Sudarshan Publication

Available online at S-epub.in/ojs/

THE PHARMA RESEARCH

An International Journal of Pharmacy Research

ANTICHOLINESTERASE ACTIVITY AND EFFECTS ON MEMORY IN ADULT RATS OF 1, 3-DISTEAROYL-2-OLEOYLGLYCEROL SUBSTANCE ISOLATED FROM PLATONIA INSIGNIS MART. (BACURIZEIRO)

Chistiane Mendes Feitosa[a,b*]; Soane Kaline Morais Chaves[a], Patricia Régia Pereira dos Santos[a]; Antonio do Nascimento Cavalcante[a]; Joaquim Soares da Costa Júnior[b]; Khaled Rashed[c]

[a]Post-graduate Programs in Chemistry and Pharmaceutical Sciences, Federal University of Piaui, Ininga, 64.049-550, Teresina, Piauí, Brazil.

[b]Department of Chemistry, Federal Institute of Piaui, 64.049-550, Teresina, PI, Brazil.

[c] National Research Centre, Pharmacognosy Department, 33 El-Bohouth st.-Dokki, Giza, Egypt- P.O.12622.

ARTICLE INFO

Published on: 15-03-2016

ISSN: 0975-8216

Keywords:

Platonia insignis; Bacurizeiro; Cream of bacuri; 1, 3-distearoyl-2-oleoylglycerol; Alzheimer's disease; Antioxidant

Corresponding author:
Dr. Khaled Nabih Zaki Rashed,
Pharmacognosy Department,
National Research Centre,
33 El-Bohouth st.-Dokki, Giza,
Egypt- P.O.12622.

ABSTRACT

Platonia insignis Mart. (bacurizeiro) is traditionally used in Brazil in folk medicine as antiinflamatory. The aim of this study was to investigate the influence of 1, 3-distearoyl-2-oleoylglycerol (TG1) obtained from the seed, on specific memory of the animal and to evaluate *in-vitro* antioxidant potential (SOD, CAT, TBARS, and determine content of nitrite) and also anticholinesterase activity in the hippocampus of adult rats. Already in the Morris water maze test in the treated group TG1 can be observed a significant decrease of 63.59% and 38.71% in the latency time when compared with the vehicle group and control (neostigmine), respectively. In neurochemical tests can be found in treated groups TG1 a significant reduction of lipid peroxidation 88.49% and 76.89% and the content of nitrite. Furthermore, it can be observed a significant increase in the activity of 51.97% catalase and 45.53% in the activity of superoxide dismutase when compared to vehicle. When determining *in vitro* inhibition of anticholinesterase activity (IC_{50} = 4.38 µg/mL) compared to rivastigmine (IC_{50} = 1.87 µg/mL). The results suggest that TG1 can be a promising molecule for the development of pharmaceuticals.

INTRODUCTION

Old people with dementia exist nearly in every country of the world. Dementia rates are predicted to increase at an alarming rate in the least developed and developing regions of the world despite mortality resulting from

malnutrition, poverty, war, and infectious diseases. World Health Organization (WHO) projections suggest that by 2030 (65.7 million) and more than triple by 2050 (115.4 million). Dementia affects people in all countries, with more than half (58%) living in low- and middle-income countries. By 2050, this is likely to rise to more than 70%. Treating and caring for people with dementia currently costs the world more than US$ 604 billion per year. In Brazilians, dementia was determined to be the most significant predictor of death, followed by age, history of stroke, complaints of visual impairment, heart failure, and severe arterial hypertension [1, 2]. Developing countries tend to retain traditional herbal medical practices and thus offer an invaluable resource for new anti-dementia therapies [3,4].

Alzheimer's disease (AD) is the most frequent age-associated neurodegenerative disease. Its cognitive and neuropsychiatric manifestations result in progressive disorder and disability [5, 6]. AD affects approximately 10% of patients more than 65 years old and 40% of patients more than 80 years old. It is estimated that, in 2050, 25% of the global population will be elderly, thus increasing the disease prevalence. The initial symptom is characterized by progressive loss of recent memory. The progressive impairment in cognitive faculties such as memory, verbal and visuospatial ability is often accompanied by behavioral disorders, such as aggressiveness, depression and hallucinations [5,6]. Important studies were published through evaluation of possible anticonvulsant effects of ethyl acetate fraction from *P. insignis* on epilepsy models [7].

Disorders of the transmission of acetylcholine and acetyltransferases occur frequently in affected individuals. Some studies have demonstrated that the hippocampus is essential for short term memory structure which is dramatically reduced in individuals affected by Alzheimer's disease. The most promising treatment for AD is obtained by increasing the circulating levels of acetylcholine in the brain using acetylcholinesterase inhibitors (IAChE). There is growing research to discover new inhibitors acetilcolinesterae starting from medicinal plants [3]. IAChE such as physostigmine, neostigmine, galantamine and tacrine have been used in the treatment of AD, however, some of these substances have undesirable side-effects, such as the hepatotoxicity. Galantamine (Reminyl ®), an alkaloid obtained from plants, has long-acting, selective, reversible, competitive to inhibit AChE, is considered more effective in treating the disease and has fewer limitations [8]. Given this context the lack of drugs used for the treatment and prevention of AD, which combine a high efficacy and low side effects, has stimulated the search for new agents that may represent an alternative therapeutic. Other studies indicate that the reduction of oxidative stress have been proven as a therapeutic strategy to attenuate the neuronal death and neurodegenerative events in AD [9]. and inflammatory processes in AD, based on the compelling evidence that inflammatory processes are involved in the pathogenesis of AD, research has looked into the use of antiinflammatory drugs as a treatment option for patients with AD [10]. Bacuri is the fruit of the specie *P. insignis* Mart., Belonging to the

