**Original Research Article**

**ACETYLCHOLINESTERASE INHIBITION, NOOTROPIC AND ANTIOXIDANT EFFECT OF AN EXTRACT FROM *AGAVE* SPECIES.**

**ABSTRACT**

**Background:**The systemic administration of 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. They possess compounds with anti-inflammatory, antioxidant, and anti-neuroinflammatory activity.

**Materials and Methods:**Extracts of *A. tequilana* (At-A), *A. angustifolia* (Aan-A), *A. americana* (Aam-A) (125 mg/kg) were administered to male ICR mice with lipopolysaccharide (LPS) to evaluate the ACh -E inhibition, the concentration of the antioxidant enzyme GR and the prooxidant enzyme 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, Vmax value, and KM. These products increased GR concentration, and in terms of NOX, only the *A. tequilana* extract decreased it. *A. tequilana* and *A. americana* species significantly improved the retention latency parameter during the passive avoidance test.

**Conclusion:***Agave* species show promising results in developing novel drugs for neurodegenerative disease therapy.

**Keywords:** Agave, Acetylcholinesterase, LPS, Neurodegeneration

**INTRODUCTION**

The Agavaceae family includes important species in Mexico, not only for their abundance but also for their use made of them. 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 world1.

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 hypertension2.

More specifically, it is mentioned that A. Americana can alleviate gastrointestinal problems such as ulcers and dysentery3. A. angustifolia for digestive disorders4and A. tequilana, a plant of important industrial use in Mexico for tequila production5.

Pharmacological reports indicate diverse biological effects for the genus, including antioxidant, antibacterial, anticancer, and anti-inflammatory properties6.

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 brain7. 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 aggression8, 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 diseases11. 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 memories12. An essential element in this neurotransmission system linked to memory is the enzyme acetylcholinesterase (ACh-E), which is responsible for the ACh hydrolysis13; 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 based14. 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 obtaining the extract.**

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 *Agave tequilana* F. A.C. Weber, *Agave angustifolia* Haw, and *Agave 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; 35 g 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 experimental groups (n=8) were defined as follows:

**1.-** Basal group (healthy animals): administered with sterile saline (SS) (i.p.) for 7 days, the following 7 days received orally pathway (o.p) the vehicle (1% Tween 20 solution).

The successive groups received a daily dose of LPS (0.25µg/kg, i.p.) for 7 days. Then, for the next 7 days, each animal received the corresponding treatment (o.p.). On the last day (d14) 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: Indomethacin (INDO, 5 mg/kg).

**4.-** Experimental Group: At-A (125 mg/kg)

**5.-**Experimental Group: Aam-A (125 mg/kg)

**6.-** Experimental Group: Aan-A (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**

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.

**Passive avoidance test.**

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 sec) was released and applied to the mice's paws. 24 hours later, 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 compared16.

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: 100 µl/10 g vehicle weight.

**3.**- Experimental Group: At-A (125 mg/kg)

**4.**- Experimental Group: Aam-A (125 mg/kg)

**5.-** Experimental Group: Aan-A a (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 Veh group in the antioxidant activity assays and the behavioral assay.

**RESULTSAND DISCUSSION**

Only a few reports refer to 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. Considering that some neurodegenerative 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 receptors14.

**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 enzyme14. Although at the moment, 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 (5mg/kg) and the acetone extract of the three Agaves, Aan-A, Aam-A, and At-A, decreased the speed compared to the VEH group.



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

The KM and Vmax values (Table 1) indicate that LPS modified the enzymatic activity with respect to the Basal group since healthy animals' brains have a lower Vmax than the VEH group, with a higher KM, 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 Vmax value, the bioavailability of ACh increased.

Regarding the KM 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 KM value, increasing the ACh bioavailability. The relation between both variables, Vmax/KM, 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 (Vmax/KM): Basal > Aan-A > INDO > Aam-A > At-A > At-A > VEH.

