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RESEARCH ARTICLE

ACETYLCHOLINESTERASE INHIBITION, NOOTROPIC AND ANTIOXIDANT EFFECT OF EXTRACTS FROM *AGAVE* SPECIES

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Abstract

Background: The systemic administration of Lipopolysaccharide (LPS) is a pharmacological model to evaluate different processes associated with neurodegeneration; it is capable of causing an increase in the oxidative state in the brain and increasing the activity of the enzyme acetylcholinesterase (ACh-E), involved in the degradation of acetylcholine (ACh). This cholinergic transmission system participates in memory processes, a crucial symptom in neurodegenerative disorders, and its modulation with scopolamine turns out to be an essential tool in the evaluation of plants. Species of the genus *Agave* are considered a natural resource of economic and cultural importance in Mexico. They possess compounds with anti-inflammatory, antioxidant, and anti-neuroinflammatory activities.
Methods: Extracts of *A. tequilana* (At-A), *A. angustifolia* (Aan-A), *A. americana* (Aam-A) at 125 mg/kg were administered to male ICR mice with LPS to evaluate the ACh-E inhibition, the concentration of the antioxidant enzyme Gluthatione Reductase (GR) and the prooxidant enzyme NADPH Oxidase (NOX). Also, the nootropic effect of these extracts on scopolamine-induced cognitive impairment was evaluated.
Results: The three *Agave* species studied decreased the ACh-E enzyme activity, V_{max} value, and K_M. These products increased the GR concentration from 0.173±0.003 (vehicle) to 0.642±0.002 µMol/mg in At-A, which was the highest one. Regarding NOX, only the *A. tequilana* extract decreased it (0.513±0.002 µMol/mg) compared with Vehicle (0.621±0.008 µMol/mg). *A. tequilana* and *A. americana* species significantly improved the retention latency parameter (260 seconds) during the passive avoidance test.
Conclusion: *Agave* species showed promising results in developing novel drugs for neurodegenerative disease therapy.
Keywords: Acetylcholinesterase, *Agave*, LPS, Neurodegeneration.

INTRODUCTION

The Agavaceae family includes important species in Mexico, not only for their abundance but also for their uses. For example, *Agave americana*, *Agave angustifolia*, and *Agave tequilana* are widely distributed across the American continent, with specimens from the south of the United States of America to South America. It is a broad group that includes 210 species, 159 of which are found in Mexico, where 129 are endemic, representing 61% of the total number in the world[1]. In traditional Mexican medicine, these species have been employed to treat wounds, rheumatoid arthritis, snake bites, cancer, extremity paralysis, postpartum abdominal inflammation, as well as diabetes and hypertension[2]. More specifically, it is reported that *A. Americana* can alleviate gastrointestinal problems such as ulcers and dysentery[3]. *A. angustifolia* for digestive disorders[4] and *A. tequilana*, a plant of important industrial use in Mexico for tequila production[5].
Pharmacological reports indicate diverse biological effects for the genus, including antioxidant, antibacterial, anticancer, and anti-inflammatory properties[6]. It has been demonstrated that *A. americana*, *A. angustifolia*, or *A. tequilana* acetonic extract can modulate the neuroinflammatory response of mice exposed to lipopolysaccharide (LPS) because they decreased IL-6 and TNF-α pro-inflammatory cytokine concentration and increased IL-10 (anti-

inflammatory) in the brain[7]. Thus, *Agave* species may be clinically valuable for treating psychiatric diseases (such as depression) and neurodegenerative diseases like Alzheimer's disease (AD) since cellular and biochemical pharmacological mechanisms of action associated with neuroinflammation have been established. Neuroinflammation is a complex mechanism involving a wide variety of receptors and intracellular signaling systems, which act together to repair damage caused by degeneration derived from environmental or aging damage, but also participate in the perpetuation of neuroinflammation during disease. The activation of microglia, cells that coordinate the immune system, is fundamental during this process. Therefore, the immunogenic stimulus that causes inflammation triggers a cell signaling cascade that aims to resolve the aggression[8], provoking the microglia to adopt an amoeboid morphology, leading to an overproduction of cytokines and reactive oxygen species (ROS)[9,10].

