GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Effect of *Leptadenia hastata* (Pers.) Decne (Asclepediaceae) on scopolamine-induced memory loss in the white mouse Mus *Musculus Swiss* (Murideae)

Renaud Nonmarmbaye [1, 2, *], Sidiki Neteydji [2] and Elisabeth Ngo Bum [2, 3]

[1] *Department of Life and Earth Sciences, Faculty of Life and Earth Sciences and Regional Planning, University of Ati, P.O. Box 20, Ati, Chad.*
[2] *Department of Biological Sciences, Faculty of Sciences, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon.*
[3] *Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814, Maroua, Cameroon.*

GSC Biological and Pharmaceutical Sciences, 2024, 29(03), 233-247

Publication history: Received on 28 October 2024; revised on 14 December 2024; accepted on 16 December 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.29.3.0464

Abstract

Ethnopharmacological relevance: *Leptadenia hastata* (Pers.) Decne (Asclepediaceae) is a plant widely used by traditional healers in several African countries to treat oxidative stress, depression, arterial hypertension, headaches and other conditions. This study aims to determine the therapeutic efficacy of *Leptadenia hastata* (*L. hastata*) against scopolamine-induced neurotoxicity.

Aim of the study: To evaluate the pharmacological effects of *L. hastata* against scopolamine-induced neurotoxicity in *Mus Musculus Swiss* white mice.

Materials and methods: *L. hastata* leaf extract was tested on the central nervous system of animals with scopolamine-induced cognitive deficits. Different doses (19; 38 and 76 mg/kg *p.o*.) of the extract and piracetam (200 mg/kg *i.p*.) were administered to the animals one hour before scopolamine (1 mg/kg i.p.) for 15 consecutive days of treatment. T-maze and object recognition tests were used for 3 days to assess the animals' behavioral performance.

After behavioral testing, all animals were sacrificed by cervical decapitation and the brains harvested for analysis of oxidative stress parameters.

Results: Administration of the plant extract resulted in a significant behavioral performance, significantly inhibited the reduction of locomotor significantly inhibited the reduction in locomotor and exploratory abilities in scopolamine-treated animals. The 19 and 38 mg/kg of the extract significantly reduced catalase (CAT), reduced glutathione (GSH) and reduced glutathione (GSH) and an increase in Malondialdehyde (MDA) levels induced by scopolamine. *L. hastata* extract significantly counteracted the increase in cerebral of acetylcholinesterase (AchE) in animals after 15 days of consecutive treatment with scopolamine.

Conclusion: *Leptadenia hastata,* leaf extract has neuroprotective properties that facilitate memorization and correct scopolamine-induced cognitive deficits in white mice. All the results obtained in this article justify the use of *L. hastata* extract in traditional medicine.

Keywords: *Leptadenia hastata*; Neuroprotection; Neurotoxicity; Oxidative stress; Memory

* Corresponding author: Nonmarmbaye Renaud

1. Introduction

Brain degeneration in the elderly is implicated in several central nervous system diseases [1, 2]. Scopolamine-induced neurotoxicity is at the root of neurodegenerative diseases such as dementia, Alzheimer's disease, Parkinson's disease, Schizophrenia, etc. [3, 4]. Nootropic agents such as vitamins C and E are mainly used to improve memory, mood and behavior [5]. They are also thought to be used to alleviate age-related neurodegenerative diseases [6, 7]. However, adverse effects associated with these agents have limited their use [8]. Despite the availability of various therapeutic molecules, natural plant products remain one of the best sources of new drugs [9]. It is therefore interesting to explore the usefulness of medicinal plants in the treatment of various cognitive disorders. Previous pharmacological studies on *L. hastata* have shown that all parts of *L. hastata* are used by people in tropical areas to treat several diseases. The leaves are used to treat patients suffering from sexual impotence, malaria, agalactia, pain, diarrhoea and trypanosomiasis [10]. *L. hastata* has demonstrated anti-inflammatory, analgesic, antioxidant and antipyretic activity [10, 11]. Phytochemical analysis of *L. hastata* leaves reveals the presence of secondary metabolites such as tannins, flavonoids, alkaloids, saponosides, coumarins, steroids and terpenoids [12]. Scientific research into the pharmacological properties of *L. hastata* has shown that aqueous extracts of trunk bark, twigs and leaves have proved effective as antioxidants, antipyretics and antidiabetics [13].

To our knowledge, the pharmacological activity of *L. hastata* on the central nervous system has not yet been the subject of extensive scientific studies. To better validate its use in folk medicine to treat brain degeneration, the present study aims to evaluate the pharmacological effect of *L. hastata* against scopolamine-induced neurotoxicity and oxidative stress.

2. Material and methods

2.1. Plant material and extract preparation

Samples of *L. hastata* were collected in the morning of May 2023 in Moundou, province of Logone Occidental in southern Chad. Species identification was carried out at the National Herbarium in Ndjamena (Chad), where a specimen was kept under number: AG1147. The leaves of *L. hastata* were ground to a powder. A volume of 2.5 g of powder was boiled in 50 ml of distilled water for 20 minutes on a hot plate set at 100°C. After cooling, the mixture was filtered through a Wattman n°1 filter paper, and the filtrate recovered constituted the stock solution.

To determine the mass yield, the filtrate obtained (25 mL stock solution) was evaporated in an oven at 40°C. A mass of 0.19 g of dry extract was obtained in 1.9% yield, with a stock solution concentration of 7.6 mg/mL. Given an administration volume of 10 mL/kg, the initial dose of the extract was 76 mg/kg. Two other solutions of different doses (19 and 38 mg/kg) were prepared from the dry extract.

2.2. Phytochemical characterization tests on *Leptadenia hastata*

Preliminary phytochemical characterization tests on *L. hastata* were carried out using qualitative colorimetric methods [14] to determine the main chemical groups.

2.2.1. Alkaloid test

1 mL of the extract, prepared at 0.3 mg/mL, was taken into a test tube, then 1 mL of HCl, prepared at 5%, and 3 drops of Dragendorff's reagent were added. The formation of a white or orange precipitate indicates a positive test [15].

2.2.2. Tannin test

1 mL of the extract, prepared at 0.3 mg/mL, was taken into a test tube and 3 drops of FeCl3, prepared at 1%, were added. The formation of a blue-blackish coloration confirmed the presence of tannins [16].

2.2.3. Flavonoid test

1 mL of the extract, prepared at 0.3 mg/mL, was taken into a test tube, then 3 magnesium chips and 1 mL concentrated hydrochloric acid were added. Orange coloration indicates the presence of flavones, red of flavonols and violet of flavonones [17].

2.2.4. Test for triterpenes or steroids

1 mL of the extract, prepared at 0.3 mg/mL, was taken into a test tube. 1 mL chloroform, 1 mL concentrated H2SO4 and 1 mL acetic anhydride were added. The formation of a violet or blackish-green coloration confirms that the test is positive [17].

2.2.5. Anthraquinone test

1 mL of the extract, prepared at 0.3 mg/mL, was taken into a test tube. 2 mL mixture (petroleum ether/chloroform) and 2 mL NaOH, prepared at 10%, were added to the extract. The formation of a red coloration indicates a positive test [17].

2.2.6. Phenolics test

1 mL of *L. hastata* extract prepared at 0.3 mg/mL was taken from a test tube and 3 drops of FeCl3 prepared at 10% were added. The formation of a green or bluish coloration confirms the presence of phenolic compounds [18].

2.3. Determination of the content of bioactive compounds in *Leptadenia hastata* extract

2.3.1. Determination of polyphenol content

Polyphenols were assessed by the spectrophotometric method using the Folin-Ciocalteu reagent described by [19]. A mass of 0.3 g of *L. hastata* was placed in a tube, to which 15 mL of 70% (v/v) ethanol was added; leave under agitation for 1 h and centrifuge at 3500 rpm for 20 min. Approximately 0.02 mL of extract (0.2 mg/mL) was added to a 10 mL test tube, followed by 1.38 mL distilled water and 0.2 mL Folin-Ciocalteu reagent. After a 3 min rest, 0.4 mL sodium carbonate (Na2CO3 7.5%) was added to the mixture. Tubes were vortexed and incubated for 20 min in a water bath at 40°C, and absorbance was read against a blank at 760 nm. Calibration was carried out using a freshly prepared aqueous solution of gallic acid (0.2 mg/mL).

