The Journal of Phytopharmacology (Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X JPHYTO 2023; 12(1): 1-10 January- February Received: 22-01-2023 Accepted: 28-02-2023 Published: 28-02-2023 ©2023, All rights reserved doi: 10.31254/phyto.2023.12101

Galba Jean Beppe

Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814 Maroua, Cameroon

Bertrand Poo Barga

Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814 Maroua, Cameroon

Donatien Albert Atsamo Department of Animal Biology, and Physiology, University of Yaoundé I, Cameroon. P.O. Box 812

Christ-Roi Fedaski

Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814 Maroua, Cameroon

Alice Irène Folefack

Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814 Maroua, Cameroon

Nanou Gael Allah-Doum

Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814 Maroua, Cameroon

Franklin Gamo Zemo

Department of Psychology, Faculty of Arts, Letters and Social Science, University of Yaoundé I, P.O. Box 7011, Yaoundé, Cameroon

Alain Bertrand Dongmo

Department of Animal Biology Faculty of Science University of Douala P.O. Box 24157 Douala, Cameroon

Correspondence:

Dr. Galba Jean Beppe Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814 Maroua, Cameroon

Email: beppe840@gmail.com

Memory-Enhancing and Antioxidant Activities of the Hydroethanolic Leave Extract of *Piliostigma reticulatum* (Fabaceae) in the Monosodium Glutamate-Induced Alzheimer's Disease Model

Galba Jean Beppe, Bertrand Poo Barga, Donatien Albert Atsamo, Christ-Roi Fedaski, Alice Irène Folefack, Nanou Gael Allah-Doum, Franklin Gamo Zemo, Alain Bertrand Dongmo

ABSTRACT

Background: Piliostigma reticulatum (Fabaceae) is a plant used in Cameroon for the treatment of madness and migraine. Aims and Objectives: This study aims to evaluate the memory-enhancing and antioxidant activities of the hydroethanolic leaves extract of PR in the monosodium glutamate-induced Alzheimer's disease model. Materials and Methods: The Y-maze, Radial-arm maze and Novel object recognition tests were used to assess short, and long-term memory. At the end of the experiments, the brains of mice were scavenged for biochemical and histological analysis. Results: It was observed that the hydroalcoholic extract of PR leaf significantly reversed the percentage of spontaneous alternation compared to the negative control group at the different doses tested (p<0.01; p<0.001) in the Y-maze test. In the radial arm maze test, the extract significantly (p<0.001) reduced the time spent consuming the bait, the number of reference memory and working memory errors at all doses. The treatment of animals with hydroethanolic extract of PR resulted in a significant increase in the discrimination index (p<0.001) in the unfamiliar object recognition test. In addition, the extract significantly (p < 0.01); p < 0.001) decreased acetylcholinesterase activity, and MDA levels at all tested doses; and significantly (p < 0.05; p < 0.001) increased SOD level and Catalase activity in comparison to the negative control group. The histological analysis showed that the extract of Piliostigma reticulatum protected the different structures of the hippocampus against damage caused by monosodium glutamate. Conclusion : The memoryenhancing induced by the hydroethanolic leaves extract of Piliostigma reticulatum could be due to his antioxidant and anticholinesterase properties. These effects could justify the use of this plant to manage nervous system affections.

Keywords: Reference memory, Working memory, Anticholinesterase, Spontaneous alternation, Alzheimer's disease.

INTRODUCTION

Memory impairment is a group of brain diseases that progressively affect cognitive functions and occur more frequently in the elderly ^[1]. The best-known types of memory loss are those leading to Alzheimer's disease, which accounts for 60-70% of cases, followed by vascular dementia, dementia with Lewy bodies and frontotemporal dementia ^[2]. It is estimated that over 50 million people worldwide have dementia, with a new case occurring every 3 seconds ^[3]. The regional distribution of new cases was 10.5 million in Europe, 22.9 million in Asia, 9.4 million in America and 4 million in Africa ^[4].

Glutamate is a neurotransmitter of the vertebrate central nervous system. It is one of the most abundant additives in the human diet and is present as monosodium glutamate ^[5]. Excessive intake of monosodium glutamate induced excessive intracellular Ca²⁺ release, which causes excitotoxicity, oxidative stress, neuroinflammation and cell death. These effects induced by monosodium glutamate could lead to Alzheimer's type dementia ^[2]. Drugs used for memory impairment are generally acetylcholinesterase inhibitors (Donepezil, Rivastigmine and Galantamine), and an NMDA receptor antagonist, memantine ^[8]. These treatments are only symptomatic and have many side effects such as diarrhoea, headache, nausea, vomiting and hallucinations ^[9]. 80% of the world's population rely on traditional pharmacopoeia for their primary care ^[10]. Some studies on natural drug have shown the beneficial effects of some plants such as *Daniellia oliveri* ^[11] and *Crassocephallum crepioides* ^[12] on the protection against memory loss. Our interest focuses on *Piliostigma reticulatum* (Fabaceae), a plant traditionally used in Cameroon for the treatment of several pathologies including madness and migraines. Previous studies done on this plant shown that it is endowed with anti-inflammatory, anti-ulcerogenic, and anticholinesterase properties ^[13].

