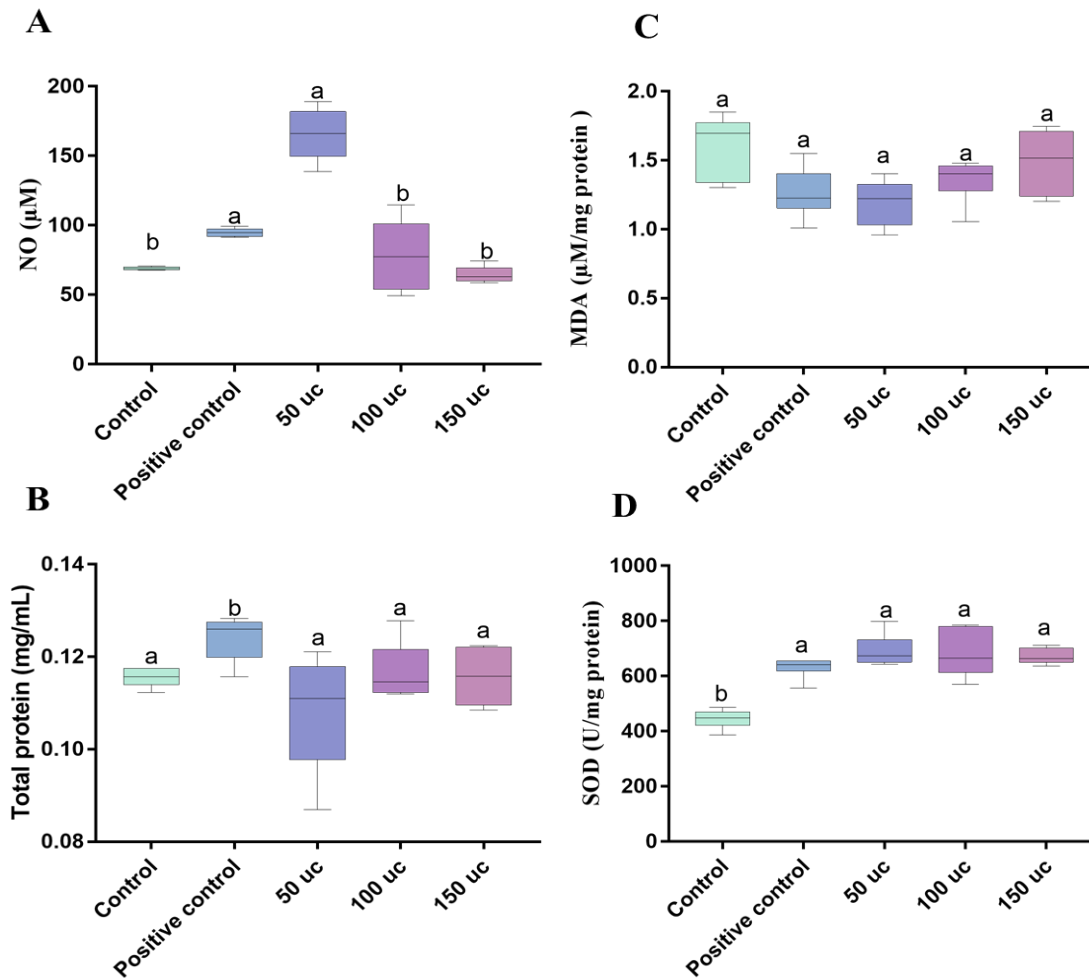


Source: Silva RN, et al., 2024. NC – Negative control; CP – Positive control of experimentally induced Alzheimer's mice; 50µl – Group induced al-Alzheimer and treated with 50µl of Cannabis extract in mice experimentally induced for Alzheimer's and treated with Cannabis sp extract. in a 2:1 THC: CBD ratio. 100µl – Group induced al Alzheimer and treated with 100µl of cannabis extract in mice experimentally induced for Alzheimer and treated with extract of Cannabis sp. in a 2:1 THC: CBD ratio. 150µl – Group induced al Alzheimer and treated with 150µl of cannabis extract in mice experimentally induced for Alzheimer and treated with extract of Cannabis sp. in a 2:1 THC: CBD ratio. Values are mean \pm SD. Different letters (a, b) = $P < 0.05$ (ANOVA and Student Newman Keuls Test).