In pharmacological assays, a model widely employed to induce neuroinflammation and cognitive impairment is that of LPS (lipopolysaccharide) systemic administration, which has been shown to cause dysregulation in the inflammatory (IL-1β, IL-6, TNF-α) and anti-inflammatory (IL-10) cytokine concentrations. This dysregulation leads to the overproduction of ROS and promotes oxidative stress resulting in cell damage and contributing to the pathogenesis of neurodegenerative diseases[11]. These cellular events cause behavioral changes, where memory loss is the most impacting symptom experienced by a patient and family because it leads to a lack of identity. So, a relevant therapeutic target is the memory associated with neurodegenerative diseases such as AD.

Cholinergic neurotransmission regulates higher cortical functions related to memory, learning, and concentration through neuronal transmission by acetylcholine (ACh) and its cholinergic receptors. It has been demonstrated that scopolamine (ESC), which acts on muscarinic receptors for ACh, impairs the encoding new memories without deteriorating the stored memory. At the same time, activating nicotinic receptors leads to improved consolidation of new memories[12]. An essential element in this neurotransmission system linked to memory is the enzyme acetylcholinesterase (ACh-E), which is responsible for the ACh hydrolysis[13]; so this protein's activity reduces the ACh levels and cholinergic transmission. Pharmacologically, ACh-E inhibition is a clinical strategy on which some of the prescribed treatments for patients with cognitive deficits, like those with AD, are based[14]. These drugs are therapeutically beneficial but with limited efficacy, making searching for new therapies an ongoing line of research.

The present work aimed to evaluate the effects of an acetonic extract of three *Agave* species: *A. americana*, *A. angustifolia*, and *A. tequilana*, on the enzymes ACh-E, nicotinamide adenine dinucleotide phosphate oxidase (NOX) and glutathione reductase (GR) activity, using as substrate the brain homogenate of mice with LPS-induced neuroinflammation, as well as to evaluate them in a scopolamine-induced memory impairment test.

MATERIALS AND METHODS

Plant material and extraction

The different *Agave* species leaves were identified at the Institute of Biology of the Universidad Nacional Autónoma de México (UNAM) by Dr. Abisaí Josue García Mendoza as *A. tequilana* F AC Weber, *A. angustifolia* Haw, and *A. americana* L. Marginata Hort. The 5-year-old leaves of *A. tequilana* and *A. angustifolia* were obtained from a traditional controlled crop in Tlaquiltenango, Morelos (18°37'48"N, 99° 10'00"W). Those from *A. americana* were collected in Toluca de Lerdo, Estado de México (19°17'29"N, 99° 39'38"W).

The material was weighed, chopped into pieces, lyophilized, ground, macerated with acetone, and concentrated under reduced pressure in a rotavapor. The extracts obtained from this method were named At-A (*A. tequilana*), Aan-A (*A. angustifolia*), and Aam-A (*A. americana*); each extract was lyophilized and refrigerated at 4°C.

Animals

Male mice (ICR; 35g weight) from the Centro Médico Siglo XXI IMSS biotherium were employed. They were kept under controlled conditions (12 h of light and 12 h of darkness), with free access to food and water. The assays were performed according to the Norma Oficial Mexicana NOM-062-ZOO-1999. The experimental protocols of the present study were approved by the IMSS research committee with reference number R-2010-1701-21.

Damage induced with lipopolysaccharide (LPS) administration

The experimental model selected was that of intraperitoneal (i.p.) administration of LPS from *E. coli*[15]. The 0.25 µg/kg, i.p. LPS dose employed, as well as that of 125 mg/kg orally pathway (o.p.) for the extracts of the *Agave* species evaluated as treatments and 5 mg/kgo.p. for Indomethacin, a non-steroidal anti-inflammatory drug used as a positive control, were taken according to the results reported in a previous work7. The experimental groups (n=8) were defined as follows:

1. Basal group (healthy animals): administered i.p. with sterile saline solution (SS) for 7 days. The following 7 days they received Tween 20 solution at 1% (o.p.) as vehicle.

The successive groups received a daily dose of LPS (0.25 µg/kg, i.p.) for 7 days. Then, for the next 7 days, each animal received the corresponding treatment (o.p.). On the last day (day 14) of the experiment, the mice were administered for the last time with LPS i.p. four hours before sacrifice.