**Table 1.** Effect of oral administration of the extract of different Agaves, on the enzymatic activity of ACh-E (Km and Vmax) in brain of mice with LPS.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Treatments | **Basal** | **VEH** | **Aan-A** | **At-A** | **Aam-A** | **INDO** |
| **KM** | 3.16 | 1.62 | 2.53 | 2.60 | 2.47 | 0.00052 |
| **Vmax** | 0.00010 | 0.00034 | 0.00009 | 0.00015 | 0.00012 | 0.00000002 |
| **Vmax/KM** | 3.16×10-5 | 2.10×10-4 | 3.55×10-5 | 5.76×10-5 | 4.86×10-5 | 3.85×10-5 |

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 pathway19. The cholinergic system, widely distributed throughout the economy, mainly participates in muscle contraction, heart rate, and conduction rate20; 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 deficits21. It has been described that ACh-E participates in inflammation processes, apoptosis, cell adhesion, and on oxidative stress22, promoting its function in neurodegenerative diseases23, 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 impairment24,25.

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 extract, 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 inhibitor26.

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 action27. The results shown in our study indicated that INDO (5mg/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 Vmax of 5.7±0.46. When these sample types were incubated with INDO, ACh-E activity increased by 21% and 54%, respectively28.

**Antioxidant effect.**

Intraperitoneal or intracerebroventricular administration of LPS activates nitric oxide synthesis in both the periphery and central nervous system, an important mediator of brain damage during systemic inflammation, promoting an oxidative, neuroinflammatory, and neurodegenerative brain environment29.

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 125, Aam-A 125, and Aan-A 125, increased the GR concentration compared to the Veh, 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 Veh 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 dexamethasone 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 days30. Likewise, the toxicity produced by INDO treatment has been reported since 197331 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 shocks32; the which highlights the importance of conducting studies to establish a dose that promotes the antioxidant pharmacological effect beyond the prooxidant one.

At-A 125 treatment (0.513± 0.002 µMol/mg) reduced NOX concentration in brains compared to the Veh group p < 0.05. The Aam-A 125 treatment (0.606± 0.003 µMol/mg) showed no statistical difference compared to the Veh group. Furthermore, Aan-A 125 induced an increase in the concentration of the prooxidant enzyme NOX (0.917± 0.002 µMol/mg) compared to the Veh group p < 0.05 (Table 2).

**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.

The antioxidant results observed when administering Ateq 125 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 glibenclamide33.

**Passive Avoidance Test in mice with cognitive impairment induced with scopolamine**

The results achieved are shown in Figure 2. In this graphic, 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 s); 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, the retention latency (RL) demonstrated that the Basal group had the longest RL, 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. Aang 125 was the treatment that exhibited the least protective effect against cognitive impairment by showing 180 s of RL. The negative control group (Veh) reduced the RL (44 s)(\*p<0.05).



**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).

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 impairment34.

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 brain35.

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 test33.

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.

**CONCLUSION**

The results reported here show the pharmacological importance of *Agave tequilana* F. A.C. Weber, *Agave angustifolia* Haw, and *Agave 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.

**ACKNOWLEDGEMENTS**

This work was supported by “Fondo de InvestigaciónenSalud-IMSS” with funding number FIS/IMSS/PROT/G10/848; as well as through the Instituto Politécnico Nacional with the grant registered under the title: "Chemical characterization of bioactive compounds with pharmacological activity" and registered as SIP 20113780 and 20120430 and through a CONAHCyT postgraduate studies grant.

**DECLARATION OF CONFLICTING INTERESTS**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**AUTHORS’ CONTRIBUTIONS**

H-R M and A-O ML obtained the funding resources;J-F E, H-R M and A-O ML designed theexperiments; H-R M and M-B N wrote the paper.M-B N performed the experiments; M-B N analyzed data.Z A did chemical analysis.All authors have read and approved the final manuscript.

