Efeito agudo do L-Triptofano e das Nanopartículas de L-Triptofano associadas ou não ao exercício físico no comportamento cognitivo e motor de modelo experimental de Alzheimer

Acute effect of L-Tryptophan and L-Tryptophan Nanoparticles associated or not with physical exercise on cognitive and motor behavior of Alzheimer's experimental model

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Meiriélly Furmann
Doutoranda em Desenvolvimento comunitário
Universidade Estadual do Centro-Oeste – Campus Irati
PR 153 - Km 07 Riozinho 84500000 - Irati, PR - Brasil
E-mail: meiry.furmann2@gmail.com

Ivo IlvanKerppers
Doutorado em Engenharia Biomédica
Universidade Estadual do Centro-Oeste – Campus Cedeteg
Rua Simão Camargo Varela de Sá, 13 vila Carli 85040-080 - Guarapuava, PR - Brasil
E-mail: ikerppers@hotmail.com

Emerson Carraro
Doutorado em Infectologia
Universidade Estadual do Centro-Oeste – Campus Cedeteg
Rua Simão Camargo Varela de Sá, 13 vila Carli 85040-080 - Guarapuava, PR – Brasil
E-mail: emersoncarraro@bol.com.br

Aristides M. Rodrigues
Doutorado em Ciências do Desporto
Universidade de Coimbra
Paço das Escolas, 3004-531 Coimbra, Portugal
E-mail: rodriguesari@hotmail.com

Luis Paulo Gomes Mascarenhas
Doutorado em Saúde da Criança e do Adolescente
Universidade Estadual do Centro-Oeste – Campus Irati
PR 153 - Km 07 Riozinho 84500000 - Irati, PR – Brasil
E-mail: lmascarenhas@unicentro.br

Camila da Luz Eltchechem
Mestranda em Desenvolvimento comunitário
Universidade Estadual do Centro-Oeste – Campus Irati
PR 153 - Km 07 Riozinho 84500000 - Irati, PR – Brasil
E-mail: camilagpv@gmail.com

Samily dos Santos Silva
Mestranda em Desenvolvimento comunitário
Universidade Estadual do Centro-Oeste – Campus Irati
PR 153 - Km 07 Riozinho 84500000 - Irati, PR – Brasil
E-mail: samilysantoss@gmail.com

RESUMO

Introdução: A DA apresenta gradativamente diversos sinais e sintomas que causam grande comorbidade e necessidade de cuidados e tratamentos alternativos. O L-triptofano, com ação relacionada à dinâmica cerebral, depressão e ansiedade é precursor da serotonina, importante neurotransmissor cerebral, que pode ter sua ação potencializada pelo uso das Nanopartículas. O exercício físico por sua vez, também pode estar relacionado à alteração de monoaminas cerebrais como a serotonina, sendo mais uma alternativa de tratamento. Portanto, este estudo tem como objetivo analisar a eficácia do L-Triptofano e das Nanopartículas de L-Triptofano associadas ao efeito agudo do exercício físico em modelo experimental de Alzheimer. Materiais e Métodos: A amostra foi composta por 20 ratos, divididos em 4 grupos (n=5) GLt (L-Triptofano), GN (Nanopartículas), GN+e (Nanopartículas+exercício) e GLt+e (L-Triptofano+exercício), foram realizados testes de ansiedade, memória espacial, memória aversiva, e comportamento motor. Resultados: De acordo com a análise estatística, houve melhora significativa em relação à ansiedade no GLt e no GN com maior força, já em relação ao comportamento motor apenas o GLt+e demonstrou efeitos positivos significativos como tratamento. Apenas por meio do *Size Effect* pode-se observar que o GLt apresentou diferença em relação ao comportamento motor, e o GN obteve diferença na memória aversiva e em diversas variáveis do comportamento motor, demonstrando melhor desempenho em relação ao GLt como método de tratamento. Porém, quando associado ao exercício físico o L-Triptofano apresentou mais valores com diferença entre as variáveis de comportamento motor do que quando associado às Nanopartículas. Todavia apenas na associação do exercício agudo às Nanopartículas houve melhora da ansiedade. Discussão: Outros tratamentos com fármacos também utilizados são as BZD, indicadas para situações agudas, por curtos períodos de tempo, porém ainda não apresenta eficácia satisfatória, assim como ainda não há um consenso em relação aos benefícios do exercício para a população portadora de DA, pois existem controvérsias em relação ao melhor tipo de exercício, intensidade e duração necessários para produzirem a redução dos sintomas depressivos. Sendo assim o L-Triptofano e as Nanopartículas surgem como uma alternativa de tratamento, necessitando de novos estudos com diferentes avaliações e duração de tratamento. Conclusão: Nanopartículas de L-Triptofano apresentam um grande potencial para ser utilizado como método de tratamento da DA, uma vez que diminuiu o sintoma de ansiedade e melhorou o comportamento motor dos animais.