family Clusiaceae. In Brazil its popular use and results of the various properties, such as antiinflammatory, antimicrobial, antitumor, cytotoxic and antioxidant. It is widely used in folk medicine to treat skin diseases in both humans and animals and the seed decoction has been used to treat human diarrheas and inflammatory diseases [7, 11-12] In a recent manuscript [13] evaluated of the cicatrizant activity of a semisolid pharmaceutical formulation obtained from *P. insignis* cream with TG1 in three concentrations demonstrated efficacy in wound healing, as evidenced by macroscopic and microscopic analyses of lesions in Wistar rats. Based on this, a further development of phytomedicines for wound care is suggested. The specie P. insignis is a fruit plant and timber, has its origin in the western Brazilian Amazon, Pará State and is found in every state in the Northern region of Brazil and Mato Grosso, Maranhão and Piauí. Outside the national territory, is also found in the Guianas, Peru, Bolivia, Colombia and Ecuador [14]. In this context, the present study aimed to verify the influence of 1,3-distearoyl-2-oleoylglycerol (TG1), cream substance extracted from the seeds of P. insignis (bacurizeiro) on specific memory in animal models, and to evaluate the potential antioxidant and anticholinesterase activity in the hippocampus of adult rats treated.

MATERIAL AND METHODS

Plant material and isolation of the substance TG1

P. insignis, fruits were collected at Barras, Piauí State, Brazil, in March 2012. A voucher specimen has been identified and deposited at the "Graziela Barroso", Herbarium of Biology Department of Federal University of Piauí, Brazil (Voucher N°.: ICN TEPB 27.164). The seeds collected from the fruits of *P. insignis* were dried at 55 °C under shade and powdered mechanically. Crush yielded of seeds (848 g) was extracted with *n*-hexane (63%, w/w), followed by 95% ethanol (5.8%, w/w) in a Soxhlet apparatus (8 h for each solvent). In the *n*-hexane extract it, which was then fractionated using polarity increasing solvents. The fraction was concentrated in a vacuum evaporator. The *n*-hexane extract was fractionated using polarity increasing solvents by classical chromatographic column on silica gel to the isolation of TG1.

Chemical elucidation of compound TG1

The *n*-hexane extract was fractionated using polarity increasing solvents by classical chromatographic column on silica gel to the isolation of TG1 identified as a derivative of triolein, soluble in nonpolar solvents. It has the molecular formula $C_{58}H_{112}O_6$, its composition is formed by structural is C (76.93%), H (12.47%) and O (10.60%) and molecular mass of 856 u.m.a. 1,3-distearoyl-2-oleoylglycerol [see **Fig. 1]**, named TG1 corresponds to a triglyceride isolated from the hexane extract from the seeds of bacuri. Analysis of the ^{13}C spectra, ^{1}H, correlation and DEPT suggest structure TG1. Assignments of ^{13}C NMR signals in the region of double bond of lipids: -129.70 ppm: Carbon 9 (cis) of oleic acid referent will chain 2 carbon of glycerol -129.73 ppm: Carbon 9 (cis) of oleic acid will respect the chain of carbons 1 and 3 of glycerol -130.03 ppm: carbon 10 oleic acid (cis) will respect the chain of carbon 2 of the glycerol -130.04 ppm: carbon 10 oleic acid

(cis) for will chain of carbons 1 and 3 of glycerol [7, 11-12,15].

1,3-distearyl-2-oleyl-glycerol (TG1) : Its spectra of NMR ^{1}H NMR, ^{13}C NMR, DEPT 135 COSY and HMBC for identification of TG1 (13): $C_{57}H_{108}O_6$. NMR spectra description of ^{1}H is 13C NMR ($CDCl_3$, ppm, 125 MHz): δ_H = 5.45-5.35 (4H, m, -CH-CH-), 5.30-5.28 (1H, m, -CH_2-CH-CH_2-), 4.34-4.30 (2H, dd, -CH_2-CH(O)-CH_2-), 4.19-4.15 (2H, dd, -CH_2-CH(O)-CH_2-), 2.35-2.31 (6H, t, -C=O-CH_2-CH_2-), 2.04-1.96 (4H, m, =CH_2-CH_2-), 1.65-1.62 (6H, m, -C(=O)-CH_2-CH_2-), 1.32-1.29 (nH, m, -CH_2-), 0.92-0.89 (9H, t, -CH_3). NMR spectra description of ^{13}C is ^{13}C NMR ($CDCl_3$, ppm, 500 MHz), δ_C = 173.31(C-1, sn 1,3); 172.87 (C-1, sn 2); 34.06 (C-2, sn 1, 3); 34.21 (C-2, sn 2); 24.88 (C-3, sn 1,3); 24.88 (C-3, sn 2); 29.22 (C-4, sn 1,3); 29.14 (C-4, sn 2); 29.50(C-5, sn 1,3); 29.50 (C-5, sn 2), 29.35 (C-6, sn 1,3); 29.35 (C-6, sn 2); 29.70 (C-7, sn 1,3); 29.70 (C-7, sn 2); 29.72 (C-8, sn 1,3), 29.72 (C-8, sn 2); 29.72 (C-9,sn 1,3); 129.69 (C-9, sn 2); 29.72 (C-10, sn 1,3); 130.03 (C-10, sn 2); 29.72 (C-11, sn 1,3); 29.72(C-11, sn 2); 29.72 (C-12, sn 1,3); 29.72 (C-12,sn 2); 29.38 (C-13, sn 1,3); 29.38 (C-13, sn 2); 31.94 (C-14, sn 1,3); 31.94 (C-14, sn 2); 22.71 (C-15, sn 1,3); 22.71 (C-15, sn 2); 31.94 (C-16); 22.71 (C-17); 14.13 (C-18); 68.88 (C-2', CHO); 62.10 (C-1' and 3', CH_2O).