The amount of polyphenol (Q) was expressed as mg gallic acid equivalent per gram of dry matter, referring to the calibration curve and the regression equation DO = ax+b according to the formula:

$$Q = Co \times Vo \times F / m$$

With Q the polyphenol content; Co the extract concentration obtained from the calibration line; Vo the final volume of the daughter solution; F the dilution factor and m the extract mass.

2.3.2. Determination of tannin content

Tannins were determined spectrophotometrically, using catechin as the standard [20, 21]. Approximately 1 g of extract sample was placed in an Erlenmeyer flask and 15 mL acetone (10% acetic acid) added. The mixture was stirred with a bar magnet for 15 min, then filtered. The filtrate obtained was diluted 20-fold with distilled water prior to assay. 1 mL of diluted filtrate was introduced into each test tube covered with aluminum foil to exclude light, followed by the addition of 3 mL of a freshly prepared solution of 4% catechin vanillin in ethanol (w/v). After shaking, 1 mL of concentrated hydrochloric acid was added to each test tube, which was then left to stand at room temperature for 15 min, and the absorbance was read at 500 nm against a blank. At the same time, catechin (5 mg/mL) was prepared as a reference sample and used to plot the calibration curve. Tannin content was expressed as mg of catechin per gram of dry matter using the tannic acid calibration curve.

2.3.3. Determination of triterpene content

The amount of triterpenes was determined according to the protocol used by [22] with a few modifications. Lupeol was used as the standard, and the stock solution was prepared at a concentration of 100 µg/mL. Test tubes were successively filled with 100, 200, 400, 600 and 800 µL of the stock solution and kept in a water bath (85°C) until dry, followed by the addition of 250 µL of Vanillin solution (50 mg/mL) and 500 µL of sulfuric acid (99.5%) in that order. Test tubes were heated in a water bath (60 ± 1°C) for 30 min, then transferred to an ice bath followed by the addition of 2500 µL acetic acid (99.7%). The resulting solutions were kept under chilling for 20 min at room temperature. The same procedure was followed after adding 75 µL of *L. hastata* decoction to test tubes. A blank solution was prepared in a similar way, but the sample and absorbances were read at 548 nm using a spectrometer and the total triterpene content was calculated by linear regression of the lupeol calibration curve and expressed as µgEqLu / 100g extract.

2.4. Determination of the free radical scavenging capacity of *Leptadenia hastata* extract

2.4.1. Diphenyl-picryl-hydrazyl method

Antioxidant capacity is determined by the ability of a given antioxidant to scavenge a free radical or donate a hydrogen atom according to the method of Zhang et [23] with a few modifications. In the analytical protocol, 2 mL of DPPH (0.1 mM prepared in methanol) was introduced into a test tube containing 0.5 mL of the extract (0.1 to 1 mg/mL). The mixture was then shaken well for 5 min and incubated in the dark for 60 min at room temperature (20°C). For the control tube, methanol was used instead of extract. The reference used was ascorbic acid at concentrations of 0.1 mg/mL to 1 mg/mL. Absorbance was read at 517 nm. The antioxidant activity of the extract was expressed as a percentage of inhibition.

$$\% IP = [(Ablanc - Aéch) / Ablanc] \times 100$$

Where IP denotes percentage inhibition, Ablanc the absorbance of the control and Aéch the absorbance of the sample.

2.5. Behavioral tests

2.5.1. Animal model

Naïve white mice, *Mus Musculus Swiss* (Murideae), aged between eight and ten weeks, of two genera and weighing between 25 and 30 g were used in this study [24]. The mice were supplied by the Laboratoire National Vétérinaire (LANAVET) du Cameroun (Garoua, Cameroon). They were acclimatized for one week at the Laboratoire des Plantes Médicinales, Santé et Formulation Galénique of the University of Ngaoundéré before the start of the experiments [24]. These animals were housed in standard cages, at room temperature, on a 12/12-hour light-dark cycle, consuming tap water and granules ad libitum. Experiments were conducted in accordance with the International Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health, USA (NIH Publication No. 85 - 23, revised 1996) and with the guidelines of the Cameroon National Ethics Committee (No. FWA-IRB00001954, October 22, 1987).

For each test, mice were divided into 6 homogeneous batches of 5 mice and treated for 15 days as follows: a first batch received distilled water (10 mL/kg *p.o.*) [25] and served as a negative control batch, a second batch received piracetam (200 mg/kg *i.p.*) and served as a positive control batch [26]. The other three batches received the different doses (19; 38 and 76 mg/kg, *p.o.*) of the plant and served as test batches [27]. All these batches received scopolamine (1 mg/kg) one hour after the first treatment, to induce neurotoxicity [28]. A sixth batch was added, and this received distilled water (10 ml/kg *p.o.*) only, serving as a normal control batch. On test day, 30 minutes after the last treatment, the animals were subjected to the various behavioral tests [27].

2.5.2. T-maze test

The effects of *L. hastata* on the level of exploration and memory in naïve mice (unmanipulated mice) placed in the T-maze were evaluated [29, 30]. Two days before the start of the experiments, the animals were progressively deprived of food to maintain them at 80-85% of their body weight. The animals were divided into 6 homogeneous batches of 5 animals each. The animals received distilled water for the negative control batch, Piracetam (200 mg/kg) for the positive control batch and different doses of *L. hastata* extract for the test batches. Mice were placed one after the other in the starting arm of the T maze one hour after administration of the various substances. This task was carried out in three phases: habituation, acquisition and retention.

In the first or habituation phase, the mice were familiarized with the device for a period of 5 minutes. Food is placed in each corridor to encourage exploration. The experimenter places the mice in the starting compartment. After 15 seconds, he opens all the guillotine doors. The animal can then choose one or other of the lanes, indicating its preference. The parameters recorded were: latency time to choose an arm (the arm that will be the animal's preferred arm throughout the test), time spent and number of entries in the preferred arm and the discriminated arm, and number of returns to the starting arm.

The second or acquisition phase begins 24 hours after the habituation phase. The corridor of the arm discriminated by the animal is closed, then a reinforcer (food) is placed in the arm chosen by the animal. The experimenter places the mouse in the starting compartment and lets it move towards the open corridor (preferred corridor during habituation). This phase takes 5 min for each animal. The following parameters are recorded: latency to retrieve food, time spent and number of entries into the preferred arms, and number of returns to the starting arm. Finally comes the retention phase,

24 hours after the acquisition phase. Each animal is placed in the device for 5 min, this time with all arms open. The experimenter places the food in both arms of the maze. The parameters recorded are: latency time to find the preferred arm, time spent and number of entries in the preferred arm and the discriminated arm, and number of returns to the starting arm. After 5 min of experimentation

2.5.3. Object Recognition Task in Open Field

The object recognition test was performed using the method described by Ennaceur and Delacour [31]. This test was conducted in an open field box (50 × 50 × 40 cm) and is comprised of three phases. At the end of the treatment, the mice were allowed to explore the open field for 5 min during the habituation phase. During the acquisition phase (T1), two identical objects (red cubes 4 × 4 × 4cm) were placed in two corners of the open field at 10cm from the sidewall. The mice were placed in the middle of the open field and allowed to explore these two identical objects for 5 minutes. After, they were put back in their cages. Subsequently, 24 hours after T1, the test "choice" (T2) was made. During T2, a new object (blue cone) has been introduced and mice were reexposed to the two objects: the familiar (F) and the new (N). The time spent by the mice in the exploration of each object during T1 and T2 was manually recorded using a stopwatch. A discrimination index (DI) was then calculated as follows:

$$DI = (TN - TF / TN + TF) \times 100$$

We used the discrimination index (DI) to evaluate the curiosity of mice towards new objects. The DI was defined as the percentage of time spent on a novel object to the total time spent on familiar and novel objects, where TN is time spent with the novel object and TF is time spent with the familiar object.