The present study aims to evaluate the effects of hydroethanolic leaves extract of *Piliostigma reticulatum* (*P. reticulatum*) on monosodium glutamate-induced Alzheimer model.

MATERIAL AND METHODS

Plant material

Collection and extraction

Powders (500 g) obtained from the dried leaves were macerated into 5 L of ethanol/water mixture (80/20) for 72 hours. The macerate was filtered by using wattman paper number 4. The filtrate obtained was concentrated in the Rotavapor (BUCHI, R-300) at 60° C and then dried in the oven at 50°C for 48 hours to obtain a crude dry extract (29 g). The extraction yield was 5.8%.

Phytochemical screening of the hydroethanolic leaves extract of *Piliostigma reticulatum*

The determination of total polyphenols, total flavonoid, tannins and saponins contents was carried out according to the methods describe by Folin-Ciocalteu ^[14], Mimica-Duckic, 1999, Bainbridge et al., 1996, Alilou et al. 2014 respectively. The references compounds used were gallic acid, quercetin, catechin and diosgenin respectively for total polyphenol, total flavonoid, tannins and saponins ^[14, 15, 16, 17].

Evaluation of the in vitro antioxidant activity of the hydroethanolic leaves extract of *Piliostigma reticulatum*

The iron-reducing antioxidant capacity (FRAP) and the anti-free radical activity at DPPH (2,2-Diphenyl-2-Picrylhydrazyl) were used to evaluated the in vitro antioxidant activity of the hydroethanolic leaves extract *of Piliostigma reticulatum*. The reducing power of iron (Fe³⁺) was determined according to the method described by Soural et al., 2022 [18]. While the effect of the extract on DPPH was measured by the procedure described by Sanchez-Moreno, (2002) ^[19].

Animal material

Male mice, Mus Musculus (Muridae) aged 6-8 weeks were used in this experiment. They were housed in plastic basins covered with wire mesh with wood shavings as bedding. Animals were fed with a standard diet and have free access to tap water. The study was approved by the Ethics Committee of the Faculty of Sciences of the University of Maroua (Ref. N°14/0261/ Uma/D/FS/VD-RC), according to the guidelines of Cameroonian bioethics committee (reg N°. FWA-IRB00001954).

Animal were divided into 6 groups of 7 mice each and received the following treatments:

- Normal control group (Nor) which received NaCl only (p.o + i.p);

- Negative control group (Neg) which received NaCl (p.o.) and MSG (4 mg/kg, i.p.);

- Positive control group (Vit C) which received vitamin C (100 mg/kg, i.p) and MSG (4 mg/kg, i.p);

- Three test groups (PR100, PR200 and PR400), which received the hydroethanolic extract of leaves of *Piliostigma reticulatum* at doses of

100, 200 and 400 mg/kg respectively (p.o.) and MSG (4 mg/kg, i.p.) each.

The treatment was carried out for 28 days and the volume of administration was 10 mL/kg. At the end of the treatment each animal was subjected to a behavioural assessment of memory using Y-maze test, Radial arm maze test and Novel object recognition test.

Behavioural assessments

Evaluation of memory impairment of the hydroethanolic extract of leaves of *Piliostigma reticulatum* using Y-Maze test

The Y-maze task measures spatial working memory through spontaneous alteration behaviour in rodents. The Y-maze used in this study was consisted of three symmetrically arms (33 cm length \times 11 cm width \times 12 cm height), separated at 120°. For the test, each mouse was individually placed in the triangular centre of the maze and allowed to freely explore it for 8 min ^[20]. Total entry number (arms visits) was used as a measure of locomotor activity and the degree of spontaneous alternation was estimated by the following calculation:

Percentage of alternanation= (Nomber of alternance)/(Total nomber of arms visited-2) X 100

After the passage of each mouse, the maze was wiped with 70% ethanol to minimize odour cues.