Animals testing

Male Wistar rats (250-300.0 g, two months-old), were used. The animals were randomly housed in appropriate cages at 26±1 ºC under 12 h light/dark cycle (lights on 06:00 am-18:00 pm) with access to food (Purina®) and water *ad libitum*. All experiments were carried out 8 am in a quiet room. Experimental protocols and procedures were approved by the Ethics Committee on Animal Experiments at the Federal University of Piauí (CEEA/UFPI # 004/2012). All procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Passive Avoidance aparatus

The inhibitory avoidance apparatus consists of made of 2 mm aluminum with epoxy paint, high impact floor in stainless steel bars with spacing of 12.5 mm, the inner area of leakage with 200 mm x 75 mm, and front door transparent acrylic drip tray waste and urine. The rat is placed on the platform inside a metallic box with a floor connected to an electrical stimulator. The rat as an animal exploration will seek to explore the rest of the box. To test a single session, the animals were initially submitted to an individual training session, in which they were gently placed on the platform fixed on the left side of the box inhibitory avoidance and allowed to explore the entire box. After a few seconds, the moment the animal down from the platform with all four paws on the grid of bronze bars eletric to explore the rest of the box, get an electric shock from 0.4 to 0.5 mA for 2-3 seconds. The animal is then removed from the test box and put back in its box residence [16]. To assess the memory formed during the session workout the animals were submitted to sessions of test, 15 minutes and 24 hours after training (to Memory of short and long duration, respectively), when they were again reattached upon the platform of box by avoidance. In test session was verified the

latency platform lowering. The more the animal retain the memory formed during the workout, the greater the latency of descent from platform in the moment of the test.

In the elevated plus-maze test

This test has been validated memory studies by Viana et al. [17]. The maze was constructed of wood, with three arms of equal dimensions (50 X 10 cm), with an arm enclosed by walls 40 cm perpendicular to the two opposite open arms. To avoid the fall of the open arms animals are surrounded by a strip of acrylic of 1 cm. The maze is elevated 50 cm above the ground. During the two days before testing the animals were handled gently for 5 minutes. Twenty four hours after the end of treatment TG1 with 30 mg / kg and 0.05% Tween 80 in 0.9% saline, the test was performed. Each rat was placed in the closed end of the arm of the maze and the time taken to exit the arm with all four paws was recorded (training). Following the same procedure was repeated on two subsequent workouts (avoidance 1 and 2) with 30 second intervals. Two days later (48 h), the animals were again placed in the maze and the time spent out of the closed arm was measured (avoidance 3). The maximum time of observation (*cut off*) during dodges was 300 sec.

Morris water Maze

This test is used to evaluate spatial memory [18]. The water maze consists of a circular plastic tank (132 cm in diameter) and walls 40 cm in height, filled (at 10 cm from the edge) in water (25 ° C) plus corn starch (to make the water opaque). The device has a platform acrylic (15 x 15 x 19 cm) placed in the northwest quadrant 2 cm below the water surface. Twenty-four hours after treatment with TG1 and 0.05% Tween 80 in 0.9% saline, the animals began training. Six were performed on two consecutive days training (learning) and after 48 hours the animals were tested for the evaluation of spatial memory (retention). During training, the animal was placed in six different locations and the tank was 54 seconds to find the platform at the end of this time, it was placed in the same manually for 10 seconds and then removed from the tank for 30 seconds. At the end of day 12, each animal received training for acquisition of memory. On the fourth day (after 48 hours), the platform was removed and the animals placed in the tank, southeast position (relative to the platform position). The animals remained up to 60 seconds and the latency to reach the site of the original platform was recorded.

Methods for determining the content of acid reactive substances to thiobarbituric acid (TBARS)

The extent of lipid peroxidation was measured by determining the level of TBARS method previously described by Draper and Hadley [19]. Homogenate was prepared at 10% (w / v) in sodium phosphate buffer 50 mM pH 7.4 with hippocampal areas of all groups, vehicle (n = 8) and TG1 30 mg / kg (n = 8). The results were expressed in nmol MDA / g of tissue.