Oxidative stress

NO levels increased by 37% and 140% in the Positive Control and CNB 50 groups, respectively (**Figure 6A**). Total Protein levels increased by 22% in the positive control group (**Figure 6B**). MDA levels did not change between the experimental groups (**Figure 6C**), and SOD activity increased by 15% in the CBN 150 group and approximately 10% in CBN 50 and 100 (**Figure 6D**).

Figure 6 - Evaluation of malondialdehyde, nitric oxide and total protein levels, and plasma superoxide dismutase activity in animals induced with Alzheimer's disease and treated with cannabis.

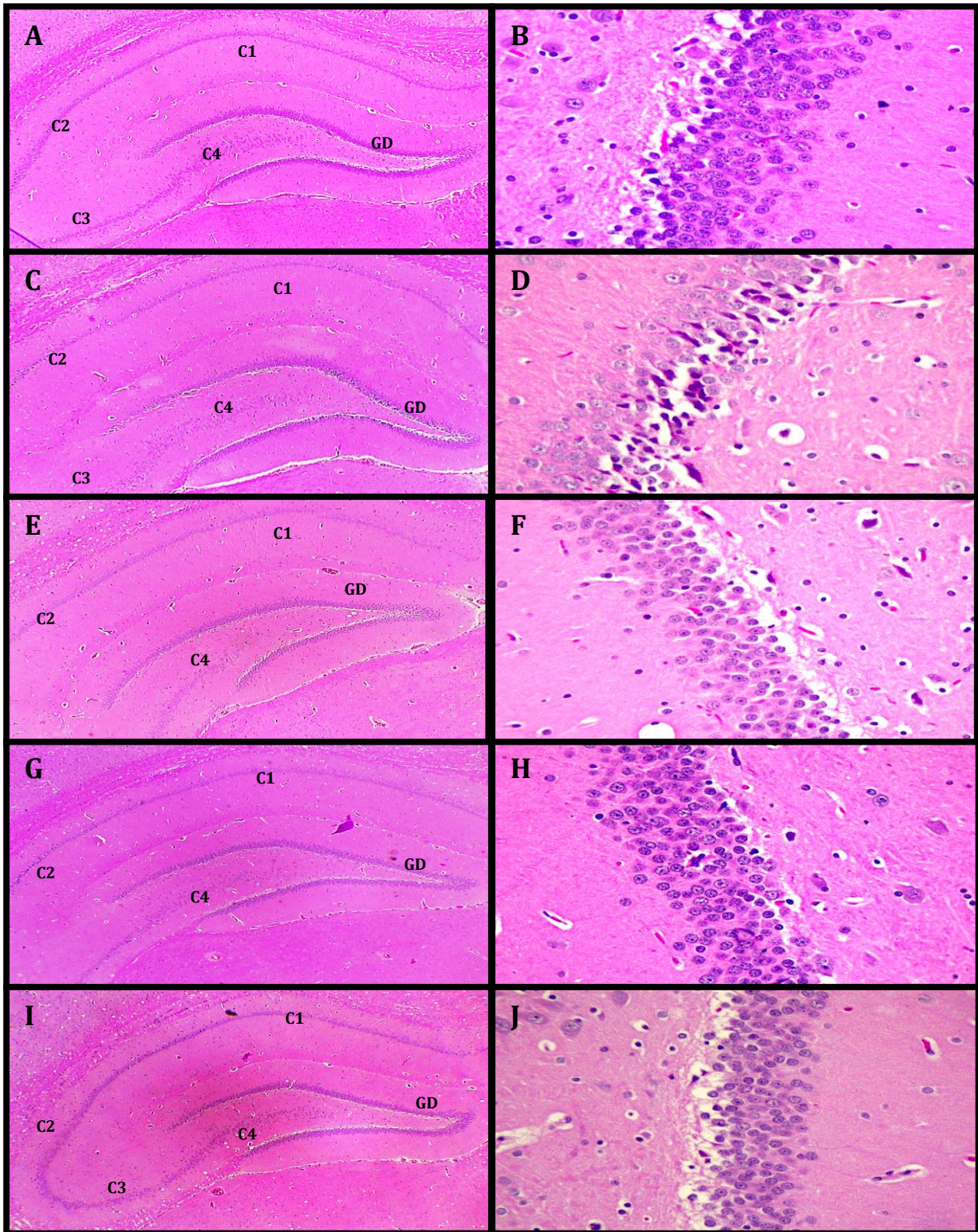


Source: Silva RN, et al., 2024. NC – Negative control; CP – Positive control of experimentally induced Alzheimer's mice; 50µl – Group induced al-Alzheimer and treated with 50µl of Cannabis extract in mice experimentally induced for Alzheimer's and treated with Cannabis sp extract. in a 2:1 THC: CBD ratio. 100µl – Group induced al Alzheimer and treated with 100µl of cannabis extract in mice experimentally induced for Alzheimer and treated with extract of Cannabis sp. in a 2:1 THC: CBD ratio. 150µl – Group induced al Alzheimer and treated with 150µl of cannabis extract in mice experimentally induced for Alzheimer and treated with extract of Cannabis sp. in a 2:1 THC: CBD ratio. MDA – Malondialdehyde, NO- nitric oxide, SOD – superoxide dismutase, PTN – Total protein. Values are mean ± SD. Different letters (a, b) = P<0.05 (ANOVA and Student Newman Keuls Test).

Histopathology

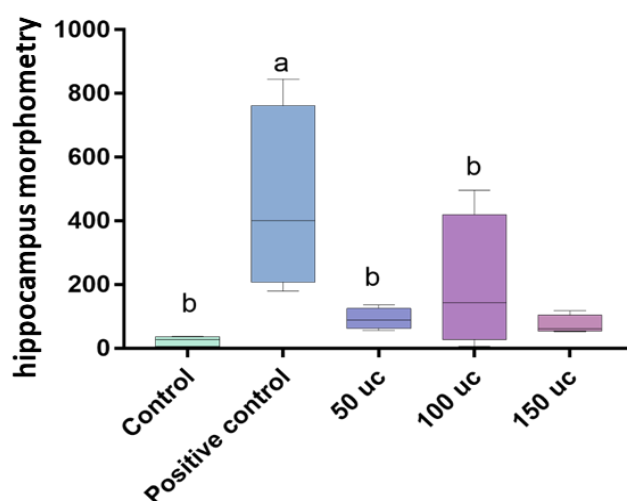