Evaluation of memory impairment of the hydroethanolic extract of leaves of *Piliostigma reticulatum* using Radial Arm Maze test

The Radial Arm Maze is a test of special learning and short and longterm memory in rodents. The apparatus used in this study was consisted of 8 arms (48 cm length x 12 cm height), numbered 1 to 8 partitioned from a central cylindrical area (32 Cm diameter). On day 16 of the experiment, the animals were subjected to a habituation phase during which each animal was allowed to explore the maze for 10 minutes (for 5 consecutive days). On day 1, baits were placed on the floor of the central platform on four of the eight arms (1, 3, 5, and 7), as well as in the feeding wells of each of these lanes, but from day 2 to day 5, baits were progressively restricted until only one bait was hidden in the feeding wells at the end of these four designated lanes. On the 21st day of the experiment (test phase), each animal was placed individually in the center, of the maze and subjected to 5minute learning tasks, in which the same arms were baited. The other arms (2, 4, 6 and 8) were never baited throughout the experiment. The parameters evaluated were the number of working memory errors (entering an arm containing food but previously visited) and reference memory errors (entering an arm that had never been baited)^[21]. The time spent to consume the four baits was also recorded.

Evaluation of memory impairment of the hydroethanolic extract of leaves of *Piliostigma reticulatum* using Novel Object Recognition Test

The experiment was conducted in an open box and in three stages: habituation, familiarisation and test phases. To habituate and reduce the animals' fear of a new environment, the mice were exposed to the device without objects, with the freedom to explore the apparatus for 10 minutes. The next day, the mice were introduced in the same environment, but with two identical objects (A1 and A2) in their presence. The exploration time of familiarisation was 5 minutes. 24

hours after the mice were suggested for test phase. In this phase, mice were placed back in the apparatus which contain a novel object (B) used to change A2. Exploration was considered when mice sniffed or made contact with the objects using their nose ^[22]. The contact time with the "novel" object (TN) and the contact time with the familiar object (TF) were recorded. At the end of each task, the apparatus was cleaned with 70% ethanol to mask any olfactory traces. To assess recognition memory, the discrimination index (DI) was calculated as follows:

$$DI = (TN-TF)/(TN+TF)$$

Sacrifice and tissue collection

On day 28 of the experiment, mice were euthanized with a combination of ketamine and diazepam (10 mg/kg and 50 mg/kg respectively); and then sacrificed. Blood was collected for biochemical analysis and brains were removed for oxidative stress assays and histological sectioning.

Biochemical analyses

For biochemical analyses, blood was used to prepare serum (3000 rpm for 15 minutes) use to evaluate acetylcholinesterase level. Seahorses were carefully isolated from the brains, homogenized, and centrifuged at 3000 rpm for 15 minutes. The supernatants were collected for malondialdehyde (MDA), and catalase (CAT) assays. The determination of MDA level was performed according to the method described by Wilbur et al., 1949 ^[23]. The catalase activities were evaluated according to the methods described by Shina, (1972) ^[24]. The level of acetylcholinesterase was estimated in serum by the method described by Ellman et al. 1961 ^[25].

Histological analyses

Whole brains were fixed in 10% formalin, dehydrated, and then impregnated in paraffin baths. The paraffin section (5 μ m thickness) for each brain was subjected to a standard protocol of haematoxylineosin staining. Hippocampal lesions were observed under a microscope at 100 x magnification for the dentate gyrus and 200 x for the Ammon's horn.

Statistical analysis

The data were expressed as the mean \pm S.E.M. The ANOVA followed by Dunnett's post-test and ANOVA followed by Bonferroni's posttest (Graph Pad Prism, version 5.03) were used to evaluate the significant difference between treated groups and Negative control as well as Normal control. A p value < 0.05 was considered significant.

RESULTS

Phytochemical analysis of the hydroethanolic extract of leaves of *Piliostigma reticulatum*

The results presented in Table 1 shown the concentration of selects compounds in the hydroethanolic extract of leaves *of P. reticulatum*. These results show that the extract contains: total polyphenols (73.15 \pm 4.02) are the most representative compound followed by flavonoids, saponins and tannins.

In vitro **DPPH** and **FRAP** antioxidant activity of the hydroethanolic extract of leaves of *Piliostigma reticulatum*

The analysis of results presented in Table 2 shown that the extract plant has a low inhibition percentage of FRAP (63.94 ± 1.62) and DPPH (48.87 ± 1.48) compared to BHT (72.05 ± 1.50).

Effects of the hydroethanolic extract of leaves of *Piliostigma reticulatum* on memory and locomotor activity evaluate by the Y maze test

The sub-chronic administration of monosodium glutamate resulted in a significant (p < 0.001) decrease in the number of arm visits (Figure 1A) and the percentage of spontaneous alternation (Figure 1B) in the Negative control group compared to the Normal control group. The treatment with the hydroethanolic extract of leaves of *P. reticulatum* at all doses of 100 and 200 mg/kg, as well as vitamin C, induced a significant (p < 0.01; p < 0.001) increased of the number of arm visits (Figure 1A) and the percentage of spontaneous alternation (Figure 1B) compared to the Negative control group.