Method of determining the content of nitrite

The content of nitrite in the experimental groups vehicle (n = 8) and TG1 30 mg / kg (n = 8) were determined based on the Griess reaction [20]. Nitric oxide was generated by decomposition of sodium

nitroprusside in 20 mM phosphate buffer (pH 7.4) by Griess reaction. The results were expressed as percentage of nitrite formed compared to the sodium nitroprusside (NPS) alone (reaction medium) and mM.

Method of determining the activity of catalase (CAT)

Activity of catalase was measured in the experimental groups vehicle (n = 8) AC 250 (n = 8) and TG1 30 mg / kg (n = 8), using the basic principle of measuring the rate of production of O_2 and H_2O. Protein concentration was determined (Lowry et al, 1951). The results were expressed in mmol / min / mg protein [21].

Method of determining the activity of superoxide dismutase (SOD)

Hippocampal homogenates 10%were centrifuged (800 × g, 20 min) and the supernatants used to determine the activities of superoxide dismutase (SOD). SOD activity in vehicle group (n = 8), TG1 30 mg / kg (n = 8) was tested by the rate of reduction of cytochrome C by superoxide radicals, using the system xanthine - xanthine oxidase as a source of superoxide anion (O_2-) (Arthur, Boyne, 1985). The results were expressed as U/mg protein. One unit (U) of SOD activity corresponds to 50% inhibition of the reaction of O_2- with cytochrome C. Protein concentration was obtained by the method of Lowry et al.[22].

Determination of in vitro anticholinesterase activity of TG1

The inhibitory effect on the TG1 acetylcholinesterase activity *in vitro* was evaluated as described by Ellman [23]adapted for Ingkaninan [8]. Used a spectrophotometer was Biosistem SP220 for the inhibitory activity quantitatively. Initially, 100 µL of the sample (concentrations of de 0.1 µg/ml, 0.05 µg/ml, 0.025 µg/ml e 0.0125 µg/ solution) in 50 mM Tris-HCl pH 8, and 10% methanol) were mixed with 100 µL AChE ¶ l of 0.22 U / ml (22 U of enzyme diluted in 100 µL of 50 mM Tris-HCl pH 8, 0.1% bovine serum albumin,BSA) and 200µL buffer (50 mM Tris-HCl pH 8, 0.1% (BSA). the mixture was incubated for 5 min at 30 ° C, then 500 µL was added acid 5,5-dithiobis (2-nitrobenzoic acid) - DTNB (in concentration of 3 mM Tris-HCl pH 8, 0.1 M NaCl, 0.02 M $MgCl_2$) and 100 µL of Acetylthiocholine iodide (ATCI, 4 mM in water). A blank was also prepared by replacing AChE with 100 µL of buffer (50 mM Tris-HCl buffer pH 8, 0.1% BSA). All samples were analyzed in triplicate. The reaction was monitored for 5 min at 412 nm. The drug neostigmine was used as a standard and was used as a negative control Buffer (0.1% methanol in 50 mmol / L Tris-HCl pH 8, 10%). The percentage of inhibition of the isolated substance and rivastigmine were calculated according to equation 1. Antiacetylcholinesterase activity (I %) was calculated as following:

$$I\ (\%) = (1 - V0\ Sample/V0\ Blank) \times 100$$

(Equation 1).

V0 Sample and V0 Blank represent the initial velocities of samples and blank. IC_{50} values were obtained though Log-Probit plotting.

Statistical Analyses

After collecting the data, they were tabulated on spreadsheets and then analyzed statistically. The results were expressed as the mean ± standard error of the mean (S.E.M) using the "Software" GraphPad Prism 5.0 Comparison between experimental groups was performed by testing one-way ANOVA, followed by t-test Newman-Keuls as post hoc test and the significance level of 5%.

RESULTS

1,3-distearyl-2-oleyl-glycerol [see **Fig. 1**] white solid,. identified as a derivative of triolein, soluble in nonpolar solvents.

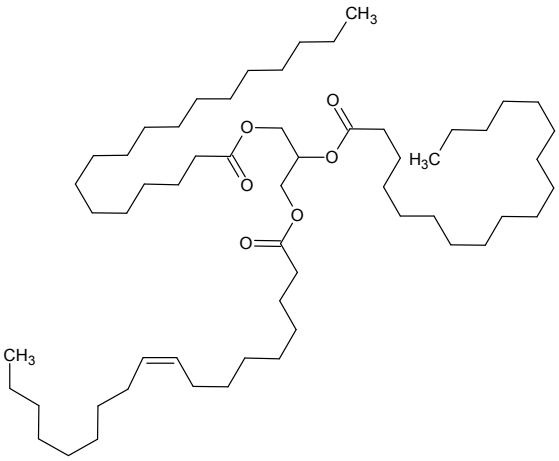

Figure 1: Chemical structure of 1,3-distearoyl-2-oleoylglycerol (TG1).