In the negative control group, more than 100 cells were not counted. Thus, it was considered without alteration. The most intense score (+++) was observed in the positive control group, justified by the positive control group. Among the treatment groups, the 50µl and 150µl groups obtained a discrete score (+), whereas, in the 100µl group, the score was moderate (++). The positive control had a 90% increase in cell death concerning the negative control group in the statistical analysis. Concerning the treated groups, the 50µl group had an 80% reduction, the 100µl group reduced by 56%, and the 150µl group decreased by 83% concerning the positive control (**Figures 7 and 8**).

Figure 7 - Hippocampus of mice experimentally induced for Alzheimer's and treated with extract of Cannabis sp. in a 2:1 THC: CBD ratio.



Source: Silva RN, et al., 2024. (A and B) Negative control. (C and D) Positive control. (E and F) Group 50µl. (G and H) Group 100µl. (I and J) Group 150µl. Staining (HE).

Figure 8 - Hippocampus morphometry in animals induced with Alzheimer's disease and treated with cannabis.



Source: Silva RN, et al., 2024. NC – Negative control; CP – Positive control of experimentally induced Alzheimer's mice; 50µl – Group induced al-Alzheimer and treated with 50µl of Cannabis extract in mice experimentally induced for Alzheimer's and treated with Cannabis sp extract. in a 2:1 THC: CBD ratio. 100µl – Group induced al Alzheimer and treated with 100µl of cannabis extract in mice experimentally induced for Alzheimer and treated with extract of Cannabis sp. in a 2:1 THC: CBD ratio. 150µl – Group induced al Alzheimer and treated with 150µl of cannabis extract in mice experimentally induced for Alzheimer and treated with extract of Cannabis sp. in a 2:1 THC: CBD ratio. Values are mean ± SD. Different letters (a, b) = P<0.05 (ANOVA and Student Newman Keuls Test).