2. Negative control group: VEH (vehicle).

3. Positive control group: INDO (Indomethacin, 5 mg/kg).

4. Experimental Group: At-A (*A. tequilana* acetonic leaves extract; 125 mg/kg).

5. Experimental Group: Aam-A (*A. americana* acetonic leaves extract; 125 mg/kg).
6. Experimental Group: Aan-A (*A. angustifolia* acetonic leaves extract; 125 mg/kg).
Once this experimental stage was concluded, the animals were sacrificed with an anesthetic overdose to dissect the brain. The homogenate of this tissue was used as an enzymatic extract to determine the activity of the Acetylcholinesterase (ACh-E), Glutathione Reductase (GR), and NADPH-oxidase(NOX).

***In vitro* assays of ACh-E, GR and NOX enzyme activity inhibition**

For the ACh-E inhibition assay, as well as the activities of the antioxidant enzyme GR and the prooxidant enzyme NOX, ELISA assay kits were purchased from Sigma-Aldrich, following the manufacturer's instructions.

Scopolamine-induced cognitive impairment

Passive avoidance test

The passive avoidance equipment (PA) consists of two chambers, one illuminated and one dark; on the floor, a metal base is connected to a stimulator that generates electric shocks that allow one to study the acquired memory. The animal is conditioned with an aversive stimulus and is subsequently evaluated if the mouse remembers the experience.

The animals were trained by placing each mouse in the illuminated chamber of the device during 30 sec. The door that separates the chambers (illuminated and dark) was opened, and the time it took for each individual to cross into the dark chamber was measured. Once the individual had crossed, the door was closed and an electrical shock (0.2 mA, for 2 seconds) was released and applied to the mice's paws. After 24 hours, the test was repeated, but without electric shocks, and the time recorded was considered as initial latency (IL). If 300 seconds elapsed without crossing, the test was concluded on the understanding that the individual achieved the desired conditioning. Subsequently, the different treatments were administered o.p. during a week. On the last day of administration of each treatment, cognitive impairment was induced by administering scopolamine (SC) i.p., 30 minutes before performing the avoidance challenge. The time recorded in this occasion was considered as retention latency (RL). Finally, IL and RL parameters were compared[16].

The groups (n=7) established for this test were the following:

1. Basal control group: animals without SC and 100 µl/10 g wt. of vehicle o.p.

The following groups were administered with the corresponding treatment v.o. daily for a week and on the day of the final test, SC was administered at 2.0 mg/kg i.p. prior to exposure to the PA apparatus.

2. Negative control group: Veh (100 µl/10 g vehicle weight).
3. Positive control group: Gal 2.0 (Galantamine, 2mg/kg).
4. Experimental Group: At-A (*A. tequilana* acetonic leaves extract; 125 mg/kg).
5. Experimental Group: Aam-A (*A. americana* acetonic leaves extract; 125 mg/kg).
6. Experimental Group: Aan-A (*A. angustifolia* acetonic leaves extract; 125 mg/kg).

Statistical analysis

The results were analyzed with the statistical software SPSS version 11.0 through an ANOVA and a Bonferroni post-test. A value of $*p<0.05$ was considered statistically different compared to the Vehicle group in the antioxidant activity assays and the behavioral assay.

RESULTS AND DISCUSSION

Only a few reports mention the anti-neuroinflammatory effect of *Agave* species; in contrast, this research group has conducted several trials where they have reported the anti-inflammatory and anti-neuroinflammatory effect of the acetonic extract of *A. Americana*, *A. angustifolia*, and *A. tequilana*, in addition to the pair of saponins isolated from the first species, cantalasaponin-17[17] and 3-O-[(6'-O-palmitoyl)-β-D-glucopyranosyl sitosterol][18]. In this work, the neuroprotective effect on memory was explored, for which the model of systemic administration of LPS was employed to elicit neuroinflammation.