2.6. Biochemical analysis

2.6.1. Brain sampling and preparation of homogenates

Homogenates were prepared on day 15. Immediately after behavioral testing, previously established animal models of neuroinflammation were sacrificed individually by cervical decapitation. Brains were harvested, rinsed in NaCl (9‰) solution, wrung out and weighed. The brains were placed in a ceramic mortar. A total of 500 µL of PBS solution was added to each brain in the mortar, and the mixture was ground. The prepared homogenate was then centrifuged at 10,000 rpm for 15 minutes at 4°C. A 20 mg volume of the supernatant was pipetted into a new tube and labeled for markers of oxidative stress and neuroinflammation.

2.7. Determination of oxidative stress markers in the brain

2.7.1. Determination of malondialdehyde levels

The concentration of malondialdehyde (MDA) in the brain was determined in the homogenate using the thiobarbituric assay (TBA). To 1 mL homogenate, 0.5 mL thiobarbituric acid (20%) and 1 mL thiobarbituric acid (0.67%) were added. The mixture was heated in a water bath at 100°C for one hour. After cooling, the mixture was centrifuged at 3000 rpm for 15 minutes and the absorbance of the homogenate was read spectrometrically at 530 nm. The amount of MDA was calculated using Beer Lambert's formula and an extinction coefficient of 1.56x105 M/cm [32].

2.7.2. Determination of catalase activity

Catalase activity (CAT) was determined by the method of Sinha [33]. 50 µL of brain homogenate sample was mixed with 750 µL of 0.1M phosphate buffer (pH 7.5) and 200 µL of H_2O_2. The reaction was stopped by adding 200 µL of acid reagent (dichromate/acetate). All tubes were heated for 10 minutes and absorbance read by spectrometry at 620 nm. CAT concentration was expressed in terms of mmol H_2O_2/mg protein.

2.7.3. Determination of reduced glutathione levels

The level of reduced glutathione (GSH) in brain was determined in homogenate following the protocol described by Ellman [34]. 20 µL of brain homogenate was mixed with 3 mL of Ellman's reagent at room temperature. After one hour, the absorbance of the yellow compound was read by spectrometry at 412 nm. The amount of glutathione was calculated using the Beer Lambert formula.

2.7.4. Determination of superoxide dismutase levels

The determination of SOD was carried out according to the method described by Beyssiri *et al* [34]. In the control tube, 1666 µL of carbonate buffer (0.05 M, pH 10.2), 0.2 mL of adrenaline solution (0.3mM) and 134 µL of distilled water were

introduced to calibrate the spectrophotometer. Next, 134 µL of homogenate and 1,666 µL of carbonate buffer are added to the spectrophotometer. Once the spectrometer has been calibrated, the reaction is started by adding 0.2 mL of adrenaline solution to the reaction mixture, followed by homogenization. The optical density (OD) is read after 20 s and at 80 s at 480 nm.

$$\textbf{SOD activity} = (\textbf{U/mg protein}) = \left(\frac{\textbf{Number unit of } \frac{\textbf{SOD}}{\textbf{mL}}}{\textbf{mg protein}} * \textbf{Dilution factor}\right)$$

SOD unit = (100 - ((Δ DOessay x 100)) / ΔDOwhite = % inhibition; 50% inhibition corresponds to 1 SOD unit; OD variation: ΔDOmin = DO20s - DO80s.

2.8. Assessment of the cholinergic system

2.8.1. Determination of acetylcholinesterase activity

Acetylcholinesterase (AchE) activity was assessed using method [35]. A volume of 0.05 mL supernatant was added to a cuvette containing 3 mL phosphate buffer (0.1 M, pH 8), 0.1 mL 5,5'dithio-bis (Ellman's reagent) and 0.1 mL acetylthiocholine iodide. The contents of the cuvette were thoroughly mixed by bubbling air. Absorbance was read at 412 nm for 2 minutes at 30-second intervals. Enzyme activity was calculated according to the Beer-Lambert law.

2.9. Statistical analysis of data

Data were analyzed by descriptive statistics: using Microsoft Excel 2016 software to determine the mean ± Standard Error on the Mean (SEM), analysis of variance (ANOVA) followed by Tukey's multiple comparison test were performed using Graph Pad Prisme software version 8.3.1. Values are considered significant at $p \leq 0.05$

3. Results

3.1. Qualitative phytochemical screening of *Leptedania hastata* extract

The results of phytochemical characterization tests on *L. hastata* extract reveal the presence of the following major chemical families: polyphenols, tannins, anthraquinones, alkaloids, saponins and triterpenes. These results are shown in Table 1 below.

Table 1 Major chemical families of *Leptedania hastata*

Chemical compounds	Absence or presence
Phenolic compounds	++
Flavonoids	-
Tannins	++
Anthraquinones	+
Alkaloids	+
Saponins	+
Triterpenes	++
Steroids	-

++: Quite abundant; +: Not abundant; -: Absent

3.2. Content of bioactive compounds in *Leptadenia hastata*

The results of the bioactive compound assay of *L. hastata* extract show a high polyphenol content of 194.75 ± 0.05 mgEAG/g extract, followed by a tannin content of 6.65 ± 0.30 mgEqCat/g extract. On the other hand, triterpene content is relatively low at 1.60 ± 0.29 mgEqLu/g extract.

3.3. Anti-free radical activity of *Leptedania hastata* extract

3.3.1. Diphenyl-picryl-hydrazyl test

The antioxidant power of *Leptadenia hastata* using the DPPH method is shown in Table 2 below.

Table 2 Percentage inhibition in (%) and IC50 in (mg/ml)

Decoction and reference compound	Percentage of Inhibition (%)	IC_{50} (mg/ml)
Vitamin C	95,78 ± 0,00	6,52
Leptedania hastata extract	89,46 ± 0,02	5,51

3.4. Effects of *Leptadenia hastata* on scopolamine-induced neurotoxicity in white mice in the T-maze test

3.4.1. Effects on latency to enter the first arm

After 15 days of treatment, scopolamine induced a significant increase ($P < 0.001$) in the latency time for preferred arm choice (Fig. 1) in all 3 phases (habituation, acquisition, retention), ranging from 9.4 ± 2.55 seconds in normal control mice to 19 ± 0.83 seconds in negative control mice. *L. hastata* extract antagonizes the effects of scopolamine, significantly ($P < 0.001$) reducing this time to 2.8 ± 0.88 seconds and 4 ± 0.83 seconds in animals treated with 76 mg/kg and piracetam (habituation); from 7.8 ± 1.88 seconds in normal control mice to 16 ± 2.16 seconds in negative control mice. *L. hastata* counteracts the effects of scopolamine by significantly ($P < 0.001$) decreasing this time to 2.4 ± 0.88 seconds and 3.2 ± 1.55 seconds in animals treated with 76 mg/kg and piracetam (acquisition); from 5.4 ± 0.88 seconds in normal control mice to 12.4 ± 0.5 seconds in negative control mice. *L. hastata* reverses the effects of scopolamine, significantly ($P < 0.001$) decreasing this time to 1.8 ± 0.66 seconds and 2.6 ± 0.44 seconds in animals treated with 76 mg/kg and piracetam (retention).