Main component analysis

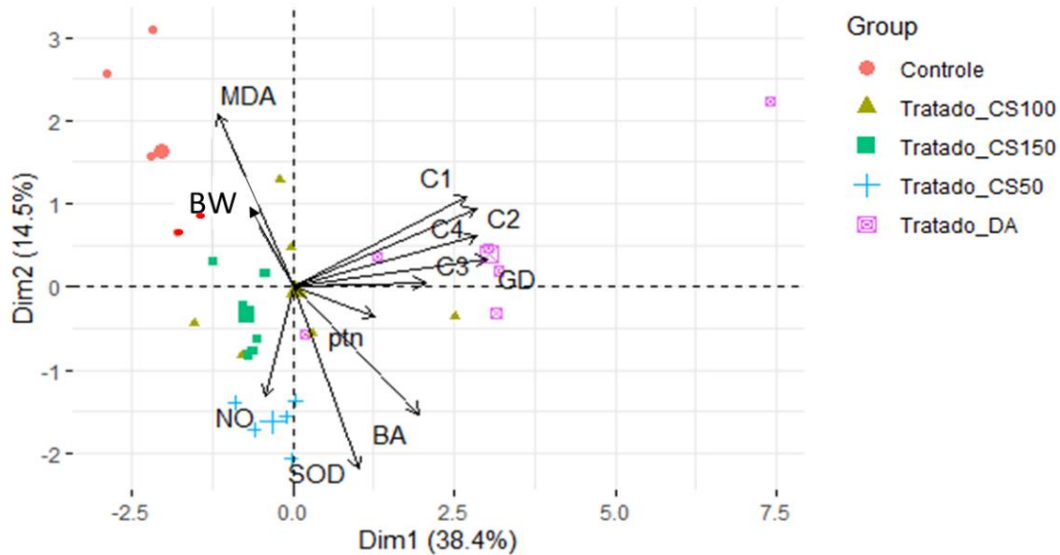
Principal component analysis (PCA) was performed to verify possible clusters and define the most critical variables when separating groups that received different treatments. Thus, the PCA of the analyzed parameters of Wistar rats submitted to AD and treated with varying doses of cannabis explained 52.9% of the data variability in the first two dimensions.

Principal component 1 (PC1) summarized 38.4% of the data and separated the cannabis-treated and negative control groups from the AD group. Central component 2 (PC2) summarized 14.5% of the data variability and separated the cannabis-treated groups from the negative control group (**Figure 9**).

The variables that most contributed to PC1 were DG, C4, C2, and C1. These variables have a positive relationship with the AD group. On the other hand, MDA, PC, and NO present a negative association with the AD group (**Figures 10A**).

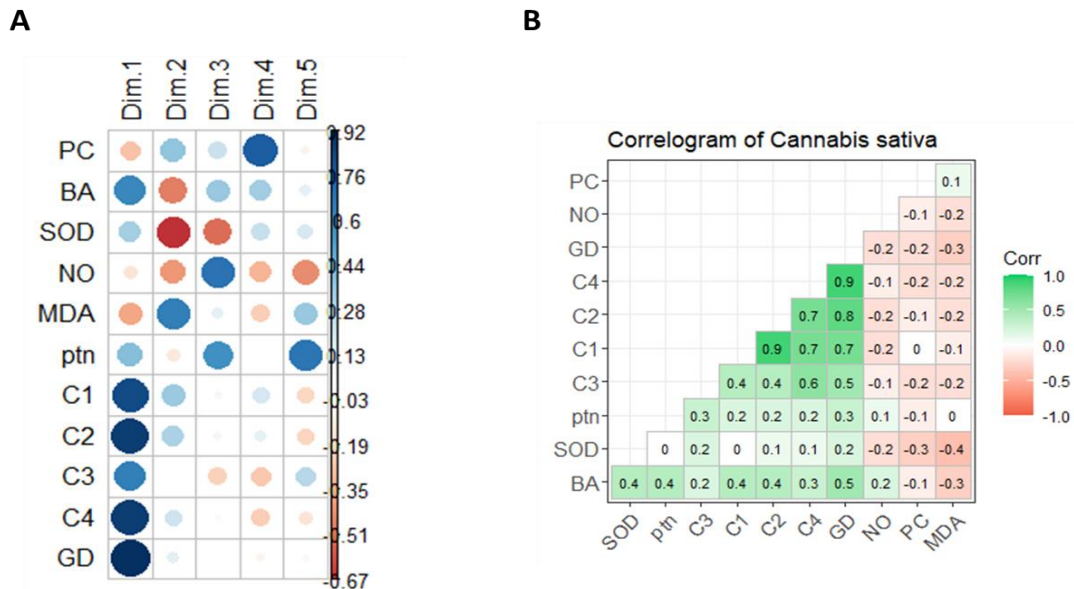
The main parameters contributing to PC2 were SOD, MDA, BA, and NO. MDA shows a positive relationship in the negative control group, and SOD, BA, and NO negatively correlate with the negative control (**Figures 10B**).