Palavras-chave: Nanopartículas, Doença de Alzheimer, Exercício físico, Triptofano

ABSTRACT

Background: Alzheimer Disease (AD) presents signs and symptoms that cause comorbidity. The L-tryptophan, wich has action related to brain dynamics is a precursor of serotonin, which may have its action enhanced using Nanoparticles. Exercise may also

be related to the alteration of cerebral serotonin. This study aims to analyze the efficacy of L-Tryptophan and L-Tryptophan Nanoparticles associated with the acute effect of physical exercise in experimental model. Methods: The sample consisted of 20 rats, divided into 4 groups (n = 5) GLt (L-Tryptophan), GN (Nanoparticles), GN+E (Nanoparticles + exercise) and GLt+E (L-Tryptophan + exercise). There were performed tests of anxiety, spatial memory, aversive memory and motor behavior. Results: The statistical analysis, there was a significant improvement in anxiety in GLt and GN, in relation to motor behavior, only the GLt+E demonstrated positive effects. Size Effect can it be observed that the GLt presented difference in relation to the motor behavior, and the GN, obtained a difference in the aversive memory and in several variables of the motor behavior. Conclusions: L-Tryptophan nanoparticles have great potential to be used as a treatment method for AD, since it has reduced the anxiety symptom and improved the motor behavior of the animals.

Keywords: Nanoparticles, Alzheimer's disease, exercise, Tryptophan.

1 INTRODUÇÃO

Alzheimer's disease (AD) is a dementia that affects the elderly and compromises their physical, mental and social integrity, leading to total dependence in the advanced stage of the disease and demanding, increasingly complex care[1, 2].

AD presents gradually several signs and symptoms that cause great comorbidity and need for care[3], making interventions necessary to improve the quality of life of this population. The use of medications, amino acids like tryptophan, and the practice of exercises for example, can be an alternative as a treatment method to reduce the symptoms that cause so manycomplications.

L-tryptophan, an essential amino acid with action related to brain dynamics, depression and anxiety is the precursor of serotonin, an important brain neurotransmitter. According to some studies[4, 5] the levels and functions of several neurotransmitters are influenced by the stock of their dietary precursors, and may influence mood and behavior, with tryptophan ingestion and cerebral serotoninergic action as the main example. This kind of substance can also be used as a nanoparticles form.

Nanoparticles can be an alternative to increase the potential action of drugs, since it has the function of increasing the bioavailability of the drugs, and with that to hide the side effects that they can cause, besides having high stability, which confers a long life in the organism, high transport capacity and the feasibility of incorporation of hydrophilic and hydrophobic substances[6]. Altering the monoamines levels of the brain.

Another way to alter the cerebral monoamines levels, such as dopamine, serotonin and noradrenaline, is aerobic physical activity, and its biological effects, wich is related an increase in blood perfusion, which improves oxygenation and consequently cerebral functioning. It is known that AD is characterized by impairments of neuropsychiatric and cognitive functions with manifestations of behavioral and personality changes, it can be affirmed that they end up interfering in the individual's abilities to perform their normal daily activities, impairing their functions[7, 8].

Therefore, this study aims to analyze the efficacy of L-Tryptophan and L-Tryptophan Nanoparticles associated with the acute effect of physical exercise in experimental Alzheimer's model.

2 METHODS

The sample consisted of 20 Rattus Norvegicus, male, Wistars strain, weighing between 200-250 grams, aged 2 months, with feed and water ad libitum throughout the study period. The cages with the animals were arranged in a shelf, kept in the room under the light/dark cycle of 12 hours (lights connected from 7:00 am to 7:00 pm) at a temperature of 23 ± 1 ° C, controlled by 7000BTU's sprint air conditioning. This study had the approval of the Ethics Committee on animal use - CEUA of the University of the MidwestState under the document 025/2016.