**REFERENCES:**

1. García-Mendoza AJ, Franco-Martínez IS, Sandoval-Gutiérrez D. Cuatro especies nuevas de Agave (Asparagaceae, Agavoideae) del sur de México. Act. Bot. Mex 2019;126:1-18. <https://doi.org/10.21829/abm126.2019.1461>
2. Herrera-Ruiz M, Gutiérrez-Nava ZJ, Trejo-Moreno C, Zamilpa A, González-Cortazar M, Jiménez-Aparicio AR, Jiménez-Ferrer E. *Agave tequilana*CounteractsChronicHypertension and Associated Vascular Damage. J MedFood 2022; 25(4):443-455. <https://doi.org/10.1089/jmf.2021.0044>
3. Argueta VA, Cano LM, Rodarte ME. Atlas de las Plantas de la Medicina Tradicional Mexicana, Volume I, II y III; Instituto Nacional Indigenista: Mexico: 1994. p.1786. ISBN 9682961327.
4. Monroy C, Castillo, P, Plantas Medicinales Utilizadas en el Estado de Morelos; 1st ed. Universidad Autónoma del Estado de Morelos: Cuernavaca, Mexico: 2007. p. 265–266. ISBN 968-878-277-7.
5. Statista. Mexico: Distilled Agave-BasedBeveragesProductionValue 2013–2020. 2022. Available online: https://www.statista.com/statistics/717349/distilled-Agave-based-beverage-production-value-in-mexico/ (accessedon 11 May 2023).
6. Herrera-Ruiz M, Jiménez-Ferrer E, González-Cortazar M, Zamilpa A, Cardoso-Taketa A, Arenas-Ocampo ML, Jiménez-Aparicio AR, Monterrosas-Brisson N. Potential Use of Agave Genus in Neuroinflammation Management. Plants (Basel) 2022; 11:2208. <https://doi.org/10.3390/plants11172208>
7. Herrera-Ruiz M, Jiménez-Ferrer E, Tortoriello J, Zamilpa A, Alegría-Herrera E, Jiménez-Aparicio AR, Arenas-Ocampo ML, Martínez-Duncker I, Monterrosas-Brisson N. Anti-neuroinflammatoryeffectof agaves and cantalasaponin-1 in a modelof LPS-induceddamage. NatProd Res 2021; 35: 884-887. <https://doi.org/10.1080/14786419.2019.1608537>
8. Woodburn SC, Bollinger JL, Wohleb ES. Thesemanticsofmicrogliaactivation: neuroinflammation, homeostasis, and stress. J Neuroinflammation 2021; 18:258. [https://doi.org/10.1186%2Fs12974-021-02309-6](https://doi.org/10.1186/s12974-021-02309-6)
9. Cornell J, Salinas S, Huang HY, Zhou M. Microgliaregulationofsynapticplasticity and learning and memory. Neural Regen Res 2022; 17(4):705-716. [https://doi.org/10.4103%2F1673-5374.322423](https://doi.org/10.4103/1673-5374.322423)
10. Xu YJ, Au NPB, Ma CHE. Functional and PhenotypicDiversityofMicroglia: ImplicationforMicroglia-BasedTherapiesforAlzheimer'sDisease. Front AgingNeurosci 2022; 26:896852. <https://doi.org/10.3389/fnagi.2022.896852>
11. Teleanu DM, Niculescu AG, Lungu II, Radu CI, Vladâcenco O, Roza E, Costăchescu B, Grumezescu AM, Teleanu RI. An overview of stress, neuroinflammation and neurodegenerative diseases. Int J Mol Sci 2022; 23:5938. [https://doi.org/10.3390%2Fijms23115938](https://doi.org/10.3390/ijms23115938)
12. Atri A, Sherman S, Norman KA, Kirchhoff BA, Nicolas MM, Greicius MD, Cramer SC, Breiter HC, Hasselmo ME, Stern CE. Blockade of central cholinergic receptors impairs new learning and increases proactive interference in a word paired-associate memory task. BehavNeurosci 2004; 118:223–236. <https://psycnet.apa.org/doi/10.1037/0735-7044.118.1.223>