Passive Avoidance Test

In the passive avoidance test animals learn to avoid the shock (training), when evaluated 15 minutes after the shock (recent memory) or 24 hours after the shock (delayed recall). Figure 2 shows that the animals in the vehicle group (0.05% Tween 80) showed a good retention of memory in the next stage memory (recent) (T0 = 102,6 ± 33,95; T15 = 164,5 ± 51,28; T24 = 94,00 ± 33,79), In the groups treated with the positive control (neostigmine 30 mg / kg) showed better retention in the consolidation phase (delayed recall), (T0 = 33,50 ± 6,11; T15 = 20,13 ± 4,84; T24 = 203,1 ± 47,50) ($p<0,05$). TG1 in groups treated with 30 mg / kg showed significant increase in latency to fall from the platform when compared to the training, meaning an improvement in learning and memory (T0 = 33,50 ± 6,118; T15 = 20,13 ± 4,846; T24 = 203,1 ± 47,50). Comparing the treated groups, can be observed 68.57% reduction in latency platform down, TG1 treated rats (30 mg / kg), evaluated in comparison with the training vehicle, as noted in recent memory a decrease of 18.3% in the treated group TG1 to the vehicle and an increase of 85% compared to the positive control, since when evaluated in delayed recall a significant increase in latency to fall in the group treated with bar TG1 30 mg / kg, with better activity than the reference drug.

In the elevated plus-maze test

Treatment with 30 mg / kg (r.o) TG1 [**see fig. 3**], did not affect the memory, since the time spent in the closed arms was similar to the control group and the time up to the time of the group treated with neostigmine (0.5 mg / kg). Aversive space exploration was greater in rats treated with neostigmine, suggesting that the TG1 can have better effects as learning and memory in rats. The elevated T-maze as a potential model for the combined study of anxiety and memory [17].

Morris water Maze

In Figure 4 demonstrated the latency to find the platform in the Morris water maze on the testing day. It can be observed a significant decrease of 63.59% and 38.71% for the latency time of treated group TG1 (30 mg /

kg, r.o) (15.25 ± 5.371 s) when compared with vehicle group (41.88 ± 2.248 s) and control (neostigmine 0.5 mg / kg) (24.88 ± 3.425 s), to find the platform respectively.

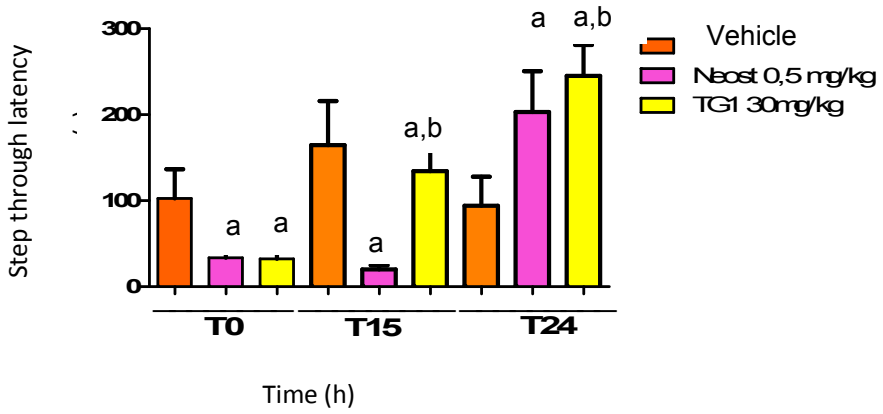

Figure 2– Effect of (TG1) 30 mg/ kg (r.o) to rats in delayed recall (n = 8) in the passive avoidance test, ($p<0.05$). a= b= c=

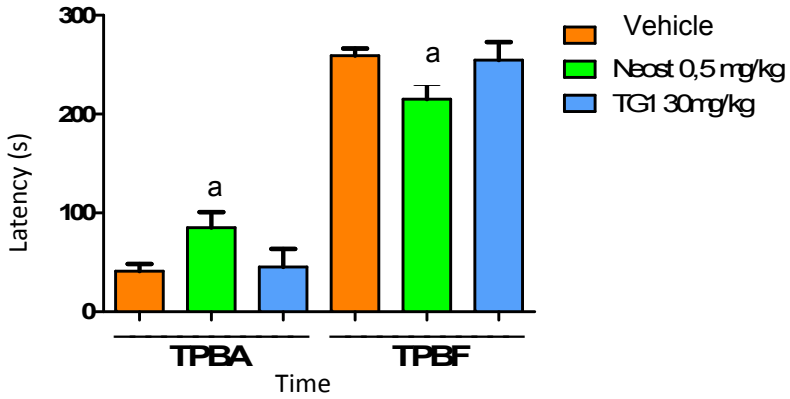

Figure 3 – Effect of treatment with (TG1) 30mg/kg (r.o) in the acquisition memory of rats (n = 8) Maze test T ($p < 0,05$).