Figure 9 - Principal Component Analysis of rats experimentally induced for Alzheimer's and treated with Cannabis sp extract. in a 2:1 ratio THC: CBD. C1, C2, C3, C4, AND GD – Hippocampal regions.



Source: Silva RN, et al., 2024. MDA – Malondialdehyde. BA-B-amyloid. SOD – superoxide dismutase. PTN – Total protein.

Figure 10 - Data correlation matrix from control and cannabis-treated Wistar rats at different concentrations. Blue indicates positive correlation and red indicates negative correlation.



Source: Silva RN, et al., 2024. The darker and larger the circle, the greater the correlation. BW – Body weight; BA – Amyloid beta; SOD – superoxide dismutase; NO – nitric oxide; MDA – malondialdehyde; C1, C2, C3, C4, and GD – cell death in different regions of the hippocampus.

DISCUSSION

This study provides information on the effect of an oily extract of Cannabis with a higher proportion of THC concerning CBD on animals experimentally induced with injuries similar to Alzheimer's disease. It was observed that the extract with higher ratios of THC is efficient in reversing oxidative damage and cell death. Cannabis sativa already has its chemical components well described and three main chemotypes are recognized for the plant, based on the proportion of the two main cannabinoids present in cannabis, Δ^9 -THC and CBD: (i) Drug type, when there is a predominance of Δ^9 -THC; (ii) Intermediate type, when there are similar amounts of both cannabinoids; and (iii) Fiber type, when CBD is prevalent (SALENTIJJN EMJ, et al., 2015).

Although THC is the primary psychoactive constituent of cannabis, CBD is primarily non-psychoactive and has been shown to attenuate the behavior and metabolic effects of THC (ENGLUND A, et al., 2013). Studies have shown that individuals who ingested foods with high Al contents were twice as likely to develop AD because prolonged exposure of rats to soluble Al salt causes selective neuronal loss and loss of cholinergic function (YIN Z, 2020).

In addition, this exposure also reduces the transmission of acetylcholine and attenuates its release, causing a decrease in reflexes and acting as a reducer of neuronal activity (YOKEL RA, et al., 1994). Other studies indicate that aluminum damages the cortex and hippocampus structures, causing oxidative stress, inducing A β accumulation and the formation of neurofibrillary tangles, affecting synaptic activities and promoting neuronal death (OHYAGI Y and MIYOSHI K, 2013). For Auti ST, et al. (2019), the injuries were more marked, with the 42-day protocol, which clarifies that the more chronic the metal administration, the more intense the injuries.

It is important to emphasize that the good results obtained in this study may be due to the initiation of treatment after 21 days of induction, which, taking into account the reality of clinical trials, may indicate that the early initiation of therapy improves the patient's prognosis. Recent studies have shown that pharmacological intervention in selective CB1 and CB2 cannabinoid receptor agonists can reduce cognitive impairment and brain pathological changes associated with A β production in animal models with AD (MARTÍN-MORENO AM, et al., 2012).

In this study, an increase in beta-amyloid levels was observed in the serum and the preparations stained with Congo red, in the positive control groups, and the lowest dose treated with the cannabis extract. In this way, the induction of AD can be confirmed since the animals that received quantities of 100 and 150 μ l of the oily extract of Cannabis were able to revert the levels of beta-amyloid to normal levels concerning the negative control.