13. Saxena M, Dubey R. Target Enzyme in Alzheimer's Disease: Acetylcholinesterase Inhibitors. Curr Top MedChem2019;19:264-275. <http://dx.doi.org/10.2174/1568026619666190128125912>
14. Walczak-Nowicka ŁJ, Herbet M. Acetylcholinesterase Inhibitors in the Treatment of Neurodegenerative Diseases and the Role of Acetylcholinesterase in their Pathogenesis. Int J Mol Sci 2021; 22:9290. <https://doi.org/10.3390/ijms22179290>
15. Lee J, Lee Y, Yuk D, Choi D, Ban S, Oh K, Hong J. Neuro-inflammationinducedbylipopolisaccharide causes cognitive impairmentthroughenhancementof beta-amyloidgeneration. J Neuroinflammation 2008; 5:37–53. <https://doi.org/10.1186/1742-2094-5-37>
16. Kruk-Slomka M, Biala G. Cannabidiol Attenuates MK-801-Induced Cognitive Symptoms of Schizophrenia in the Passive Avoidance Test in Mice. Molecules 2021; 26:5977. <https://doi.org/10.3390/molecules26195977>
17. Monterrosas-Brisson N, Ocampo ML, Jiménez-Ferrer E, Jiménez-Aparicio AR, Zamilpa A, Gonzalez-Cortazar M, Tortoriello J, Herrera-Ruiz M. Anti-inflammatoryactivityofdifferent agave plants and thecompound cantalasaponin-1. Molecules 2013; 18:8136-46. <https://doi.org/10.3390/molecules18078136>
18. Hernández-Valle, E.; Herrera-Ruiz, M.; Salgado, G.R.; Zamilpa, A.; Ocampo, M.L.; Aparicio, A.R.; Tortoriello, J.; Jiménez-Ferrer, E. Anti-inflammatoryeffectof 3-O-[(6’-O-Palmitoyl)-β-DglucopyranosylSitosterol]), from Agave angustifolia onear edema in mice. Molecules 2014; 19:15624–15637. <https://doi.org/10.3390/molecules191015624>
19. Fronza MG, Baldinotti R, Fetter J, Rosa SG, Sacramento M, Nogueira CW, Alves D, Praticò D, Savegnago L. Beneficial effects of QTC-4-MeOBnE in an LPS-induced mouse model of depression and cognitive impairments: The role of blood-brain barrier permeability, NF-κB signaling, and microglial activation. BrainBehavImmun 2022; 99:177-191. <https://doi.org/10.1016/j.bbi.2021.10.002>
20. Crick SJ, Wharton J, Sheppard MN, Royston D, Yacoub MH, Anderson RH, Polak JM. Innervation of the human cardiac conduction system. A quantitativeimmunohistochemical and histochemicalstudy. Circulation 1994; 89:1697–1708. <https://doi.org/10.1161/01.cir.89.4.1697>
21. Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. [Alzheimer's disease: Targeting the Cholinergic System.](https://pubmed.ncbi.nlm.nih.gov/26813123/)CurrNeuropharmacol 2016; 14:101-15. <https://doi.org/10.2174/1570159x13666150716165726>
22. Ruz C, Alcantud JL, Montero FV, Duran R,Bandres-Ciga S. Proteotoxicity and Neurodegenerative Diseases. Int J Mol Sci 2020; 21:5646.<https://doi.org/10.3390/ijms21165646>
23. Moss DE. Improving Anti-Neurodegenerative Benefits of Acetylcholinesterase Inhibitors in Alzheimer's Disease: Are Irreversible Inhibitors the Future?.Int J Mol Sci. 2020;21:3438.<https://doi.org/10.3390/ijms21103438>
24. Tyagi E, Agrawal R, Nath C, Shukla R. Influence of LPS-induced neuroinflammation on acetylcholinesterase activity in rat brain. J Neuroimmunol 2008; 205:51-6.<https://doi.org/10.1016/j.jneuroim.2008.08.015>