Effects of the hydroethanolic extract of leaves of *Piliostigma reticulatum* on memory evaluate by the radial maze test

The analysis of Figure 2 shown that from day 2 onwards of the experiment, the sub-chronic administration of monosodium glutamate significantly (p < 0.01; p < 0.001) increased bait consumption time (Figure 2A), the number of errors in reference memory (Figure 2B) and the number of errors in working memory (Figure 2C) in the Negative control group compared to animals in the Normal control group. Compared to the Negative control group, the extract administration, at all tested doses, as well as vitamin C, improves memory by inducing a significant (p < 0.05; p < 0.01; p < 0.001) decrease of the time of bait consumption (Figure 2A), the number of errors (Figure 2B) in reference and working memory (Figure 2C).

Effects of the hydroethanolic extract of leaves of *Piliostigma reticulatumon* memory evaluate by the Novel Object Recognition test

As shown on Figure 3, the administration of monosodium glutamate induced a significant (p < 0.001) decrease of the novel object exploration time (Figure 3A) and the discrimination index (Figure 3B) in Negative control animals compared to Normal control. The treatment of the animals with the extract at the dose of 100 and 200 mg/kg as well as Vitamin C, induced a significant (p < 0.01; p < 0.001) increase of the novel object exploration time (Figure 3A) and the discrimination index (Figure 3B) in comparison to the Negative control group.

In vivo effects of the hydroethanolic extract of leaves of *Piliostigma reticulatumon* some parameters of oxidative stress

Compared to the Normal control group, administration of monosodium glutamate induced a significant (p < 0.001) increase of the MDA level (Figure 4A) and a significant (p < 0.01; p < 0.001) and Catalase (Figure 4B) activities in hippocampus of the negative control group. The hydroethanolic extract of *P. reticulatum* at all tested doses, as well as Vitamin C, significantly (p < 0.05; p < 0.01; p < 0.001) decreased the concentration of MDA (Figure 4A) and Catalase (Figure 4B) activities compared to the Negative control group.

Effects of the hydroethanolic extract of leaves of *Piliostigma reticulatum* on acetylcholinesterase activity in serum

Results presented in Figure 5 shown that administration of MSG resulted in a significant (p < 0.001) increase of acetylcholinesterase activity in the serum of mice of the negative control group compared to the Normal control. The extract at the doses of 100, 200 and 400 mg/kg, as well as vitamin C, significantly (p < 0.01; p < 0.001) decreased acetylcholinesterase activity in the serum of mice which received the extract plant compared to the Negative control (Figure 5).

Pearson correlation between MDA level and discrimination index, and number of arms visits

The Pearson's regression showed a significant negative correlation (r = -0.628; p < 0.001) between the decrease in discrimination index and the increase in MDA concentration in mouse hippocampal homogenates (Figure 6A) and a significant negative correlation (r = -0.437; p < 0.01) between the decrease in the number of visits and the increase in MDA concentration (Figure 6B).

Effects of the hydroethanolic extract of leaves of *Piliostigma* reticulatum on the hippocampus microarchitecture

The analysis of the microarchitecture of hippocampal sections (Figure 7) of mice brain of the Normal control group (Figure 7A) showed a structure with normal-looking neurons in the different layers (GD, CA1, CA2, and CA3). Compared to Normal group, administration of monosodium glutamate to mice in the Negative control group (Figure 7B), of, resulted in histomorphological changes marked by a disorganization of microarchitecture of hippocampus (Pn), the presence of necrotic (Cn) and vacuolated cells (Vn). The animal which received the extract at all tested doses (Figure 7D, E and F) and the one receiving vitamin C (Figure 7C), showed a microstructure of the hippocampus close to the normal.

DISCUSSION

To study the memory improvement of the hydroethanolic leaves extract of *Piliostigma reticulatum* (*P. reticulatum*) on monosodium glutamate-induced neuronal excitotoxicity and memory loss, several tests were performed in mice: the Y-maze test, the radial arm maze test and the object recognition test. Some markers of oxidative stress (MDA, and CAT) and acetylcholinesterase activity were also assessed as well as the histopathology of hippocampus.