The animals were randomly divided into 4 groups described in table 1.

Table 1 - Animals Group Division

GROUPS (n=5)	DESCRIPTION	INTERVENTION
GLt	Group L-Tryptophanwith induction of senile plaques	Ingestion of L-Tryptophan
GN	GroupNanoparticles, with induction of senile plaques	Ingestion of L-TryptophanNanoparticles
GLt+E	Group L-Tryptophan plus exercise, with induction of senile plaques	Ingestion of L-Tryptophan + Exercise Protocol
GN+E	Group Nanoparticles plus Exercise, with induction of senile plaques	Ingestion of L-Tryptophan Nanoparticles + Exercise Protocol

EXPERIMENTAL SURGERY

The animals (n = 20) were anesthetized intra-abdominal with a solution in the ratio of 80mg/kg Ketamine Hydrochloride (Ketamine, 10ml) to 15mg/kg Xilasine Hydrochloride (Dopaser, 10 ml) via intraperitoneal and taken to an INSIGHT® stereotaxic device, where their heads were fixed by the temporal rock and upper incisors. The coordinates used were: AP = 0.0 mm, ML = ± 0.8 mm and DV = 4.0 mm, respectively, taking the bregma as reference, being the lambdoide and bregmatic sutures in the same horizontal plane, being injected 3 microliters Beta Toxin -Amyloid1-42 by means of a 10 microliter Halminton syringe, 0.02 microliters being injected every minute totaling 9 minutes of induction, following the coordinates of the atlas of Paxinos and Watson, 1997. For the analgesia was used the hydrochloride of tramadol at a dose of 10mg/kg, every 12 hours, intraperitoneally for 7 (seven) days[9].

After induction of the senile plaque process and neurofibrillation, the animals were rested for a period of 30 days for the inflammatory and neurodegenerative processes of the neurons in the hippocampus to occur. An euthanasia of an animal was performed to verify the presence of plaques and neurofibrils in the hippocampus, and only after this verification the other animals received their respective treatment protocols.

TREATMENT

L-TRYPTOPHAN ANDNANOPARTICLES OF L-TRYPTOPHAN

The treatment was done by gavage of 1.5 mg/kg of the nanoparticles of Tryptophan and L-tryptophan for a period of six consecutive days and was administered at the same time.

OBTAINING THE L-TRYPTOPHAN NANOPARTICLES

The nanoparticlescontainingtryptophanwere prepared by emulsificationevaporation method of the solvent, using a simple emulsion. Initially, 5.0 mg of tryptophan was dissolved in appropriate solvent, and the polymer to obtain the nanostructured system (poly-epsiloncaprolactone, 50 mg) dissolved in dichloromethane (1000 μL), thus forming the oily phase of the formulation. The oily phase was poured into the aqueous phase composed of 10 mL of 0.5% polyvinyl alcohol (PVA), which proceeded under sonication for 3 minutes at 60 Hz. After sonication, the formed emulsion

was washed for evaporation in a rotary evaporator with negative pressure to remove the organic solvents. The nanoparticles formed were separated by centrifugation (15,000 rpm / 30 minutesand the precipitate was resuspended in water, followed by further centrifugation. The supernatant resulting from these two centrifugations was separated for analysis of the tryptophan encapsulation efficiency. The nanoparticle precipitate was resuspended in 5% sucrose and lyophilized for further storage. The final production of nanoparticles used was 90mg, divided among the animals that received the dose of 1.5mg, where the nanospheres presented approximate size of 171.6nm.

The polymer used to encapsulate tryptophan was zein (corn protein) with sodium caseinate (milk protein), which makes the compound more hydrophilic, making it even faster to release the drug, since tryptophan has slower controlled releasecharacteristics. Among all the analyzes of the quality control of nanoparticle production, only the Z-potential was not realized because it is an indirect measure, which reflects the electrical potential of the suspended particles.

EXERCISE PROTOCOL

The animals were submitted to daily training with 30 minutes duration without interval; during the period of 6 consecutive days, with 3 days of adaptation to the aquatic environment before the beginning of the treatment. A lead training overload was used, representing 5% of the animal's body mass.