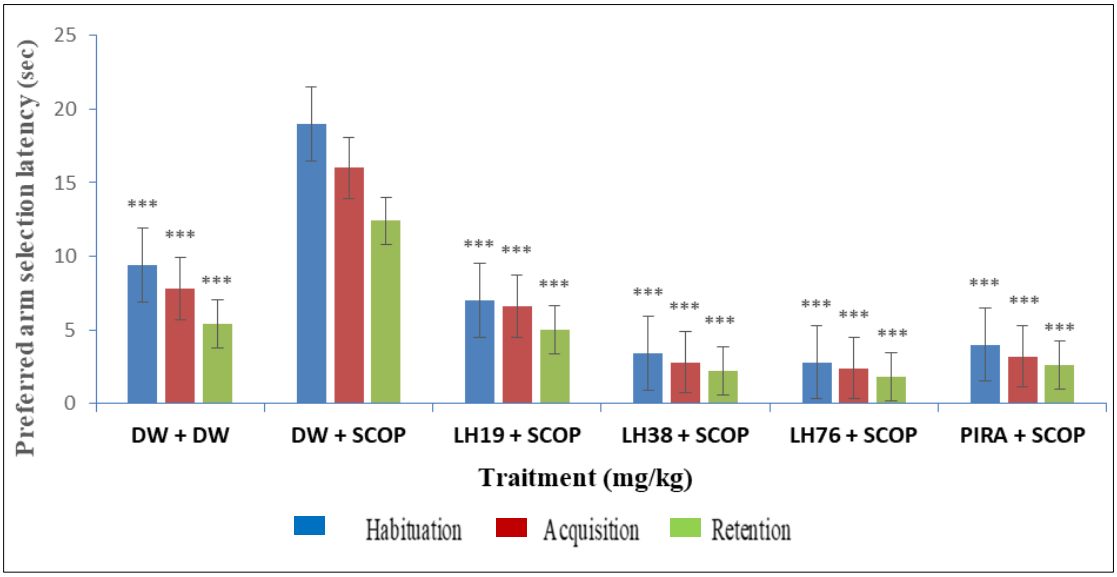

Figure 1 Effects of *Leptadenia hastata* extract on latency of preferred arm choice in the T-maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one-way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001 vs. Scopolamine treated group (DW + SCOP); DW: distilled water; LH19, LH38, LH76: Doses of *Leptadenia hastate;* SCOP: Scopolamine; PIRA: Piracétam.

3.4.2. Effects on the number of returns to the starting arm

Similarly, scopolamine significantly ($P < 0.001$) increases the number of returns to the starting arm (Fig. 2) from 5.2 ± 1.66 in normal control animals to 7.6 ± 1.83 in negative control animals. *L. hastata* extract inhibits the effects of scopolamine, significantly ($P < 0.001$) reducing this number to 2.4 ± 1.11 and 2.6 ± 1.14 in mice treated with 38 mg/kg and piracetam (habituation); from 8.8 ± 1.50 in normal control animals to 10.8 ± 1.50 in negative control animals. *L.*

hastata inhibits the effects of scopolamine by significantly (P < 0.001) reducing this number to 1.8 ± 0.66 and 3.2 ± 1.33 in mice treated with 38 mg/kg and piracetam (acquisition); from 11 ± 0.55 in normal control animals to 14.4 ± 0.88 in negative control animals. *L. hastata* inhibits the effects of scopolamine, significantly (P < 0.001) reducing this number to 1.4 ± 0.5 and 1.8 ± 0.66 in mice treated with 38 mg/kg and piracetam (retention).

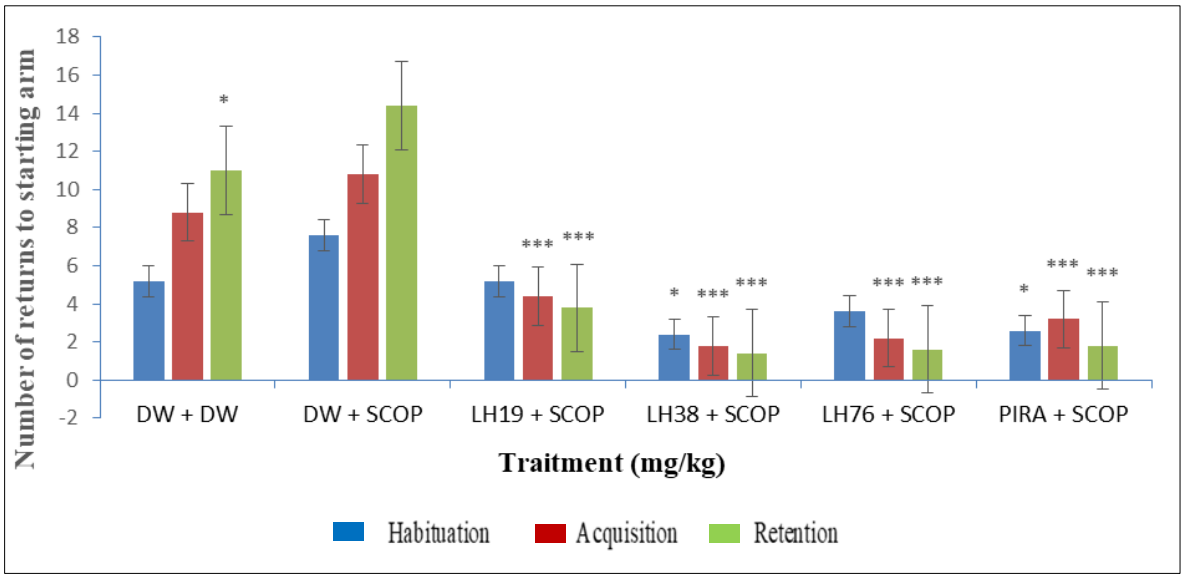

Figure 2 Effects of *Leptadenia hastata* extract on the number of returns in the T-maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one-way ANOVA followed by Turkey multiple comparisons test. *p ≤ 0,05, ***p < 0,001 vs. Scopolamine treated group (DW + SCOP); DW: distilled water; LH19, LH38, LH76: Doses of *Leptadenia hastata*; SCOP: Scopolamine; PIRA: Piracétam.

3.4.3. Effects on time spent in the preferred arm

After 15 days of treatment, it was found that scopolamine significantly (P < 0.001) decreased the time spent in the preferred arm (Fig. 3) from 117.4 ± 1.44 seconds in normal control animals to 88.2 ± 1.22 seconds in negative control animals. *L. hastata* inhibits the effects of scopolamine by significantly (P < 0.001) increasing this time to 172 ± 0.55 seconds and 150.4 ± 0.66 seconds in mice treated with 76 mg/kg and piracetam (habituation); from 78.6 ± 1.33 seconds in normal control mice to 69 ± 1.33 seconds in negative control mice. *L. hastata* counteracts the effects of scopolamine by significantly (P < 0.001) increasing this time to 195.4 ± 0.66 seconds and 159 ± 0.66 seconds in animals treated with 76 mg/kg and piracetam (acquisition); from 5.4 ± 0.88 seconds in normal control mice to 12.4 ± 0.5 seconds in negative control mice. *L. hastata* reverses the effects of scopolamine, significantly (P < 0.001) reducing this time to 1.8 ± 0.66 seconds and 2.6 ± 0.44 seconds in animals treated with 76 mg/kg and piracetam (retention); from 57.8 ± 0.64 seconds in normal control animals to 49 ± 0.66 seconds in negative control animals. *L. hastata* inhibits the effects of scopolamine by significantly (P < 0.001) increasing this time to 203.6 ± 0.44 seconds and 186 ± 0.83 seconds in mice treated with 76 mg/kg and piracetam (retention).