Y-maze test is one of the very useful models for the evaluation of behavior, learning, memory function, neurodegenerative disorders related to aging, Alzheimer's, drug screening and phenotyping. Rodents typically prefer to investigate a new arm of the maze rather than returning to one that was previously visited ^[26]. In Y-maze test, spontaneous/correct alteration has been embraced by behavioral pharmacologists as a quick and relatively simple test of spatial working memory, devoid of fear, reward of reinforces [27] Monosodium glutamate by reducing the percentage of spontaneous/correct alternation caused memory loss in mice. The treatment with the extract at the dose of 100, 200 and 400 mg/kg resulted in a significant increase in the percentage of spontaneous/correct alternation of mice compared to the Negative control group. Lee and Goto, (2015) [24, 27] had shown that the increase in the percentage of alternation is evidence of the improvement of learning process and working memory in rodents. Monosodium glutamate also induced a significant decrease in the number of arms entries in the Y maze compared to Control group. Compared to monosodium glutamate-treated group, the hydroethanolic leaves extract of P. reticulatum at the dose of 200 and 400 mg/kg induced a significant increase in the number of arm entries in the Y maze. According to Zemo et al. (2021) a substance which increases the percentage of spontaneous alternation and does not affect the number of arms entries in the Y maze could improve spatial working memory of mice without affecting their motor activity ^[26, 28].

Y-maze test and the radial maze test is widely used model for assessing spatial memory in rodents ^[29]. Administration of the extract at all tested doses improved memory performance and learning in mice by significantly decreasing the number of errors in working memory and the number of errors in reference memory compared to the Negative control group. Beppe et al. 2015 had shown that the decrease in the number of errors in working memory and reference memory in amnesic mice would be linked to an improvement in spatial memory ^[30]. These observations shows that the hydroethanolic extract of *P. reticulatum* could play an important role in the memory process.

The brain is a vulnerable organ to the adverse effects of oxidative stress because of its high energy consumption resulting in high oxidative metabolism, its high concentration of polyunsaturated fatty acids, its scarcity of antioxidant enzymes, and the fact that neurons are post-mitotic cells ^[31]. Several studies have shown that monosodium glutamate creates oxidative stress damage in the brain of mice, resulting in memory impairment ^[32]. In this study, administration of monosodium glutamate significantly increased MDA level in hippocampi compared to Normal control group. Considered an important indicator of lipid peroxidation, MDA is a major oxidation product of polyunsaturated fatty acids which are the primary target of reactive oxygen species. The increase in MDA levels suggests that monosodium glutamate-induced lipid peroxidation in the hippocampi of Negative control group. A significant decrease in MDA level was observed in the hippocampi of mice which receive the sub-acute treatment of the hydroethanolic extract of P. reticulatum at all tested doses in comparison to the Negative control group. Indeed, Beppe et al. 2020 had shown that the decrease in MDA level in the hippocampi of mice suggest that the extract could ameliorate the memory impairment and lipid peroxidation induced by neurotoxic [11]. In addition, the monosodium glutamate induced a significant decrease in Catalase activity in the hippocampi of mice compared to the Normal control group. As an antioxidant, Catalase allows the detoxification of hydrogen peroxide by converting it into oxygen and water molecules and also plays a role in the defence against cellular oxidative damage as a free radical scavenger [33]. As results in this study, a significant increase in CAT activity was observed in the hippocampi of mice treated with the extract at all tested doses compared to Negative control group. These results suggest that the extract enhancing learning and memory capacities by his antioxidant properties. Thus, the extract is able to inhibit the creation of oxidative stress. In this study, the in vivo antioxidant properties of the hydroethanolic extract of P. reticulatum were confirmed in vitro by DPPH and FRAP antioxidant assays. The results obtained suggest that the extract is endowed with compounds which possess antioxidant capacity.

Acetylcholinesterase is an enzyme that plays an important role in cholinergic functions by ensuring the termination of nerve impulse transmission within neuromuscular junctions and cholinergic synapses ^[34]. Administration of monosodium glutamate significantly increased acetylcholinesterase activity in the serum of Negative control mice compared to the Normal control group. It is well known that the increase in acetylcholinesterase activity may affect the cholinergic transmission process and lead to learning and memory deficits that

cause Alzheimer's disease ^[35]. Compared to animals treat with monosodium glutamate only, the treated with the extract plant at a dose of 100, 200 and 400 mg/kg induced a significant decrease in acetylcholinesterase activity These results corroborate with those obtained by Kim et al. 2007 who found an elevated level of acetylcholinesterase in animals receiving a neurotoxic only (scopolamine) compared to the Normal control as well as those treated with ethanolic extract of *Terminalia chebula* ^[36]. These results suggest that the hydroethanolic extract of *P. reticulatum* possesses anticholinesterase properties. Indeed, a significant negative correlation was obtained between the concentration of MDA and the discrimination index on the one hand and between the concentration of MDA and the number of visits arms.