BEHAVIOR EVALUATION

ANXIETY

In the analysis of the anxiety-related factors, the Cross-Labyrinth test was used, consisting of a cross-shaped structure composed of two open arms perpendicular to two closed arms. This structure is 60 cm from the ground. The mouse is placed in the center of the labyrinth facing one of the open arms where the four arms intersect, from where it can choose any of the four arms. Two of the arms are opened, allowing the mouse to see the ground. The other two arms are closed. The rats prefer closed arms, but can explore open arms. It is considered that the animal is in one of the arms when it has all four legs in that same arm of the labyrinth, registering its behavior for five minutes. After each session, the labyrinth was cleaned with a 50% ethanol solution to minimize the odor traces

left by the previous animal. Behaviors such as freezing, number of fecal cakes and longer stay in the closed arms are related to higher anxiety index[10]. All analyzes were done by an observer after DVR recording.

SPATIAL MEMORY

The Morris WaterMaze test was applied to assess the animal's ability to acquire spatial memory, by measuring latency for the animal to locate a submerged platform in a tank with opaque water[11]. A circular glass fiber tank, 134.0 cm in diameter and 40.0 cm high, was used with a square stainless steel platform, with dimensions of 15x15cm width and 28.5cm height, located in the center of a room of 12 m^2, with an upper window at the south point, and the training and tests are performed from 8am. To perform the test, water was added to the tank until the level exceeded 2.0 cm the platform height. Then, was addicted corn starch to make the water opaque. The test consists of 2 days of training, and 48 hours after the last training session, the test session is performed. In the training phase, the rats had 5 daily trials, with a maximum time of 54 seconds for each trial and 30-second intervals to find the platform located in the center of the tank. For each test the animal was released facing the tank wall, leaving pseudo-random points. All analyzes were done by an observer after DVR recording.

AVERSIVE MEMORY

Initially the rats were trained in the task of conditioned fear. Briefly, this task uses a training chamber (model MED-VFC2-SCT-R, Med Associates Inc., St. Albans, Vermont 05478), consisting 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 sits inside a larger and acoustically isolated box, to attenuate the interference of external sounds.

The mnemonic performance is measured and expressed in the time that the mouse remains in a state of paralysis ("freezing"). This behavior is associated as an index of fear in rats[12].

MOTOR BEHAVIOR

For the evaluation of motor activity, the Open Field Test (OF) was used, it is an apparatus consisting of a round arena (100 cm in diameter and 45 cm in height), with the

soil divided into 9 quadrants. The animals were carefully placed in the center of the OF and allowed to explore it freely for 5 min. Three motor parameters were registered: number of quadrants (the number of crossings of the four legs of the animal from one quadrant to another), frequency of lifting (the number of times the animals remained on the hind legs), number of fecal cakes and time of immobility (time in seconds that the animal remains static)[13]. The animals were carefully placed in the southwest position of the OF and allowed to freely explore the area for 5 min. The OF was cleaned with a 5% water-ethanol solution prior to testing each animal in order to eliminate possible bias caused by odors and substances left by the previous rats, and to prevent them from being induced to perform the route that the previous animal realized. All analyzes were done by an observer after DVR recording

EUTHANASIA

The animals were anesthetized with 80 mg / kg of Ketamine and 15 mg / kg of Xylazine, after anesthesia, they received lethal dose of Thiopental 20 mg / kg intraperitoneally.

STATISTICAL ANALYSIS

For the analytical treatment of the data, the normality test of Shapiro-Wilk and homogeneity variances test of Levene were initially performed. In situations in which there was normality and homogeneity, the comparison of groups was performed through factorial analysis of variance (ANOVA), with post hoc per pair and Bonferroni correction. In situations in which there was no normality or homogeneity, the comparison of groups was performed through the non-parametric analysis of Kruskal-Wallis (H variance), with post hoc by means. Size effect analysis was also used by the Cohen test with a 95% confidence interval (CI).All tests adopted $p<0.05$.

3 RESULTS

Table 2 shows the values related to anxiety behavior, which increased after the induction of senile plaques, and all groups presented significant difference in relation to freezing, the main variable of this analysis. Both L-Tryptophan and Nanoparticles demonstrate efficacy in decreasing freezing. Nanoparticles, on the other hand, also

obtained an improvement over the time of permanence in the closed arm (TBF). When associated to physical exercise, no variable presented a significant difference.