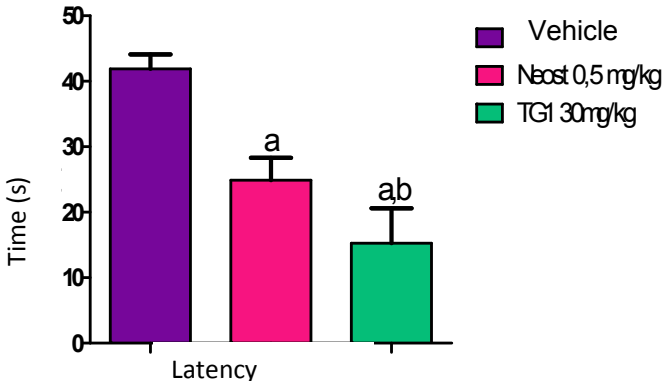

Figure 4 - Latency (s) on test day (day 4) to reach the location on training days the platform was located. Data are expressed as mean ± standard error. [a]$p \leq 0,05$ compared to the vehicle group and [b]$p \leq 0,05$ compared to the control group.

Evaluation of antioxidant activity

Table 1 shows the results of neurochemical TG1 groups treated with 30 mg / kg (r.o) and vehicle. Was observed in the group treated with TG1 a significant decrease of 88.49% in the lipid peroxidation and 76.89% in the content of nitrite and a significant increase of 51.97% in catalase activity and 45.53% in the enzyme activity superoxide dismutase compared to vehicle.

Determination of in vitro anticholinesterase activity of TG1

The results of *in vitro* determination verified the inhibition of AChE activity 95.9, 86.92 and 77.12% when used rivastigmine (Exelon®) (positive control) at concentrations of 0.1, 0.05, 0 025%, respectively. When evaluated the inhibitory activity of the compound was verified TG1 following results: 0.1% (77.12%), 0.05% (72.34%), 0025% (61.17%) and 0.0125% (20.21%). Based on these values were also determined the IC_{50} corresponding to 4.38 µg / mL (confidence interval 95% [1.27 to 4.56 g / mL], r^2 = 0.1592). The choice of an experimental model for evaluation of learning and memory of a rodent The choice of an experimental model for evaluation of learning and memory of a rodent, must take into account the animal's ability to learn the job and your ability to execute it. In the assessment of cognition in rats, the experimental procedure of the Morris water maze is appropriate, since these animals are shown to be good swimmers and have a good capacity for spatial localization required in this test because the water be an aversive, the trend of this kind is to seek escape this means [24].

Table 1- Determination of the levels of reactive substances to TBARS, nitrite content, activities of catalase and SOD in hippocampus of rats treated with TG1.

Groups	Parameters			
	TBARS (mmol/MDA/µg protein)	**NITRITE (µM)**	**CATALASE (U/µg of proteín)**	**SOD (U/µg of proteín)**
Vehicle	1.39 ± 0.15	90.41 ± 1.22	14.45 ± 0.13	2.35 ± 0.14
TG1	0.16 ± 0.01[a]	20.90 ± 0.79[a]	30.09 ± 2.23[a]	3.42 ± 0.28[a]

The results represent the mean ± S.E.M of levels of reactive substances with thiobarbituric (TBARS), nitrite content, catalase and superoxide dismutase activity in male Wistar rats acutely treated orally with vehicle (0.05% Tween 80 dissolved in 0.9 % saline) at a dose of 0.1 ml /10g body weight and TG1 at a dose of 30 mg / kg (n = 8 for both groups). After 30 days of treatment the animals were euthanized with sodium pentobarbital at a dose 40 mg / kg (i.p) for removal and dissection of the brain neurochemistry for evaluation of the hippocampus.

DISCUSSION

In experiments with water maze and young Wistar rats, were the first researchers to study the correlation between the activity of the enzyme acetylcholinesterase (AChE) in rat brain and performance on a spatial task. In this study the authors evaluated the activity of the enzyme AChE in 43 different brain regions of rats. Individual learning and retention rates were found and correlated significantly with the level of AChE in specific regions such as the striatum and hippocampus [25]. It is widely believed that the hippocampus plays a temporary role in the retrieval of episodic and contextual memories. Initial studies indicate that damage to this hippocampus structure produced amnesia for newly acquired memories but did not affect those formed in the distant past. A number of recent studies, however, have found that the hippocampus is required for the retrieval of episodic and contextual memories regardless of their age. These findings are currently the subject of intense debate and a satisfying resolution has yet to be identified. Studies suggest that detailed context memories always require the hippocampus (independent of their age) while memories that lose precision can be retrieved without this structure. These findings account for inconsistencies in the literature - memories of our distant past can be either lost or retained after hippocampus damage depending on their quality – and provide a new framework for understanding memory consolidation [26]. Treatment with TG1 caused improvement in learning and memory, as evidenced by the significant decrease in the time required for animals to find the location of the platform in the Morris water maze. Similarly, in the model of emotional memory in which the animal learns to avoid a clash (passive avoidance) involving the limbic system, which comprises the hippocampus, amygdala, medial septum, olfactory bulb and thalamic areas prior interconnected forming the papez circuit. The TG1 was also able to improve memory deficits in a dose tested (30 mg / kg) at term memory (learning after a few minutes), and delayed memory (one day after learning), as shown in our experiments. Acetylcholinesterase inhibitors used in the treatment of Alzheimer's disease (tacrine, rivastigmine, donepezil and galanthamine) alter the central cholinergic function by inhibiting acetylcholine-degrading enzymes as the enzyme AChE, thus increasing the ability to stimulate acetylcholine receptors brain nicotinic and muscarinic [27]. The substance TG1 presented the inhibition of AChE next rivastigmine (positive control) presenting thereby an effective cholinergic stimulation same reference drug concentrations. Research indicates that oxidative damage may play an important role