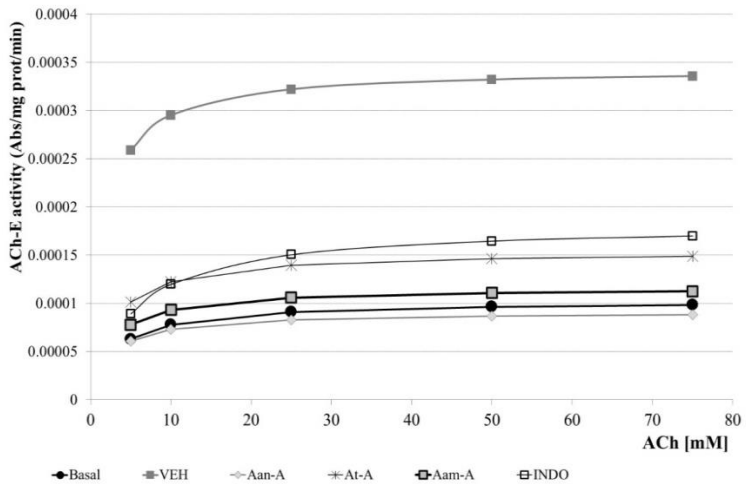

Figure 1: Effect of different *Agave* treatments on ACh-E activity in brain homogenates from mice with LPS-induced neuroinflammation.

Considering that some neuro-degenerative diseases are associated with the disruption of the cholinergic system, either through the activation of the enzyme ACh-E where ACh is degraded, leading to various cellular actions that cause, among other effects, cognitive impairment, or by interruption of the transmission mediated by muscarinic or nicotinic receptors[14].

Evaluation of ACh-E inhibition

One of the parameters associated with the neurodegeneration of cholinergic cells is the activation of the ACh-E enzyme; therefore, part of the accepted therapeutics for AD and cognitive impairment is based on drugs that attempt to improve the transmission of this system. ACh-E inhibitors enhance the impaired cholinergic transmission caused by ACh-synthesizing neuron death. Also, they reduce the associated inflammation and improve memory, a cardinal symptom in some neurodegenerative diseases, such as AD. Extracts and fractions of medicinal plants from various families and genera have been shown to inhibit the activity of this enzyme[14]. Although now, there are no data in the literature about the effect of *Agave* plants with this property6. The speed of ACh degradation was evaluated as a measure of enzymatic activity. As shown in Figure 1, the brains of mice that did not receive LPS (Basal group) showed a lower degradation rate than the damage group (VEH). The mice treated with INDO (5 mg/kg) and the acetone extracts of the three Agaves, Aan-A, Aam-A, and At-A, decreased the speed compared to the VEH group.

Table 1: Effect of oral administration of the extract of different Agaves, on the enzymatic activity of ACh-E (Km and V_{max}) in brain of mice with LPS.

Treatmen	Basal	VEH	Aan-A	At-A	Aam-A	INDO
K_M	3.16	1.62	2.53	2.60	2.47	0.00052
V_{max}	0.00010	0.00034	0.00009	0.00015	0.00012	0.00000002
V_{max}/K_M	3.16×10^{-5}	2.10×10^{-4}	3.55×10^{-5}	5.76×10^{-5}	4.86×10^{-5}	3.85×10^{-5}

The K_M and V_{max} values (Table 1) indicate that LPS modified the enzymatic activity with respect to the Basal group since healthy animals' brains have a lower V_{max} than the VEH group, with a higher K_M, which indicates that LPS increases 3.4 times the activity of the enzyme promoting the degradation of ACh. While administration of the different Agaves extracts (Aan-A, At-A, and Aam-A) in mice reversed the anticholinergic effect of that substance by decreasing the V_{max} value, the bioavailability of ACh increased.

Regarding the K_M variable, LPS decreased by 1.95 times its value when compared to healthy animal brains, reinforcing the point mentioned above that this bacterial component has anticholinergic activity and increases ACh degradation activity. Whereas Aan-A, At-A, or Aam-A treatments decreased the K_M value, increasing the ACh bioavailability. The relation between both variables, V_{max}/K_M, was used to define the impact on ACh bioavailability and, therefore, to define it as a parameter of the cholinergic effect (Table 1). It could be appreciated that according to the value obtained in this way, the cholinergic activity had the following sequence (V_{max}/K_M): Basal > Aan-A > INDO > Aam-A > At-A > VEH.

Table 2: Effect of different Agaves treatments on Glutathione Reductase (GR) and NADPH oxidase (NOX) enzymatic activity.