25. Tyagi E, Agrawal R, Nath C, Shukla R. Effect of melatonin on neuroinflammation and acetylcholinesterase activity induced by LPS in rat brain. Eur J Pharmacol 2010;640:206-10.<https://doi.org/10.1016/j.ejphar.2010.04.041>
26. Friedli MJ, Inestrosa NC.Huperzine A and Its Neuroprotective Molecular Signaling in Alzheimer's Disease. Molecules 2021;26:6531. <https://doi.org/10.3390/molecules26216531>
27. Parmar HS, Assaiya A, Agrawal R, Tiwari S, Mufti I, Jain N, Manivannan E, Banerjee T, Kumar A. Inhibition of Aβ(1-42)Oligomerization, Fibrillization and Acetylcholinesterase Activity by Some Anti-Inflammatory Drugs: An *in vitro* Study. AntiinflammAntiallergyAgentsMedChem 2017;15:191-203. <https://doi.org/10.2174/1871523015666161229143936>
28. Taisne C, Norel X, Walch L, Labat C, Verriest C, Mazmanian G. Brink C. Cholinesterase activity in pig airways and epithelial cells. Fundam ClinPharmacol1997; 11:201-205.<https://doi.org/10.1111/j.1472-8206.1997.tb00186.x>
29. Decandia D, Gelfo F, Landolfo E, Balsamo F, Petrosini L, Cutuli D. Dietary Protection against Cognitive Impairment, Neuroinflammation and Oxidative Stress in Alzheimer's Disease Animal Models of Lipopolysaccharide-Induced Inflammation. Int J Mol Sci 2023;24:5921.<https://doi.org/10.3390/ijms24065921>
30. Condino-Neto A, Whitney C, Newburger PE.Dexamethasone but not indomethacin inhibits human phagocyte nicotinamide adenine dinucleotide phosphate oxidase activity by down-regulating expression of genes encoding oxidase components. J Immunol 1998; 61:4960-7.<https://doi.org/10.4049/jimmunol.161.9.4960>
31. Burns CA. Indomethacin Induced Ocular Toxicity*.* Am J Ophthalmol1973;76:312–313*.*https://doi.org/10.1016/0002-9394(73)90185-2
32. Mazumder S, De R, Debsharma S, Bindu S, Maity P, Sarkar S, Saha SJ, Siddiqui AA, Banerjee C, Nag S, Saha D, Pramanik S, Mitra K, Bandyopadhyay U. Indomethacin impairs mitochondrial dynamics by activating the PKCζ-p38-DRP1 pathway and inducing apoptosis in gastric cancer and normal mucosal cells. J BiolChem 2019;294:8238-8258. <https://doi.org/10.1074/jbc.ra118.004415>
33. Aleem A, Shahnaz S, Javaid S, Ashraf W, Rasool MF, Ahmad T, F Alotaibi A, Albeshri KS, Alqahtani F, Imran I. Chronically administered *Agave americana var*. marginata extract ameliorates diabetes mellitus, associated behavioral comorbidities and biochemical parameters in alloxan-induced diabetic rats. SaudiPharm J 2022; 30:1373-1386. <https://doi.org/10.1016/j.jsps.2022.06.003>
34. Renner UD, Oertel R, Kirch W. Pharmacokinetics and pharmacodynamics in clinical use of scopolamine. TherDrugMonit 2005; 5:655-65. <https://doi.org/10.1097/01.ftd.0000168293.48226.57>
35. Kim SJ, Lee JH, Chung HS, Song JH, Ha J, Bae H. Neuroprotective Effects of AMP-Activated Protein Kinase on Scopolamine Induced Memory Impairment. Korean J PhysiolPharmacol 17(4):331-8.<https://doi.org/10.4196/kjpp.2013.17.4.331>