in the pathogenesis of Alzheimer's disease (AD) in at least some key regions of the brain. Further indicating that oxidative damage was observed in brains with AD in response to senile plaques and neurofibrillary tangles, but the most recent hypothesis states that oxidative damage is observed in brains in individuals with mild cognitive impairment, which is considered as phase preclinical AD [28-31]. Thus, the present study investigated the influence of TG1 lipid peroxidation, nitrite content and activity of catalase and superoxide dismutase in the hippocampus of rats submentidos the memory tests. Nitric oxide (NO) is involved in diverse cellular functions, depending on the production site acts as agent vasorrelaxante, neurotransmitter and immunomodulatory. Lipid peroxidation can be defined as the biological damage caused by free radicals which are formed under oxidative stress [32-33] Our results suggest a potent antioxidant activity of the compound against Reactive oxygen species and / or nitrogen, suggesting that the TG1 can be used as an alternative therapy for various diseases related to oxidative stress, particularly for diseases related to the central nervous system, such as Alzheimer's disease. The SOD and CAT are antioxidant enzymes that act on different sites in the metabolic pathway of free radicals, when there is a change in the activity of this enzyme without compensatory changes in the body can result in oxidative stress [34]. Given that SOD and CAT can be considered the endogenous enzymatic antioxidant defenses of the organism most important in combating free radicals production, increased their activity suggested that antioxidant TG1 has a potential related to the modulation of enzymatic activity. However, more studies should be performed to determine if this compound is able to alter the gene expression of these enzymes. One of the best sources of new substances to treat AD are natural products and their derivatives. Traditionally, plants have been used to enhance memory and to alleviate other symptoms associated with AD [35]. Alzheimer's disease (AD) is the most common neurodegenerative disorder to date. Neuropathological hallmarks are β-amyloid (Aβ) plaques and neurofibrillary tangles, but the inflammatory process has a fundamental role in the pathogenesis of AD. Supplementation of these natural compounds may provide a new therapeutic line of approach to this brain disorder. These studies should involve supplementation, a natural strategy, with relatively high doses of specific purified flavanoids for exemple, to shed light to the apparent inverse risk relationship with AD (and whether this occurs by reducing inflammation) and also to determine if such compounds are therapeutically beneficial [36].

CONCLUSION

According to the results presented in this study may suggest that acute treatment with 1,3-distearoyl-2-oleoylglycerol (TG1) showed a beneficial effect on the deficit of learning and memory, and can modulate the activity of enzymes that are antioxidants that reduce oxidative stress and formation of nitrite, and may even provide protection against brain injury. The results suggesting that TG1 is a promising molecule for the development of pharmaceuticals. However, tests on the process memory in rats must be made to

justify the development of a new formulation for the treatment of neurodegenerative diseases.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGMENT

The authors thank CNPq for financial support.

REFERENCES

1. Kalaria RN, Maestre GE, Arizaga R, Friedland RP, Galasko D, Hall K. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. Lancet. Neurol. 2008; 7:812-826.
2. Nitrini R, Caramelli P, Júnior EH. Mortality from dementia in a community-dwelling Brazilian population. Int. J. Geriatr Pschiatry 2005; 20:247-253.
3. Feitosa CM, Freitas RM, Luz NNN, Bezerra MZB, Trevisan MTS,. Acetylcholinesterase Inhibition by somes promising Brazilian Medicinal Plants. Braz.. J. Biol. 2011; 71: 108-110.
4. Perry E. Commentary: botanical potentials in Alzheimer's disease. . J. Alter.n Complement. Med. 2007; 13: 345–346.
5. Del Bo R, Ghezzi S, Scarpini E, Bresolin N, Comi. GP. genetic variability is associated with increased risk of developing Alzheimer`s disease. J. Neurol. Science. 2009; 286:66-68.
6. Ito K, Ahadieh S, Corrigan B, French J, Fullerton T, Tensfeldt T. Disease progression meta-analysis model in Alzheimer`s disease. Alzheimer`s & Dementia.. 2010; 6: 39-53.
7. Costa Júnior JS. Phytochemistry Analysis, Pharmacology and Toxicology of *Platonia insignis* Mart. (bacuri) seeds, (doctoral thesis), Luterana University of Brazil. Brasil, ULBRA, Brazil, 2011.
8. Ingkaninan K, De Best CM, Van Der Heijden, R., Hofte, Karabatak A. J., B, Irth, H. High-performance liquid chromatography with on-line coupled UV, mass spectrometric and biochemical detection for identification of acetylcholinesterase inhibitors from natural products. J. Chromatogr. A. 2000; 872: 61-73.
9. Moore AH, O'Banion MK. Neuroinflammtion and anti-inflammatory therapy for Alzheimer's disease", Ad. Drug. Deliver. Rev. 2002; 54: 1627-1656.
10. Heneka MT, O'Banion MK. Inflammatory processes in Alzheimer's disease J. Neuroimmunol. 2007; 184: 69-91.
11. Costa Júnior JS, Ferraz AB.F, Sousa TO, Silva RAC, Lima SG, Feitosa CM, Investigation of Biological Activities of Dichloromethane and Ethyl Acetate Fractions of *Platonia insignis* Mart. Seed. Basic. Clin. Pharmacol. Toxicol. 2013; 112: 34–41.
12. Costa Júnior JS, Almeida AAC, Tomé AR, Citó AM.GL, Saffi J, Freitas RM.. Evaluation of possible anticonvulsant effects of ethyl acetate fraction from *Platonia insignis* Mart. (Bacuri) on epilepsy models. Epilepsy Behav. 2011; 22:678-684.