Table 2 - Anxiety analysis in the different treatment groups

ANXIETY		*Freezing*$^{\Omega}$	*Grooming*$^{\Omega}$	TBA$^{\Omega}$	TBF$^{¥}$
L- Tryptophan	Preinduction	11,8±8,16^{A}	0,6±0,8	1,0±2,23	286,4±11,9
	Postinduction	103,8±44,4^{B}	0,4±0,54	3,0±6,7	287,2±14,8
	Post Treatment	69,4±20,8	1,0±1,0	8,0±13	289,6±17,3
Nanoparticles	Preinduction	3,6±4,9^{A}	2,4±1,51	513,4±41,9	130,2±99,7^{A}
	Postinduction	79,0±18,8^{B}	2,2±2,2	4,0±8,9	287,8±1,08^{B}
	Post Treatment	74,6±14,2	2,0±1,5	30,0±41,4	268,0±40,8
L-Tryptophan+ Exercise	Preinduction	12,0±6,2^{A}	2,4±1,14	0±0	230,0±56,5
	Postinduction	83,0±40	2,6±1,51	54,0±120	184,0± 121,1
	Post Treatment	46,0±40,3	2,6±0,57	10,0±17,3	285,0±13,6
Nanoparticles + Exercise	Preinduction	8,6±6,2^{A}	2,2±0,9	1,6±3,5	171,8±6,6
	Postinduction	63,6±39	3,4±2	3,0±6,7	278,4±20,4
	Post Treatment	47,0±41,4	1,0±0,7	0±0	288,0±5,7

A= difference between pre and post induction; B= difference between post induction and post treatment; ¥= parametrics (Anova), Ω= nonparametrics (Krukal Wallis), ($p \leq 0,05$).

In relation to the spatial memory, whose values are described in table 3, no group presented significant statistical difference.

Table 3 - Spacial Memory analisys in the different treatment groups

SPATIAL MEMORY		Latence$^{\Omega}$
L- Tryptophan	Preinduction	33,6±28,6
	Postinduction	9,6±7,12
	Post Treatment	11±6,9
Nanoparticles	Preinduction	49,8±39,9
	Postinduction	9,4±6,6
	Post Treatment	6,2±2,7
L-Tryptophan+ Exercise	Preinduction	33,2±19,2
	Postinduction	16,6±13,3
	Post Treatment	33,6±54,8
Nanoparticles+ Exercise	Preinduction	84,8±73
	Postinduction	12,6±8
	Post Treatment	10,4±7,5

A= difference between pre and post induction; B= difference between post induction and post treatment; ¥= parametrics (Anova), Ω= nonparametrics (Krukal Wallis), ($p \leq 0,05$).

The aversive memory, with its values presented in Table 4, showed a statistically significant difference only in relation to the induction protocol of senile plaques in the L-Triptophan group.

Table 4 - Aversive Memory Analisys in the different treatment groups

AVERSIVE MEMORY		*Freezing*$^{\Omega}$
L- Tryptophan	Preinduction	17,8±8,6^{A}
	Postinduction	4,2±4,9
	Post Treatment	11,4±6,9
Nanoparticles	Preinduction	12±6,2
	Postinduction	8±2,5
	Post Treatment	5,8±1,7
L-Tryptophan+ Exercise	Preinduction	10,6±3
	Postinduction	6,6±1,1
	Post Treatment	6±3,6
Nanoparticles + Exercise	Preinduction	11,2±5,6
	Postinduction	9,8±5,3
	Post Treatment	10,6±3,5

A= difference between pre and post induction; B= difference between post induction and post treatment; ¥= parametrics (Anova), Ω= nonparametrics (Krukal Wallis), (p≤0,05).

As for the motor behavior according to Table 5, all groups showed a difference in relation to the induction of senile plaques, but in relation to the treatments, only the L-tryptophan + Exercise group showed improvement in the latency variable, which refers to the time spent in the center of the arena, inversely related to the exploration of the environment.