The histopathological analysis of the microarchitecture of the hippocampi of the mice showed that the sub-chronic administration of monosodium glutamate caused neurodegeneration in the Negative control group compared to the Normal control group. This neurodegeneration induced by monosodium glutamate was marked by a hight level of neuronal loss (necrotic cells) and neuronal vacuolation in Negative control group compared to Normal control. The neuronal loss could be leads to the activation of certain enzymes such as endonucleases that cause nuclear DNA degradation ^[37]. Animals receiving the extract at all tested doses showed a microstructure of the hippocampus close to the normal one. The neuroprotection induced by the hydroethanolic extract of *P. reticulatum* could be due to polyphenolics compound found in the extract plant which inhibit lipid peroxidation or nuclear DNA degradation.

The phytochemical screening of the hydroethanolic extract of *P. reticulatum* revealed the presence of flavonoids, tannins and saponins. The observed beneficial effects of this plant extract in monosodium

glutamate-induced changes in behaviour and brain injury may thus be attributed to these diversified chemical components. Many of these compounds have proven their potential as antioxidants in various oxidative stress models as scavengers of free radicals as reported in prior studies ^[38].

Table 1: Phenolics, tannins and saponins content in mg/100 g DM in the hydroethanolic extract of leaves of *Piliostigma reticulatum*.

Compounds	Concentration in <i>P. reticulatum</i> extract	Units
Total Polyphenols	73.15 ± 4.02	mg eq Gallic acid / g DM
Flavonoids	41.12 ± 1.61	mg eq Quercetin / g DM
Tannins	$4,24\pm0,92$	mg eq Catechin / g DM
Saponins	38,80 ± 2,34	mg eq Diosgenin / g DM

Results are expressed as the mean of 3 replicates \pm S.E.M., DM = dry matter; eq = equivalent.

Table 2: In vitro DPPH and FRAP antioxidant activity of the hydroethanolic extract of leaves of *Piliostigma reticulatum*

Parameters	Percentage of inhibition
DPPH	48.87 ± 1.48
FRAP	63.94 ± 1.62
BHT	72.05 ± 1.50

Results are expressed as the mean of 3 replicates ± S.E.M. DPPH: 2,2-Diphenyl-2-Picrylhydrazyl; FRAP: Iron Reducing Antioxidant Power; BHT: Butylated hydroxytoluene.



Figure 1: Effects of the hydroethanolic extract of leaves of *Piliostigma reticulatum* on the number of arm visits (A) and the percentage of spontaneous alternation (B). Each bar represents the mean \pm S.E.M (n = 7). ^{###}p < 0.001 vs. Normal control. ^{**}p < 0.001 vs. Negative control. PR = *Piliostigma reticulatum* extract; MSG = Monosodium glutamate; Vit C = Vitamin C.



Figure 2: Effects of the hydroethanolic extract of leaves of *Piliostigma reticulatum* on bait consumption time (A), reference memory error (B), and working memory error (C). Each bar represents the mean \pm S.E.M (n = 7). * p < 0.05; *** p < 0.01; *** p < 0.01 vs. Normal control. ** p < 0.01; *** p < 0.05; *** p < 0.001 vs. Negative control. PR = *Piliostigma reticulatum* extract; MSG = Monosodium glutamate; Vit C = Vitamin C.



Figure 3: Effects of the hydroethanolic extract of leaves of *Piliostigma reticulatum* on exploration time (A) and discrimination index (B). Each bar represents the mean \pm S.E.M (n = 7). """ p < 0.001 vs. Normal control. "" p < 0.01; """ p < 0.001 vs. Negative control. PR = *Piliostigma reticulatum* extract; MSG = Monosodium glutamate; Vit C = Vitamin C.



Figure 4: Effects of the hydroethanolic extract of leaves of *Piliostigma reticulatum* on the concentration of MDA (A), and CAT (B) activities in hippocampi. Each bar represents the mean \pm S.E.M (n = 7). ###p < 0.001 vs. Normal control. *p < 0.05 **p < 0.01; ***p < 0.001 vs. Negative control. PR = *Piliostigma reticulatum* extract; MSG = Monosodium glutamate; Vit C = Vitamin C.



Figure 5: Effects of the hydroethanolic extract of leaves of *Piliostigma reticulatum*on acetylcholinesterase activity in serum. Each bar represents the mean \pm S.E.M (n = 7). ^{###}p < 0.001 vs. Normal control. ^{**}p < 0.01; ^{***}p < 0.001 vs. Negative control. PR = *Piliostigma reticulatum* extract; MSG = Monosodium glutamate; Vit C = Vitamin C.