13. Mendes MCS, Oliveira GAL., Lacerda JS, Rezende Junior LM, Silva MLG, Coelho ML. Evaluation of the cicatrizant activity of a semisolid pharmaceutical formulation obtained from *Platonia insignis* Mart. Afr. J. Pharm. Pharmacol..2015; 9: 154-164.
14. Santos PRF, Carvalho RBF., Costa Júnior JS, R. M. Freitas, C. M. Feitosa. Survey of physicochemical and pharmacological properties of extracts and compounds isolated from *Platonia insignis* Mart. a perspective for developing phytomedicines. Braz. J. Pharm. 2013; 94: 161-168.
15. Santos Júnior R Q, Soares L. C., A. L. M. Maia Filho, K. S. Araújo, I. M. S. Santos, P., J. S. Costa Junior. Histologic study of skin of wounds healing using the cream of bacuri (*Platonia insignis* Mart). ConScientiae Saúde. 2010; 9: 575-581.
16. Vianna M. R., Szapiro G., J. L. Mcgraugh, J. H. Medina, I. Izquierdo. Retrieval of memory for fear-motivated training initiates extinction requiring protein synthesis in the rat hippocampus. Proc. Natl. Acad. Sci. USA. 2001; 98:12251-12254.
17. Viana MB, Tomaz C, Graeff FG. The elevated T-maze: A new animal model of anxiety and memory. Pharmacol. Biochem. Behav. 1994; 49: 549-544.
18. Morris RGM. Spatial localization does not require the presence of local cues. Learn. Motiv. 1981; 12 :239-260.
19. Draper HH, Hadley M. Malondialdehyde determination as an index of lipid peroxidation. Methods Enzymol. 1990; 186: 421-431.
20. Green L C, Tannenbaum S, Goldman RP. Nitrate synthesis in the germfree and conventional rat", Sci. 1981; 212: 56-58.
21. Chance B, Maehly AC. Assay catalases and peroxidases. Methods Enzymol. 1955; 2 :764-768.
22. Lowry O H, Roebrough N J, Farr A L, Randal RJ. Protein measurement with follin phenol reagent", J Biol Chem. 1951 193: 265-275.
23 Ellman GL, Courtney KD, Andres V, Featherstone RM.. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7:88-95.
24. Pettenuzzo, L. F. "Effect of administration of two propionic and methylmalonic acid on Morris (Water Maze) and on biochemical parameters of oxidative alguns estresse em hippocampus. [master's thesis]. 2001
25. Segal M, Greenberger V, Israeli M, Biegon A. A. Correlation between regional acetylcholinesterase activity in rat brain and performance in a spatial task, Behav.. Brain Res. 1988; 30: 215-19.
26. Wiltgen BJ, Zhou M Y, Cai, J, Karlson MG, Parivash SN. The hippocampus plays a selective role in the retrieval of detailed context memories. Curr Biol. 2010; 20:1336–1344.
27. Grossberg GT. Cholinesterase inhibitors for the treatment of Alzheimer's disease: Getting On and Staying On. Curr Ther Res Clin Exp. 2003; 64: 216-235.
28. Bonda DJ,. Wang X, Perry G., Nunomura A.., Tabaton M., Zhu X. Oxidative stress

in Alzheimer disease: A possibility for prevention. Neuropharmacology. 2010; 59: 290-294.

29. Keller JN, Schmitt FA, Scheff SW., Ding Q, Chen Q., Butterfield DA. .Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurol. 2005; 64 :1152–1156.
30. Migliore L, Fontana I, Trippi F, Colognato R, Coppedè F, Tognoni G. Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. Neurobiol. Aging. 2005; 26:567–573.
31. Schulz JB, Lindenau J, Seyfried J, Dichgans J. Glutathione, oxidative stress and neurodegeneration, Eur J Biochem. 2000; 267: 4904–4911.
32. Migliorea L, Coppedeb F. Environmental-induced oxidative stress in neurodegenerative disorders and aging. Mutat. Res. 2009; 674: 73-84.
33. Zin ZM, Abdul-Hamid A, Osman A. Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf., Food Chem. 2002; 78:227-23.
34. Sarandol A, Sarando E, Eke SS, Erdinc S, Vatansever E, Kirli S. Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative–antioxidative systems. Hum Psychopharm. Clin. 2007; 22:67-73.
35. Houghton PJ, Howes MJR. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacol. Biochem. Behav. 2003; 75:513-27.
36. Rubio-Perez JM, Morillas-Ruiz JM. A Review: Inflammatory Process in Alzheimer's Disease, Role of Cytokines. The. Scientific. World. J. 2012; 20:1-15.