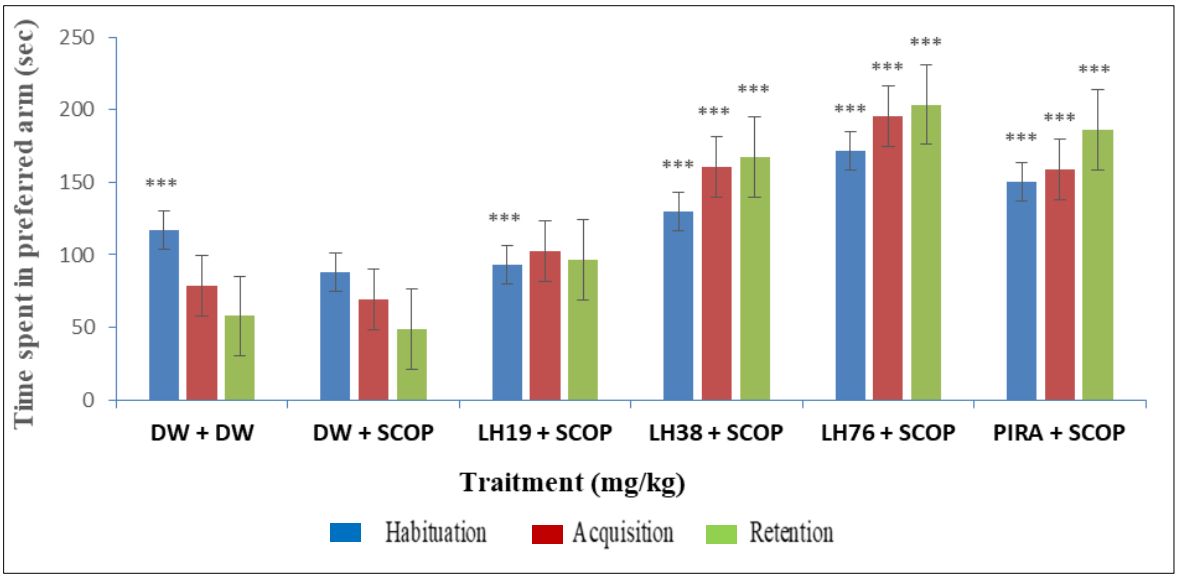

Figure 3 Effects of *Leptadenia hastata* extract on time spent in the preferred arm of the T-maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one-way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001 vs. Scopolamine treated group (DW + SCOP); DW: distilled water; LH19, LH38, LH76: Doses of *Leptadenia hastata*; SCOP: Scopolamine; PIRA: Piracétam.

3.4.4. Effects on the number of entries into the preferred arm

The number of entries into the preferred arm (Fig. 4) fell from 18.4 ± 0.77 in normal control animals to 4.4 ± 0.66 in negative control animals. *L. hastata* inhibits the effects of SCOP by significantly (P < 0.001) increasing this number to 21.2 ± 0.44 and 13 ± 0.83 in mice treated with 205 mg/kg and PIRA (habituation); from 15.6 ± 0.83 in normal control mice to 7.9 ± 0.77 in negative control mice. *L. hastata* counteracts the effects of scopolamine by significantly (P < 0.001) increasing this number to 33.2 ± 0.61 and 16.8 ± 0.28 in animals treated with 102.5 mg/kg and piracetam (acquisition); from 10 ± 0.33 in normal control animals to 4.2 ± 0.66 in negative control animals. *L. hastata* inhibits the effects of scopolamine, increasing this number to 40.6 ± 0.66 and 19.2 ± 0.61 in mice treated with 102.5 mg/kg and piracetam (retention).

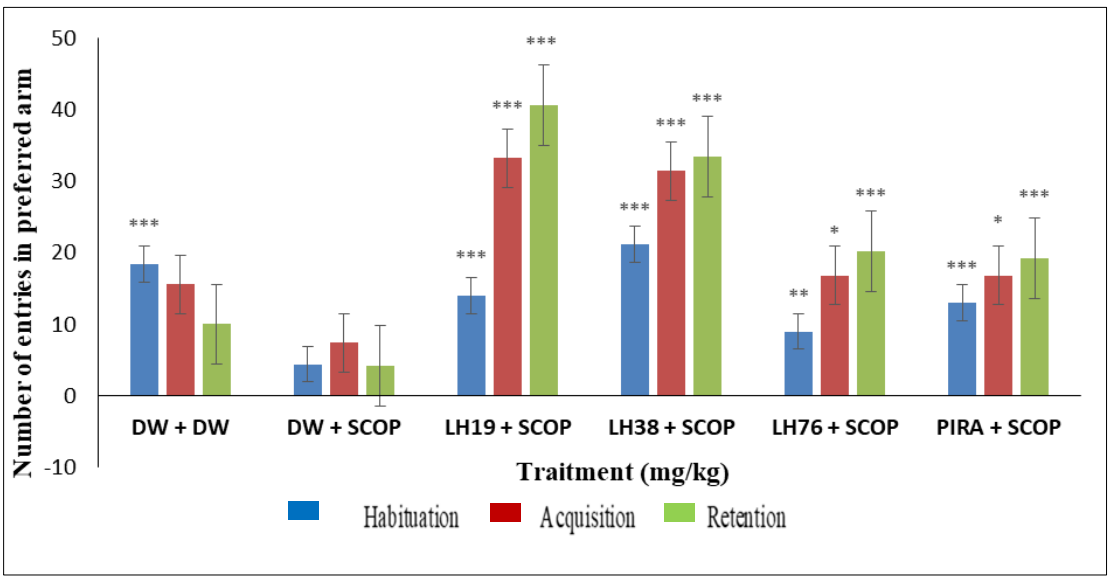

Figure 4 Effects of *Leptadenia hastata* extract on the number of entries in the preferred arm of the T maze

Each column represents mean ± SEM of five animals. The data analysis was performed using one-way ANOVA followed by Turkey multiple comparisons test. *p ≤ 0,05, ***p < 0,001 vs. Scopolamine treated group (DW + SCOP); DW: distilled water; LH19, LH38, LH76: Doses of *Leptadenia hastata*; SCOP: Scopolamine; PIRA: Piracétam.

3.5. Effects of *Leptadenia hastata* on scopolamine-induced neurotoxicity in white mice in the open field object recognition test

3.5.1. Effects on exploration time for objects A and B in the open field

Following 15 days of treatment, scopolamine produced a significant (P < 0.001) increase in exploration time for familiar object A, from 9.8 ± 0.54 seconds in normal control mice to 17.8 ± 0.64 seconds in negative control mice. Conversely, a significant decrease (P < 0.001) for novel object B, from 26 ± 0.40 seconds in normal control animals to 11.4 ± 0.88 seconds in negative control animals. *L. hastata* extract inhibits the effects of scopolamine by significantly (P < 0.001) decreasing this time by 4.8 ± 0.96 and 8.6 ± 0.48 seconds in animals treated with 38 mg/kg and piracetam for familiar object A. In parallel, *L. hastata* extract significantly (P < 0.001) increased this time by 39.4 ± 0.48 and 22.8 ± 0.64 seconds in both 38 mg/kg and piracetam-treated mice (Fig. 5).

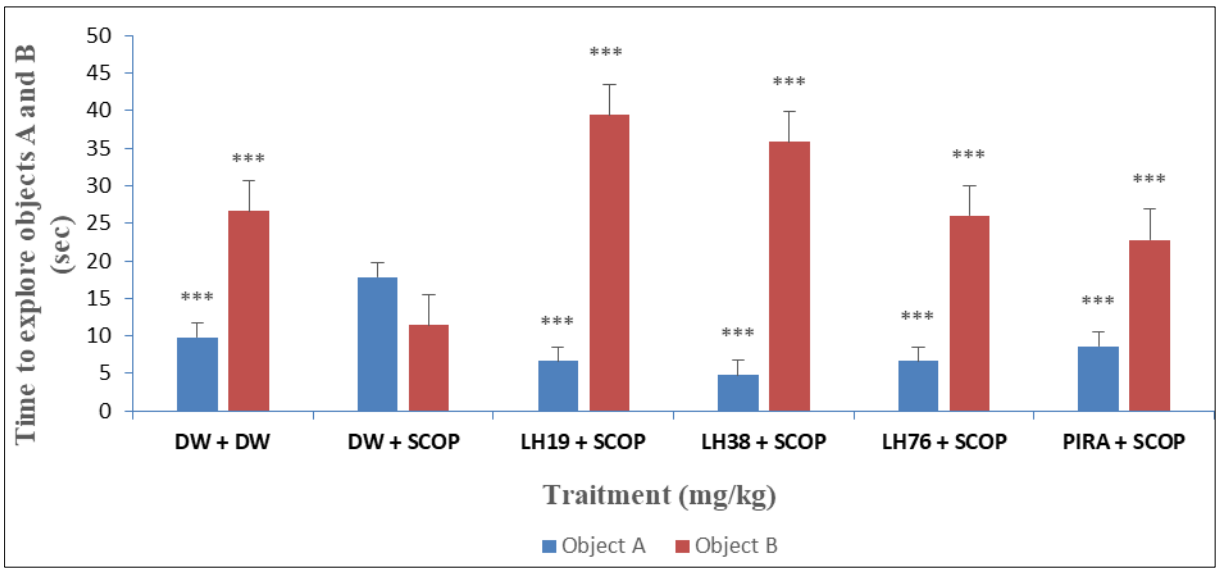

Figure 5 Effects of *Leptadenia hastata* extract on the exploration time of objects A and B in the open field

Each column represents mean ± SEM of five animals. The data analysis was performed using one-way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001 vs. Scopolamine treated group (DW + SCOP); DW: distilled water; LH19, LH38, LH76: Doses of *Leptadenia hastata*; SCOP: Scopolamine; PIRA: Piracétam.