According to Casarejos MJ, et al. (2013), the association of THC and CBD phytocannabinoids is capable of promoting a reduction in the deposit of β -amyloid peptide in the cortex and hippocampus of transgenic mice in an Alzheimer's model, corroborating the present study, where a decrease was observed a significant number of lesions in the treated groups, concerning the positive control group. Other data that reinforce the similarity between the results of the studies is the special staining in Congo red and the serum quantification of β -amyloid, which showed a decrease in the treated groups. Some authors have studied isolated phytocannabinoid compounds; one of them was Cao C, et al. (2014). The latter, through cell culture, concluded that THC could reduce β -amyloid levels in cells, in addition to preventing its aggregation, without causing no toxicity.

Another study using isolated phytocannabinoids that was also carried out from cell culture showed that CBD has neuroprotective properties, being able to reduce oxidative stress and prolong cell life (IUVONE T, et al. 2004). The degeneration of neurons, observed in AD, can also occur due to oxidative stress. Refers to conditions such as hypoxia, characterized by impairment of protective mechanisms, so that neurons become more susceptible to cytotoxic injury, oxidative stress is induced in Al-exposed brains (HP H, et al., 2001). Although AD is associated with oxidative stress, MDA levels were not altered as in other studies (OZCANKAYA R and DELIBAS N, 2002).

However, many authors attribute the development of stress caused by AD to nitric oxide (NO) levels (AKBAR M, et al., 2016), which were found to be increased in the positive control group and in the group that received the lowest dose. Of Cannabis sp extract. NO is a molecule that acts on cell signaling as a functional mediator and is directly related to oxidative stress and consequently cells death (TRIPATHI P, et al., 2007). The reduction in NO levels in the groups with higher doses of cannabis can be explained by the ability of CBD to regulate the expression of nitrotyrosine and inducible nitric oxide synthase (iNOS) (ESPOSITO G, et al., 2006). On the other hand, the increase in the activity of the antioxidant enzyme superoxide dismutase (SOD) shows a reaction of the organism to the growth of reactive oxygen species.

The increase in SOD has already been observed in other studies with AD (MARKESBERY WR, 1997). Cannabis extract acted as an antioxidant, helping to reduce NO levels and activate SOD, as CBD is known to act as an antioxidant through target molecules associated with the redox system, such as superoxide dismutase (SOD) and glutathione (GSH), peroxidase (COSTA B, et al., 2007). Furthermore, thanks to its ability to reduce reactive oxygen species (ROS), CBD maintains the correct levels of GSH, necessary for the antioxidant activity of vitamins A, C, and E (PAN H, et al., 2009). This study shows the antioxidant activity of the oily extract of Cannabis, where the groups treated with the Cannabis extract express an increase of up to 15% concerning the untreated. The redox imbalance and oxidative stress that occurs in AD ultimately causes cell death, which NO can promote, since (BLAISE GA, et al., 2005), it was with high levels in the negative control groups and with the lowest cannabis dose. Chronic aluminum chloride intoxication announced 90% more hippocampal neurons death than animals in the negative control group. On the other hand, treatment with the highest dose of the oily extract of Cannabis sp. reduced 83% of AlCl₃ induced neuronal deaths in the hippocampus.

CONCLUSION

We conclude that intoxication by aluminum chloride induces nitrosative stress, increased total proteins, and increased cell death due to the rise in β -amyloid. Subchronic treatment with oily extract of Cannabis sp. 2:1 (THC: CBD) reduced plasma nitrite levels with doses above the 100 μ l dose and removed plasma total protein levels and cell death from the 50 μ l dose. In this way, this study provides evidence that THC: CBD combinations are valid candidates for treating Alzheimer's disease. However, further studies on the mechanisms involved in this process need to be carried out.

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AUTHOR CONTRIBUTIONS

Raissa Nunes da Silva performed experiments, samples collection, analyzed data and wrote and prepared the manuscript; Fernanda Carolina Ribeiro Dias- Oxidative stress analysis and quantification of beta-amyloid, wrote and prepared the manuscript; Sandra Maria de Torres - performed experiments and histological processing; Alluanan Adelson da Silva - Oxidative stress analysis and quantification of beta-amyloid; Amanda de Deus Ferreira Alves - Collection of biological material; Antônio José Alves - designed and directed the study; Valdemiro Amaro da Silva Júnior - created and directed the study. All authors provided feedback and helped to shape the manuscript.

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