Treatment (mg/kg)	GR (µMol/mg)	NOX (µMol/mg)
Basal	0.443±0.0002*	0.246±0*
Veh	0.173±0.003	0.621±0.008
INDO (5.0)	0.032±0.001*	1.843±0.004*
Aam-A (125)	0.508±0.007*	0.606±0.003
Aan-A (125)	0.480±0.004*	0.917±0.002*
At-A (125)	0.642±0.002*	0.513±0.002*

Data represent mean ±SD (n=10), evaluated with an ANOVA post hoc Bonferroni statistical test, (*p<0.05) statistically different.

ACh-E is a highly efficient protein, expressed centrally and peripherally, and its activity causes the degradation of ACh, which performs its actions by interacting with muscarinic and nicotinic receptors. The participation of this neurotransmitter in inflammation has been defined, and it is known to decrease inflammation by modulating the NF-κB pathway[19]. The cholinergic system, widely distributed throughout the organism, mainly participates in muscle contraction, heart and conduction rate[20]; at the central level, it is critical in attention, learning, memory, and stress response mechanisms, among others. Some medical conditions associated with the ACh actions show that cholinergic neurotransmission is enhanced or promoted because ACh-E inhibitor drugs can improve attention and decrease cognitive deficits[21]. It has been described that ACh-E participates in inflammation processes, apoptosis, cell adhesion, and on oxidative stress[22], promoting its function in neurodegenerative diseases[23], where neuroinflammation is a crucial process for its progression. It has been shown that the administration of LPS to rodents causes an increase in ACh-E activity in the brain, rising chemical mediators such as IL-1 β-cytokines, TNF-α, which create a pro-inflammatory and oxidative environment leading to cognitive impairment[24,25].

The effect of medicinal plant-derived treatments on the LPS model is essential because it allows us to visualize new therapeutic strategies against neurodegeneration. In a previous study, it was observed that the administration of Agaves extracts, Aan-A, Aam-A, and At-A, decreased the concentration of cytokines IL-6, IL-1β, and TNF-α in the brain of mice exposed to LPS, indicating an anti-neuroinflammatory effect7. The present work observed that the LPS effect on the brain is antagonized by the administration of different Agaves (125 mg/kg). The chemical complexity of the extracts may lead to actions on different targets, in this case, on the enzyme ACh-E, which is over-activated by the action of the pro-inflammatory agent. So far, no reports indicate that species of the *Agave* genus can inhibit this enzyme. However, these results provide evidence of the potential use of these plants to counteract neurodegenerative pathologies6. The enzyme inhibition is an important therapeutic target for treating neurodegenerative diseases, which eventually cause cognitive impairment. There are studies where plants are natural suppliers of chemicals that may have such action, such as huperzine A (HupA), an alkaloid isolated from the moss *Huperzia serrata*, commonly employed in Chinese folk medicine to treat dementia. The effects of this alkaloid have been attributed to its ability to act as an ACh-E inhibitor. One of the main neuroprotective effects of HupA is the amelioration of Aβ accumulation and neurotoxicity through different cholinergic mechanisms, mainly through disruption of AChE-Aβ interaction[26], probably the *Agave* species act at that level as well, however more assays are still required to determine the precise mode of action exerted to inhibit AChE.

INDO, the positive control drug used in the present study, is a non-steroidal anti-inflammatory and induces multiple actions of clinical importance. Furthermore, it is also useful as a control in various experimental models, including those disorders linked to the central nervous system. There are numerous conflicting reports on the effects of anti-inflammatory drugs, which include beneficial effects (such as inhibition of the ACh-E enzyme) and some adverse effects that also depend on the chronicity of use for diseases such as AD. However, more studies are needed because the therapeutic effect seems to depend on the underlying disease stage, drug dose, and mechanism of action[27]. The results shown in our study indicated that INDO (5 mg/kg) causes an increase in enzyme activity when compared with the basal group (healthy animals). In congruence with these results, there is a study from 1997, where the activity of the ACh-E enzyme was evaluated on bronchi and homogenates of pig bronchial epithelial cells, finding a V_{max} of 5.7±0.46. When these sample types were incubated with INDO, ACh-E activity increased by 21% and 54%, respectively[28].