3.5.2. Effects on discrimination index

Daily administration of scopolamine causes a significant (P < 0.001) increase in the new object recognition index, which ranges from 57.8 ± 0.83% in normal control mice to 60 ± 0.70% in negative control mice. *L. hastata* extract counteracts the effects of scopolamine by significantly (P < 0.001) increasing the new object recognition index at all doses, with optimal values of 91.6 ± 0.54 and 91.6 ± 0.89% in animals treated with 410 mg/kg and piracetam (Fig. 6).

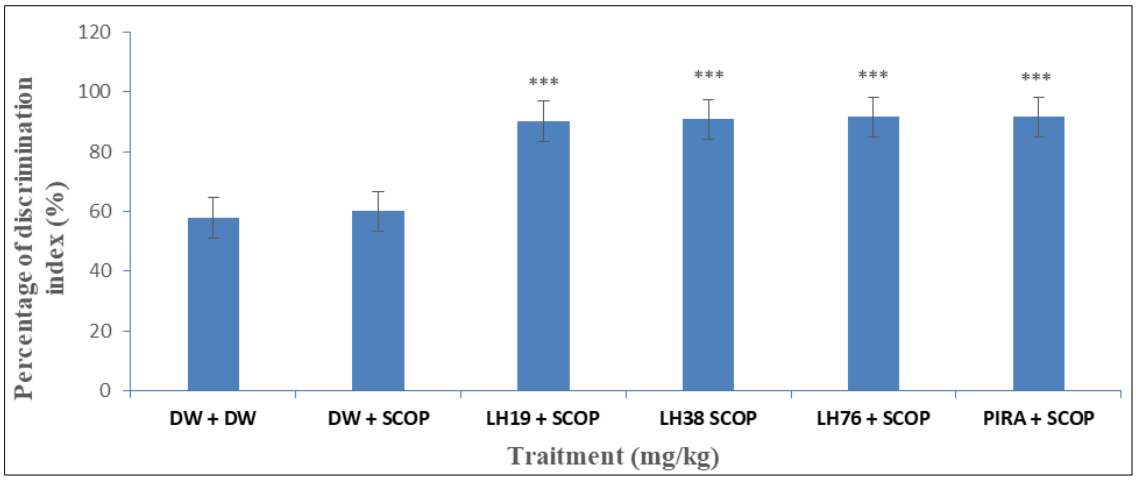

Figure 6 Effects of *Leptadenia hastata* extract on the recognition index of the new object in the open *field*

Each column represents mean ± SEM of five animals. The data analysis was performed using one-way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001 vs. Scopolamine treated group (DW + SCOP); DW: distilled water; LH19, LH38, LH76: Doses of *Leptadenia hastate;* SCOP: Scopolamine; PIRA: Piracétam.

3.6. Effect of *Leptadenia hastata* on oxidative stress parameters

Daily administration of scopolamine for 15 days causes a significant (P < 0.001) increase in MDA concentration from 4.95 ± 0.04 mol/g tissue in normal control mice to 10.54 ± 0.12 mol/g tissue in negative control mice. *L. hastata* extract inhibits the effects of scopolamine, significantly (P < 0.001) lowering this concentration to 8.54 ± 0.14 and 5.24 ± 0.08 mol/g tissue in 19 mg/kg and piracetam-treated animals (Table 3). At the same time, scopolamine significantly (P < 0.001) decreased CAT levels from 13.55 ± 0.70 mol/g tissue in normal control mice to 8.89 ± 1.55 mol/g tissue in negative control mice; GSH from 0.06 ± 0.00 mol/g tissue in normal control mice to 0.04 ± 0.00 mol/g tissue in negative control mice and SOD from 4.57 ± 0.31 SOD/mg in normal control mice to 4.16 ± 0.38 SOD/mg in negative control mice. *L. hastata* extract reverses the effects of scopolamine and significantly (P < 0.001) increases CAT levels to 15.55 ± 1.29 and 14.53 ± 1.25 mol/g tissue in 19 mg/kg and piracetam-treated animals, GSH at 0.06 ± 0.00 mol/g tissue in animals treated with 38 mg/kg and piracetam, and SOD of 5.25 ± 0.08 and 4.97 ± 0.14 SOD/mg in animals treated with 19 mg/kg and piracetam (Table 3).

Table 3 Effects of *Leptadenia hastata* on oxidative stress parameters

TRAITMENT (mg/kg)	MDA (mol/g tissue)	CAT (mol/g tissue)	GSH (mol/g tissue)	SOD (SOD/ mg protein)
DW + DW	4,95 ± 0,04***	13,55 ± 0,70***	0,06 ± 0,00***	4,57 ± 0,31
DW + SCOP	10,54 ± 0,12	8,89 ± 1,55	0,04 ± 0,00	4,16 ± 0,38
LH19+SCOP	8,54 ± 0,14***	15,5 ± 1,29***	0,05 ± 0,00***	5,25 ± 0,08***
LH38+SCOP	9,69 ± 0,07***	11,69 ± 0,91**	0,06 ± 0,00***	4,8 ± 0,31*
LH76+SCOP	10,51 ± 0,20	11,32 ± 0,94*	0,04 ± 0,00	5,09 ± 0,38***
PIRA + SCOP	5,24 ± 0,08***	14,53 ± 1,25***	0,06 ± 0,00***	4,97 ± 0,14**

Results are expressed as mean ± SEM for five animals. The data analysis was performed using one-way ANOVA followed by Turkey multiple comparisons test. *p<0,05, **p<0,01, ***p < 0,001 vs. Scopolamine treated group (DW + SCOP). DW, distilled water; LH, *L. hastate;* SCOP, Scopolamine; PIRA, Piracétam.

3.7. Effect of *Leptadenia hastata* on the scopolamine-induced cholinergic system

3.7.1. Effect on acetylcholinesterase concentration

Following 15 days of treatment, Fig.7 shows that scopolamine significantly (P < 0.001) increases AchE concentration from 0.10 ± 0.01 µM/mg/min in normal control mice to 0.17 ± 0.01 µM/mg/min in negative control mice. *L. hastata*

decoctate inhibits the effects of scopolamine by significantly ($P < 0.001$) decreasing this concentration at all doses, with minimum values of 0.10 ± 0.01 and 0.08 ± 0.01 µM/mg/min in animals treated with 38; 76 mg/kg and piracetam compared with negative control.

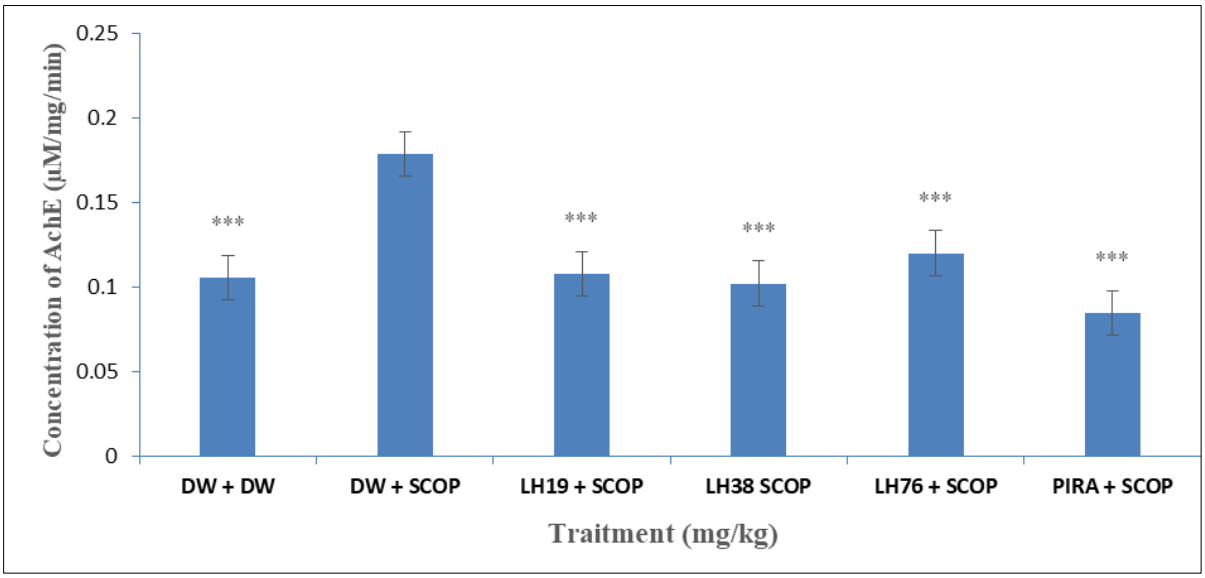

Figure 7 Effects of *Leptadenia hastata* extract on acetylcholinesterase levels in mice