Figure 6: Pearson correlation between MDA level in hippocampus and discrimination index (A), and between MDA level in hippocampus and number of arms visits (B).



Figure 7: Microphotograph of the Gyrus dente and areas CA1, CA2, and CA3 of the Ammon's horn of hippocampus.

Haematoxylin-Eosin staining, scale bar = A = Normal control; B = Negative control; C = Positive control; D, E, F = Test groups receiving the extract at doses of 100, 200, and 400 mg/kg respectively; GD = Dentate Gyrus (X100); CA1, 2, 3 = Ammon's horn 1, 2 and 3 (X200); Pn = Neuronal loss; Vn = Neuronal vacuolation; Cn = Necrotic cells.

CONCLUSION

The aim of the previous study was to evaluate the memory-enhancing and antioxidant activities of the hydroethanolic leaves extract of *Piliostigma reticulatum* in the monosodium glutamate-induced Alzheimer's disease model. At the end of this study, it appears that the extract improved the memory of amnesic mice by modulating acetyl cholinesterase activity and inhibiting the action of oxidative stress at the level of hippocampal components. These results would be due to the presence in the extract of secondary metabolites such as flavonoids. These effects induced by *P. reticulatum* extract could justify the traditional use of this plant in Cameroonian traditional medicine to manage central nervous system diseases and warrant further studies, including in other mechanism of action.

Acknowledgments

Authors are grateful to the Head of the Laboratory of the Department of Biological Sciences, Faculty of Sciences, University of Maroua, Cameroon, for providing facilities. Authors are also grateful to the Head of the Laboratory of Animal Physiology, Department of Biology and Animal Physiology of the Faculty of Sciences, University of Yaounde I, Cameroon, for providing facilities for histological assay.

Conflict of Interest

The authors declare that they have no conflicts of interest related to the publication of this study.

Financial Support

The author's state that this work was not funded by any organization or institution.

ORCID ID

Galba Jean Beppe: http://orcid.org/0000-0002-4328-5566

Allah-Doum Nanou Gael: http://orcid.org/0000-0001-7727-6951

Dongmo Alain Bertrand: http://orcid.org/0000-0002-6354-4318

REFERENCES

- 1. Hassan ZA, Arafa MH, Soliman WI, Atteia HH, Al-Saeed HF. The effects of monosodium glutamate on thymic and splenic immune functions and role of recovery (biochemical and histological study). J of cytol histol. 2014;5:283.
- Schmitt WB, Deacon RM, Seeburg PH, Rawlins JN, Bannerman DM. A within-subjects, within-task demonstration of intact spatial reference memory and impaired spatial working memory in glutamate receptor-A-deficient mice. J Neurosci. 2003;23(9):3953–3959.
- Ling Y, Morgan K, Kalsheker N. Amyloid precursor protein (APP) and the biology of proteolytic processing : relevance to Alzheimer's disease. Int. J. Biochem. Cell. Biol. 2003;35(11):1505-35.
- 4. Bros P. Developpement de methodes de reference pour spectrometrie de masse pour le dosage de biomarqueurs de la maladie d'Alzeimer Medecine humaine et pathologie. Thèse de Doctorat. Montpelier. France. 2016;124.
- 5. Reitz C, Brayne C, Mayeux R. Epidemiology of Alzeimer disease. Nat Rev Neurol. 2011;7(3):137-52.