Table 5 - Motor Behavior Analisys in the different treatment groups

MOTOR BEHAVIOR		Lifting¥	*Freezing*¥	Feces$^{\Omega}$	Latence$^{\Omega}$	Q1¥	Q2¥	Q3¥	Q4¥
L-Tryptophan	Preinduction	33,4±8,3^{A}	14,8±5,6^{A}	4,8±1,3	10,4±4,5	7,6±2,4^{A}	5,6±2,1	4±1,8	6,6±2,4^{A}
	Post induction	12,4±7,6	105,4±26,1	3,2±3,8	18±11,5	2,2±1,6	2,6±0,8	1,8±1	2±1,2
	Post Treatment	7,2±4,08	115,6±69,2	2,8±1,9	16,2±18	1,6±1,5	1,4±0,5	1,4±0,8	1±0,7
Nanoparticles	Preinduction	33,6±4,2^{A}	9,8±6,01^{A}	4,8±2,1	9,8±4,5	6,4±1,8^{A}	7,2±1,7^{A}	7,2±3,4^{A}	8±2,9^{A}
	Post induction	10,6±4,3	102,6±17,7	1,4±1,5	4,8±4,7	3±2,4	3,4±2,3	2,6±1,5	2±1,7
	Post Treatment	12,8±1,3	72,2±11,6	0,8±0,4	0±0	2±1	1,6±0,8	1±0,7	1,2±1
L-Tryptophan + Exercise	Preinduction	34,2±7,7^{A}	14,8±5,6 ^{A}	4±1,3	10,4±4,5^{A}	7,6±2,4^{A}	5,6±2,1^{A}	4±1,8	6,6±2,4^{A}
	Post induction	9,8±7,8	89,4±37,7	2,2±2,6	88,4±63,1^{B}	2±2,3	3±3,5	2,4±2,7	1,6±2,5
	Post Treatment	2±1	106,6±23,6	3,3±4,1	8±10,5	0,6±0,5	0,3±0,5	0,3±0,5	0,6±0,5
Nanoparticles + Exercise	Preinduction	49,8±1,48^{A}	1,6±2,3	4,6±1,34	4,4±1,6	10,2±1,3^{A}	10,6±1,3^{A}	9,2±1,7^{A}	11,4±1,3^{A}
	Post induction	12,2±6,3	94,4±25,2	3±2,7	11,6±3,2	3±2,2	3±2,2	2,2±2,7	2,2±3,2
	Post Treatment	8,6±6,4	41,4±22,1	2±1	14±26	1,2±1,3	1,8±1,9	1,2±0,8	1,8±1,6

A= difference between pre and post induction; B= difference between post induction and post treatment; ¥= parametrics (Anova), Ω= nonparametrics (Krukal Wallis), (p≤0,05).

Table 6 shows the values of the size effect of the treatment by the size of the sample. The L-Tryptophan group presented difference only in relation to the motor behavior, whereas the group Nanoparticles obtained difference in the aversive memory and in several variables of the motor behavior, demonstrating a better performance in relation to L-Tryptophan as a treatment method. However, when associated with physical exercise, L-Tryptophan had more difference between the motor behavior variables than when associated to the Nanoparticles. Howsoever, in the association of acute exercise with Nanoparticles, which in turn had a difference in *grooming*, with a direct relation to anxiety improvement.

Table 6 - Size Effect analyzed for all variables in the different groups

		L-Tryptophan		Nanoparticles		L-Tryptophan + Exercise		Nanoparticles+ Exercise	
Size effect		D	R	d	R	d	r	d	R
ANXIETY	*Freezing*	0,99	0,44	0,26	0,13	0,92	0,41	0,41	0,2
	Grooming	-0,74	-0,3	0,1	0,05	0	0	1,6	**0,62** ŧ
	TBA	-0,48	-0,2	-0,9	-0,39	0,51	0,24	0,63	0,3
	TBF	-0,14	-0,1	0,66	0,31	-1,17	-0,5	-0,64	-0,3
SPATIAL MEMORY	Latence	-0,19	-0,1	0,63	0,3	-0,42	-0,28	0,28	0,14
AVERSIVE MEMORY	*Freezing*	-1,2	-0,5	1	**0,45** ŧ	0,22	0,11	-0,17	-0,08
MOTOR BEHAVIOR	Lifting	0,85	0,39	-0,7	-0,32	1,4	**0,57** ŧ	0,56	0,27
	Freezing	-0,19	-0,1	2,03	**0,71** ŧ	-0,54	-0,26	2,23	**0,74** ŧ
	Feces	0,13	0,06	0,54	0,26	-0,32	-0,15	0,49	0,23
	Latence	0,11	0,05	1,44	**0,58** ŧ	1,77	**0,66** ŧ	-0,12	-0,06
	Q1	0,38	0,18	0,54	0,26	0,84	0,38	0,99	0,44
	Q2	1,79	**0,66** ŧ	1	**0,46** ŧ	1,08	**0,47** ŧ	0,58	0,28
	Q3	0,46	0,21	1,36	**0,56** ŧ	1,08	**0,47** ŧ	0,5	0,24
	Q4	1	**0,45** ŧ	0,56	0,26	0,55	0,26	0,15	0,07

d= difference mean; r= size effect; IC= 95%; Ŧ= high effect (≥0,80); ŧ= medium effect (0,40-0,70); *negative values = no effect.