Each column represents mean ± SEM of five animals. The data analysis was performed using one-way ANOVA followed by Turkey multiple comparisons test. ***p < 0,001 vs. Scopolamine treated group (DW + SCOP); DW: distilled water; LH19, LH38, LH76: Doses of *Leptadenia hastata*; SCOP: Scopolamine; PIRA: Piracétam.

4. Discussion

Scopolamine is a competitive inhibitor of muscarinic acetylcholine receptors, inducing depressive effects on cognitive functions and the learning process [28]. It also causes degeneration of the cholinergic system in the cerebral cortex [36, 37]. Scopolamine has been implicated in the pathophysiology of Alzheimer's disease through its ability to aggravate oxidative stress and neurotoxicity [37]. The neurotoxicity associated with scopolamine in human subjects is also supported by animal studies indicating behavioral alterations caused by prolonged scopolamine administration [37]. One of these animal studies demonstrated that daily administration of scopolamine causes cognitive dysfunction and particularly central nervous system neurotoxicity [36]. The present study demonstrated that prolonged administration of scopolamine results in loss of spatial memory and object recognition memory assessed in the T-maze and open field respectively. The results obtained from this study reveal, in the T-maze test, a decrease in latency to enter the preferred arm in mice given *L. hastata* decoctate. The decrease in latency indicates an improvement in working memory [38]. The increase in time spent in the preferred arm and the decrease in the number of returns to the starting arm suggest an increase in exploration and hence in memory faculties [39, 40]. The object recognition test revealed a significant increase in the time spent exploring the novel object, as well as in the discrimination index in mice treated with *L. hastata* extract. However, the increase in time to explore the novel object and the discrimination index show the curiosity of rodents to develop a performance towards the novel object [41]. This analysis suggests an improvement in episodic memory. According to Koutseff (2011), *L. hastata* extract has memetic effects that can improve learning capacity and memory recall. Assays of scopolamine-induced oxidative stress markers show a decrease in MDA levels and an increase in GSH, SOD and CAT concentrations in mice treated with *L. hastata* extract, characterizing the protection of brains against oxidative stress [42]. Thus, *L. hastata* extract is thought to possess antioxidant effects that may counteract the neurodegenerative effects associated with scopolamine. Numerous clinical studies have reported that oxidative stress is closely involved in the pathogenesis of Alzheimer's disease [43]. Acetylcholinesterase, a fundamental enzyme identified in the process of memory loss [44]. The current therapeutic strategy is to seek drugs against memory loss based on AchE inhibition [45]. AchE inhibition, diet in the form of fruit and vegetables rich in vitamins C and E, carotenoids, ubiquinone, flavonoids or lipoic acid play an important role in the prevention and treatment of memory loss [46,47]. In this study, *L. hastata* extract was shown to significantly reduce acetylcholinesterase levels in mice compared with negative control batches. The decrease in acetylcholinesterase levels in mice treated with *L. hastata* extract indicates neuroprotection of the brain [48]. These results confirm those

observed by Lee *et al,* (2016) in their work on the effects of Aronia melanocarpa against scopolamine-induced memory impairment. *L. hastata* extract therefore has neuroprotective effects.

5. Conclusion

At the end of this study, we can conclude that *L. hastata* extract counteracts the effects of neurotoxicity, altered behavior and oxidative stress induced by scopolamine. These results may at least explain the widespread use of this plant in traditional medicine for the treatment of central nervous system disorders. Nevertheless, to understand the exact mechanism by which this plant exerts mnesic and neuroprotective effects, investigations on other animal models specific to cognitive disorders would be necessary.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained

References

[1] Pasha S. A, Sindhu C. H, Nayak P. Assail of aluminum in reference to neurological disorders. In: Jelenkovi´c A, editor. Aluminum neurotoxicity: From subtle molecular lesions to neurological diseases. Nova Science Publishers. 2016; 91-110.

[2] Mirza M. R. et al., Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. New England Journal of Medicine. 2016; 375(22), 2154-2164.

[3] Khan N. et al., Physicochemical investigation of the drinking water sources from Mardan, Khyber Pakhtunkhwa, Pakistan. International journal of physical sciences. 2013; 8(33), 1661-1671.

[4] Bonfill-Teixidor E, Otxoa-de-Amezaga A, Font-Nieves M, Sans-Fons M.G, & Planas A.M. Differential expression of E-type prostanoid receptors 2 and 4 in microglia stimulated with lipopolysaccharide. Journal of neuroinflammation, 2017; 14, 1-13.

[5] Schever K. et al., "Piracetam improved cognitive performance by restoring neurochemical deficits of the aged rat brain," Pharmacopsychiatry. 1999; 32(1), 10–16.

[6] Lanoiselée H. M, Nicolas G, Wallon D, Rovelet-Lecrux A, Lacour M, Rousseau S, ... & collaborators of the CNR-MAJ project. APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. PLoS medicine, 2017; 14(3), 002270.

[7] Parnetti L, Senin U, Mecocci P, "Cognitive enhancement therapy for Alzheimer's disease," Drugs. 1997; 53(5), 752– 768.

[8] Blazer D. G. et al., "The risk of anticholinergic toxicity in the elderly—a study of prescribing practices in two populations," The Journals of Gerontology. 1983; 38(1), 31–35.

[9] Ngoupaye G. T, Ngo Bum E, Daniels W. M. U, "Antidepressant-like effects of the aqueous macerate of the bulb of Gladiolus dalenii Van Geel (Iridaceae) in a rat model of epilepsy-associated depression," BMC Complementary and Alternative Medicine. 2013; 13, 272–289.

[10] Cisse, A., Gueye, M., Ndiaye, A. Ka, F., Koma, S. et Akpo, L. E. « Ethnobotanique des plantes médicinales chez les bergers peuls de Widou Thiengoly de la commune de Téssékéré (Ferlo-Nord Sénégal) », J. Appl. Biosci., 2016; 98, 9301-9308.

[11] El-Fitiany, R. A. et Khasawneh, M. A. « Leptadenia Pyrotechnica (Forsk.) Decne: from edibility to drug discovery (comprehensive review) », Food Rev. Int., 2023; 39(9), 6834-6886.

[12] Sani, I. H., Abubakar, A. R., Yaro, A. H. et Malami, S. « Antidepressant-like effects of methanol leaves extract of *Leptadenia hastata* (Asclepidiaceae) in mice », Bayero J. Pure Appl. Sci., 2019; 12(1), 105-111.

[13] Nejad-Alimoradi, F., & Rezanejad, F. (). The study of phenolic compounds and habitat soil of Leptadenia pyrotechnica. Applied Biology, 2024; 37(3).

[14] Harborne J. B, Phytochemical methods. A guide to modern techniques of plant analysis. 1973; 2, 5-11

[15] Mort J. S et al., Early cathepsin K degradation of type II collagen in vitro and in vivo in articular cartilage. Osteoarthritis and cartilage. 2016; 24(8), 1461-1469.