Antioxidant effect

Intraperitoneal or intracerebroventricular administration of LPS activates nitric oxide synthesis in the periphery and central nervous system, an important mediator of brain damage during systemic inflammation, promoting an oxidative, neuroinflammatory, and neurodegenerative brain environment[29]. In the present work, antioxidant activity was evaluated in brain homogenates from mice with damage induced by i.p. administration of LPS and subsequently treated with extracts of *Agave* species (125 mg/kg; v.o.), INDO (5 mg/kg; v.o.) and Vehicle for the negative control. For this purpose, the concentration of the antioxidant enzyme Glutathione Reductase (GR) and the pro-oxidant enzyme NADPH oxidase (NOX) were measured. The results of the antioxidant enzyme GR in the Basal group showed that the concentration of this enzyme is 0.443±0.0002 µMol/mg, higher than that of the damage group (Veh) 0.173±0.003 µMol/mg. When damage was induced, and mice were treated with INDO 5.0, the concentration reduces to 0.032±0.001 µMol/mg. The administration of *Agave* extracts: At-A, Aam-A, and Aan-A, increased the GR concentration compared to the Vehicle, showing values of 0.642±0.002 µMol/mg, 0.508±0.007 µMol/mg and 0.480±0.004 µMol/mg, respectively $p<0.05$ (Table 2).

The analysis of the pro-oxidant enzyme NOX indicates that in the brain of the Basal group (Healthy animals), the concentration was 0.246±0 µMol/mg. The damage induced by LPS increased the concentration of this protein to 0.621±0.008 µMol/mg. When treated with INDO, the value increased significantly compared to the Vehicle group to 1.843±0.004 µMol/mg ($p<0.05$). The use of non-steroidal anti-inflammatory drugs such as INDO to treat patients with AD requires further studies focused on determining the effective dose according to the impairment grade of each patient. The data obtained in the INDO group are consistent with a report that evaluated the possible effect of this drug and dexamethasone on the NOX enzyme and found that in nuclear run-on assays, only dexametha-sone down-regulated the NADPH oxidase system, at least in part by inhibiting the transcription of the gp91-phox and p47-phox genes. INDO inhibited PMA-stimulated superoxide release in THP-1 cells differentiated with IFN-γ and TNF-α for 7 days[30]. Likewise, the toxicity produced by INDO treatment has been reported since 1973[31] moreover, in an investigation where the effect of this non-steroidal anti-inflammatory drug in the treatment of gastric cancer was studied, they found that it altered mitochondrial dynamics, promoting fusogenic activation and mitochondrial recruitment of DRP1 in rat gastric mucosa, producing defective mitochondria, metabolic and bioenergetic shocks[32]; the which highlights the importance of conducting studies to establish a dose that promotes the antioxidant pharmacological effect beyond the prooxidant one.

At-A treatment (0.513±0.002 µMol/mg) reduced NOX concentration in brains compared to the Vehicle group $p<0.05$. The Aam-Atreatment (0.606±0.003 µMol/mg) showed no statistical difference compared to the Vehicle group. Furthermore, Aan-A induced an increase in the concentration of the prooxidant enzyme NOX (0.917±0.002 µMol/mg) compared to the Vehicle group $p<0.05$ (Table 2). The antioxidant results observed when administering At-A can be supported by the antioxidant activity reported in this species by Herrera-Ruiz and collaborators in 2021; in that study, antioxidant activity was evaluated through the

evaluation of lipid peroxidation of the whole extract and an ethyl acetate fraction of *A. tequilana* in male mice strain ICR or CD1 with angiotensin-II-induced damage and reported that these Agave treatments significantly reduced malondialdehyde (MDA) concentration7.

Chronic administration of *A. americana* methanolic extract protects against oxidative stress in diabetic rat brains at 400 and 600 mg/kg by increasing the levels and activity of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), in a similar way to the positive control glibenclamide. In the same work, the MDA concentration, an indicator of lipid peroxidation due to oxidative stress, was measured, and it was observed that the administration of methanolic extract of *A. americana* (400 and 600 mg/kg) significantly reduced compared to the damage group, similar to glibenclamide[33].