4 DISCUSSION

The increase in the incidence of Alzheimer's disease has been increasing with the growing up of the elderly population, reaching between 50 and 70% of the population[14], and with this, there is a need for new studies aimed at improving the quality of life of this population with different treatment methods.

L-Tryptophan and L-Tryptophan Nanoparticles in the analysis of anxiety presented significant values with a decrease in freezing behavior (Table 2). As in the

study where the flavonoid Morina (which has an important antioxidant and neuroprotective action of the Central Nervous System) was used in the treatment of AD and an improvement in anxiety was also observed[15]. Other pharmacological treatments which are commonly prescribed are benzodiazepines (BZDs), indicated for relatively acute situations, for short periods of time, however, it still do not presenting satisfactory efficacy for this symptom so marked by dementia[16]. Thus, L-Tryptophan appears as a new alternative.

Still, when the analysis was performed by means of the effect size (Table 6), there were no significant positive responses in relation to the treatments (Table 3), but a significant improvement was observed with all treatments, especially the isolated nanoparticles, which showed an improvement in a greater number of variables in relation to the displacement within the arena. Corroborating with the study where used L-Tryptophan for 21 days and found improvement in the motor behavior of rats after treatment, which in our study had a beneficial effect even in a shorter period of time, without counting the potentiation through Nanoparticles[17].

The memory that is also very affected during the development of the disease, described in spatial and aversive memory in this study, did not present any significant improvement, which may not have occurred due to the influence of the external factors of the environment, such as temperature, noise or luminosity, or even for the short time of treatment[18]. In the study which used the essential oil of Citrus sinensis, as an AChE inhibitor, improved the performance of treated animals when compared to a control group, the study also observed high levels of AChE in certain regions which prevented a poor performance of the mice in the aquatic labyrinth[19]. Showing, this way, the importance of new studies with different types of drugs that inhibit these events. Nevertheless, the aversive memory presented improvement after the treatment with the Nanoparticles when the analysis of the sample size was done, strengthening the justification that the sample number was small.

Aerobic physical exercise in turn, with its physiological benefits[20] may be an alternative to optimize treatment. Even though, when associated with physical exercise none of the treatments showed a statistically significant improvement. But when analyzed by Size effect the L-Tryptophan + Exercise group showed improvement at motor behavior (Table 6). However, the review hat analyzed studies that used the practice of physical

activity as a therapeutic intervention, concludes that there is still no consensus regarding the benefits of exercise for the elderly population with AD[21]. In addition, there is controversy regarding the best type of exercise, intensity, and duration required to reduce depressive symptoms in AD patients.

The use of alternative systems with greater action potential has been widely used in several areas, including neurological diseases. Considering the results obtained in this study, we can point out that because the drug release system is conventionally faster acting, due to the concentration of the drug that reaches a peak soon after administration, then declining there was a significant improvement in the groups that received this form of treatment, being further potentiated with the effects of physical exercise. When the modified nanoparticle drug is used, the development of a system capable of maintaining the concentration of the drug in the blood stream within the desired therapeutic range, aiming at maintaining the concentration of the drug between these two, is sought. levels for a long time the results were positive, but not so hypothetically satisfactory, as they would be in the long term benefiting from the action of Nanoparticles associated with the benefits of exercise also in the long term[22,23,24].

In this perspective, new studies with different analyzes, longer treatment time and even other exercise protocols are necessary to verify the best alternative to improve the quality of life and potentiate the treatment and minimize Alzheimer's symptoms.

5 CONCLUSIONS

According to this study, it can be stated that L-Tryptophan Nanoparticles present a great potential to be used as a treatment method for AD, since it diminished the anxiety symptom an+d improved the motor behavior of the animals. The L-Tryptophan + Exercise also showed a great effect size in motor behavior. This is due to its physical and chemical characteristics, which potentiate the action of the drug and aerobic exercise, which in turn also promote significant improvement in the symptoms of Alzheimer's disease.

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