[16] Buvaneswari K, Ramamoorthy D, Velanganni J. Preliminary phytochemical and antimicrobial activity studies on the leaves of the Indian plant Thevetia neriifolia Juss. World Journal of Agricultural Sciences. 2011; 7(6), 659-666.

[17] Fankam A, Kuete V, Voukeng I, Pagès M. J. Antibacterial activities of selected Cameroonian spices and their synergistic effets with antibiotics against phenotypes. Complementary and Alternative Medicine. 2011; 11, 104-108.

[18] Ambe A.S, Ouattara D, Tiebre M.S, Vroh B.T.A, Zirihi G.N. & N'guessan K.E. Diversité des plantes médicinales utilisées dans le traitement traditionnel de la diarrhée sur les marchés d'Abidjan (Côte d'Ivoire). Journal of Animal & Plant Sciences, 2015; 26(2), 4081-4096.

[19] Hebi M, & Eddouks M. Évaluation de l'activité antioxydante de Stevia rebaudiana. Phytothérapie, 2016; 14(1), 17-22.

[20] Bainbridge Z, Tomlins K, Wellings K. & Westby A. Methods for assessing quality characteristics of non-grain starch staples. (Part 3. Laboratory methods.), 1996.

[21] Enneb H, Belkadhi A, Cheour F. & Ferchichi A. Comparaison des composés phénoliques et du pouvoir antioxydant de la plante de henné (Lawsonia inermis L.). Journal of New Sciences, 2015; 20(2), 788-793.

[22] Fankam A, Kuete V, Voukeng I, and Pagès M. J, Antibacterial activities of selected Cameroonian spices and their synergistic effets with antibiotics against phenotypes. Complementary and Alternative Medicine, 2011; (11), 104-108.

[23] Hamauzu Y. & Suwannachot J. Non-extractable polyphenols and in vitro bile acid-binding capacity of dried persimmon (Diospyros kaki) fruit. Food Chemistry, 2019; 293, 127-133.

[24] Ashfaq A, Sant'Anna A, Lingman M. & Nowaczyk S. Readmission prediction using deep learning on electronic health records. Journal of biomedical informatics, 2019; 97, 103256.

[25] Gasso V. Y, Hahut A. N, Yermolenko S. V, Hasso I. A, Agca C. A, Nedzvetsky V. S. & Sukharenko E. V. Local industrial pollution induces astrocyte cytoskeleton rearrangement in the dice snake brain: GFAP as a biomarker. Biosystems Diversity, 2020; 28(3), 250-256.

[26] Fanta, Y. S. A. Effets neuroprotecteurs du décocté des feuilles de Carissa edulis (Forssk.) Valh (Apocynaceae) sur des modèles expérimentaux de la perte de mémoire et de neurotoxicité de type Alzheimer, Thèse de Doctorat/PhD, 2020; 1(5).

[27] Semaan D. G, Igoli J. O, Young L. A. I, Rowan E. G &. Marrero E. In vitro anti-diabetic effect of flavonoids and pheophytins from Allophylus cominia Sw. On the glucose uptake assays by HepG2, L6, 3T3-L1 and fat accumulation in 3T3-L1 adipocytes. Journal of ethnopharmacology, 2018; 216, 8-17.

[28] Saraf, M. K., Prabhakar, S., Khanduja, K. L., & Anand, A. Bacopa monniera attenuates scopolamine-induced impairment of spatial memory in mice. Evidence-Based Complementary and Alternative Medicine, 2011; 1 - 10.

[29] Lacasse J. M, Patel S, Bailey A, Peronace V. & Brake W. G. Progesterone rapidly alters the use of place and response memory during spatial navigation in female rats. Hormones and Behavior, 2022; 140, 105137.

[30] Cogram P, Deacon R. M, Warner-Schmidt J. L, Von Schimmelmann M. J, Abrahams B. S. & During M. J. Gaboxadol normalizes behavioral abnormalities in a mouse model of fragile X syndrome. Frontiers in Behavioral Neuroscience, 2019; 13, 141.

[31] Ennaceur A. Object novelty recognition memory. In Handbook of behavioral neuroscience 2018; 27, 1-22.

[32] D. B. K. Nkontcheu, A. L. Fotio, A. D. Kenfack, G. C. T. Taboue, D. A. Acha & E. B. Fokam. Ecological risk assessment of pesticides based on earthworms in soils on the southeast slopes of Mount Cameroon. Soil & Environmental Health, 2023; 231(4), 100047.

[33] Jain A. K. & Sinha A. K. General Health in Organizations: Relative Relevance of Emotional Intelligence, Trust, and Organizational Support. International journal of stress management, 2005; 12(3), 257.

[34] Ellman G. L Tissue sulfhydryl groups. Archives of biochemistry and biophysics. 1959; 82(1), 70-77.

[35] Ellman, G.L., Courtney, K.D., Andres, J.V., & Feather-Stone, R.M. (). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 1961; 7, 88-95.

[36] Chaparro. H. V., Rivera, C. M. C., Torres, M. B. M., & Beas, Z. C. Neuronal death and tumor necrosis factor-a response to glutamate-induced excitotoxicity in the cerebral cortex of neonatal rats. Neuroscience letters, 2002; 333 (2), 95 - 98.

[37] Mason, L. H., Harp, J. P. & Han, D. Y. Neurotoxicity: Neuropsychological Effects of Lead Toxicity. Biomed Research International, 2014; 2014, 1 - 8.

[38] Farshchi A, Ghiasi G, Farshchi S. & Khatabi M. P. Effects of Boswellia Papyrifera Gum Extract on Learning and Memory in Mice and Rats. Iranian Journal of Basic Medical Sciences, 2010, 13 (2), 9 - 15.

[39] De-Mello, & Carobrez. Elevated T-maze as an animal model of memory: effects of scopolamine. Behavioural Pharmacology, 2002; 13, 139-148.

[40] Chapouthier, G., Lepicard, E. M., Rössler, A. S., & Venault, P. L'anxiété, un pont entre l'épilepsie et la mémoire? Philosophia scientiae, 2002; 6(1), 75-91.

[41] Ennaceur A. One-trial object recognition in rats and mice: methodological and theoretical issues, Behavioural brain research, 2010, 215 (2), 244 - 254.

[42] Shila, S., Kokilavani, V., Subathra, M., & Panneerselvam, C. Brain regional responses in antioxidant system to α-lipoic acid in arsenic intoxicated rat. Toxicology, 2005; 210(1), 25-36.

[43] Lee, H. Y., Weon, J. B., Jung, Y. S., Kim, N. Y., Kim, M. K., & Ma, C. J. Cognitive-enhancing effect of Aronia melanocarpa extract against memory impairment induced by scopolamine in mice. Evidence-Based Complementary and Alternative Medicine, 2016; (6145926), 1-8.

[44] Srikanth, J., Kavimani, S., Uma, M. R. C., & Chitra, K. Comparative Molecular Docking Study of Phytoconstituents Identified in Morinda Citrifolia Linn on Acetylcholinesterase and Butyrylcholinesterase. Journal of Chemical and Pharmaceutical Research, 2017; 9(4), 121-125.

[45] Arpaci, O., Meryem, T., Fatma, S. S., & Ilkay, E. O. Synthesis and Biological Evaluation of Some Benzoxazole Derivatives as Inhibitors of Acetylcholinesterase / Butyrylcholinesterase and Tyrosinase. International Journal of Pharmacological and Pharmaceutical Sciences, 2017; 4(9), 745.

[46] Lien, A. P. H., Hua, H., & Chuong, P. H. Free Radicals, Antioxidants. In Disease and Health.Biomedical science, 2008; 2, 89-96.

[47] Kuk, E. B., A. Ra, J., Seo, I. O., Hee, S. S., Su, H. S., Anupom, R., Jae, S. C., & Hyun, A. J. Anti-Alzheimer's disease activity of compounds from the root bark of Morus alba. Archive Pharmacology Resource, 2017; 40, 338–349.

[48] Haider, A., Inam, W., Khan, S. A., Mahmood, W., & Abbas, G. β-glucan attenuated scopolamine induced cognitive impairment via hippocampal acetylcholinesterase inhibition in rats. Brain research, 2016; 1644, 141-148