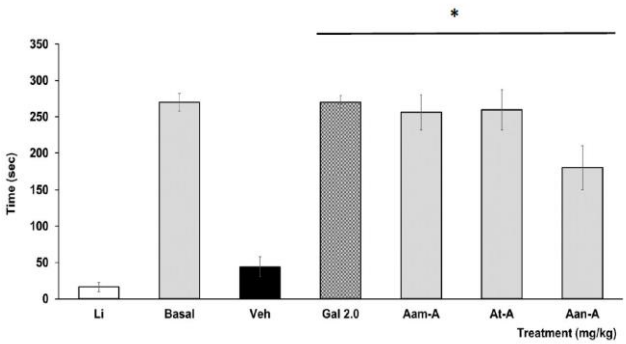

Figure 2: Effect of extracts of *A. americana* (Am-A), *A. angustifolia* (Aan-A), *A. tequilana* (At-A) on cognitive impairment induced with SC (scopolamine).

Positive control Galantamine (Gal 2.0) and negative control (Veh). Bonferroni post hoc ANOVA, significatively difference is when *p<0.05 compared to the negative control (Veh).

Passive Avoidance Test in mice with cognitive impairment induced with scopolamine

The results achieved are shown in Figure 2. Each treatment is compared according to the time the mice cross from one chamber to another. During the training stage, the positive control group (Gal 2.0) was the one with a statistical difference since it took the longest time to cross from the illuminated chamber to the dark one (28 seconds); the rest of the treatments showed similar behavior with an average of 10 seconds. During the IL phase, all groups showed behavior with an average crossing time of 270 seconds, confirming that learning was present. The retention latency (RL) after treatment administration and SC-damage induction, demonstrated that the Basal group had the longest RL (270 seconds), showing that learning was acquired and retained a week later. At-A and Aam-A had similar behavior, with an RL average of 260 seconds, even longer than Gal 2.0, a drug used to treat cognitive impairment that promotes cholinergic synapses, for which RL was 239 seconds. Aan-A was the treatment that exhibited the least protective effect against cognitive impairment by showing 180 seconds of RL. The negative control group (Veh) reduced the RL (44 seconds) (*p<0.05).

Scopolamine (SC) is a cholinolytic drug that induces a decline in learning and memory processes through different mechanisms, such as increasing AChE and butyrylcholinesterase (BuChE) and decreasing AChE-mediated transmission. It is also a product that causes neuroinflammation and oxidative stress by increasing molecules like IL-1β, TNF-α, NO, and iNOS, leading to atrophy and neurodegeneration in rats, which contributes to cognitive impairment[34]. It has been shown that administration of 2 mg/kg i.p. reduces the number of AChE-reactive hippocampal neurons in almost 80% of Sprague-Dawley rats, while 1 mg/kg decreases the ACh level in the BAlb-c mice brain[35].

There is only one report in the literature mentioning that *A. americana* methanolic extract administered at 400 and 600 mg/kg in rats with alloxan-induced hyperglycemia provoked a significant improvement in diabetes-related dementia in the Morris water maze test[33]. According to the results, the *Agave* species can interact with SC to inhibit its deleterious effects on memory associated with cholinergic transmission. These results are consistent with those obtained in the previous sections, where Agaves increase the bioavailability of the neurotransmitter ACh and allow us to propose a broader study to evaluate compounds from the three *Agave* species with less chemical complexity and isolate those responsible for this activity, to perform pharmacological tests on complex models of neurodegenerative diseases, in order to have an alternative treatment proposal to the current ones.

Limitations of the study

After COVID-19, financial funding is delayed and has affected the schedule of research assays and experiments.

CONCLUSIONS

The results showed the pharmacological importance of *A. tequilana* F. A.C. Weber, *A. angustifolia* Haw, and *A. americana* L. Marginata Hort. as potential treatments for neurodegenerative diseases. Because they could inhibit ACh-E, in a model of LPS-induced damage, promoting the *in vitro* activity of the antioxidant enzyme GR and decreasing that associated with the detonation of oxidative stress, NOX. In addition, they significantly improve SC-induced cognitive impairment.

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AUTHOR’S CONTRIBUTIONS

Maribel HR: obtained the funding resources. **Enrique JF:** designed the experiments. **Alejandro Z:** chemical analysis. **Ruperto JAA:** performed the experiments. **Lucía AOM:** designed the experiments. **Nayeli MB:** analyzed data. Every author gave their approval to the manuscript's final draft.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

None to declare.

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