- Alzeimer Disease International (ADI). The state of the art of dementia research: new frontiers. United states of America: World Alzeimer Report. 2018, 46.
- Alzeimer Disease International (ADI). The global impact of dementia: an anlisis of prevalence, incidence, cost and trends. United State of America: world Alzheimer Report. 2015, 20.
- Birks J. Cholinesterase inhibitors for Alzheimer's disease The Cockrane Library. Dementia and Cognitive Improvement. 2006;1:1468-1858.
- Fourmier H. Alzheimer et Benzodiazépines : Thèse de Doctorat en Pharmacie, Université Toulouse III Paul Sabatier, France. 2016, 100.
- Farnsworth N.R, Akerele O, Bingel A, S, Soejarto D.D et Guo Z,. Places des plantes médicinales dans la thérapeutique.Bulletin de l'organisation mondiale de la santé. 1986;64(2):159-164.
- Beppe GJ, Kenko DBL, Wado K E, Ngatanko AH, Nkwingwa BK, Damo KLJ, Rebe NR, and Foyet HS. Aqueous root bark extact of Daniella Oliveri protects neurons against Diazapaminduced Amnesia. EB Complementary and Alternative Medicine. 2020;2020:7815348.
- 12. Beppe Galba Jean, Folefack Alice Irène, Atsamo Albert Donatien, Ngatanko Aïbassou Hervé-Hervé, Barga Mpoo Bertrand, Allah-Doum Nanou Gael, Nguedia Ymele Merline, Zemo Gamo Franklik, Dongmo Alain Bertrand, Dimo Théophile, "Neuroprotective Effects of the Ethanolic Leaf Extract of Crassocephalum crepidioides (Asteracaeae) on Diazepam-Induced Amnesia in Mice", Adv Pharmacol Pharm Sci. 2022;2022:1919469.
- Aderrogba MA, Okoh EK, Okele IN, Olajide AO, and Ogundani AO. Antimicrobial and Anti-inflammatory Effects of *Piliostigma reticulatum* Leaf Extract. J. Med. Sci.. 2006;2(1):70-74. DOI: 10.3923/ijp.2006.70.74
- Vermerris W, Nicholson R. Isolation and identification of phenolic compounds. In: Phenolic compound biochemistry. Springer, Dordrecht New York, 2006, 35-62.
- Mimica-Duckic N, Mira P, Vida J, Anna S. and Olga G. "Pharmacological Studies of Mentha longifolia Phenolic Extract on Hepatoprotective Activity". Pharm. Biol. 1999;37(3):221-224.
- Bainbridge Z, Tomlins K, Wellings K, Westby A. Methodes of Assessing Quality Characteristics of Non-Gain Starch Staples. Natural Ressources Institute, University of Greenwich. 1996;39.
- Alilou H, Bencharki B, Hassani LMI, Barka N. Screening phytochimique et identification spectroscopique des flavonoïdes d'Asteriscus graveolens subsp. Odorus. Afrique SCIENCE. 2014;10(3):316-328
- Soural I., Svestkova P., Hik P., and Balik J., Different values obtained by the FRAP method for the determination of slowly and rapidly reacting phenols. 2022;51(1):84-92.
- Sanchez-Moreno C. Review: Methods Used to Evaluate the Free Radical Scavenging Activity in Food and Biological Systems. Int. J. Food Sci. 2002;3:121-137.
- Saka VP, Babu SP, and Himaja V. "Effect of aqueous piper betle leaf extract against scopolamine induced amnesia on albino rats". J. Chem. Pharm. Sci. 2017;10:116-120.
- Hritcu L; Foyet HS; Stefan M; Mihasan M; Asongalem AE; Kamtchouing P. Neuroprotective effect of the methanolic extract of Hibiscus asper leaves in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. J. Ethnopharmacol. 2011;137(1):585-91.
- Beppe GJ, Dongmo AB, Foyet HS, Tsabang N, Olteanu Z, CioancaO, Hancianu M, DimoT; et Hritcu L. Memory-enhancing activities of the aqueous extract of Albizia adianthifolia leaves in

the 6-hydroxydopamine-lesion rodent model of Parkinson's disease. BMC Complementary and Alternative Medicine. 2014;14:142.

- 23. Wilbur KN, Bernheim F, Shapiro OW. Determination of lipid peroxidation. Arch. Biochem. Biophys. 1949;24:305-310.
- Shina AK, Calorimetric assay of catalase. Anal. Biochem. 1972; 47(2):389-394.
- 25. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961;7(2):88-95.
- 26. Zemo GF, Amany DL, Ketcha WGJM, Oluwabusayo RF, Djiogue S, Taidinda GT, Njamen D, Olopade OJ. Ameliorative effects of the aqueous extract of Khaya anthotheca (Welw.) C.DC (Meliaceae) in vanadium induced anxiety, memory loss and pathologies in the brain and ovary of mice. J.ethnopharmacol. 2021;275:114099.
- Lee YA, and Goto Y. Chronic stress effects on working memory, association with prefrontal cortical tyrosine hydroxylase; Behavioural brain Research. 2015;286:122-127.
- Prieur EAK, Jadavji NM. Assessing Spatial Working Memory Using the Spontaneous Alternation Y-maze Test in Aged Male Mice. Bio Protoc. 2019;9(3):3162.
- Beppe GJ, Dongmo AB, Foyet HS, Dimo T, Mihasan M, and Hritcu L, The aqueous extract of Albizia adianthifolia leaves attenuates 6-hydroxydopamine-induced anxiety, depression and oxidative stress in rat amygdala. BMC Complement Altern Med. 2015;15:374.
- Foyet SH, T DE, and Ngatanko AHH. Enhancing Spatial Memory: Anxiolytic and Antidepressant Effects of Tapinanthus dodoneifolius (DC) Danser in Mice. Neurol Res Int. 2014;2014:974308.
- Shukitt-Hale, B and Carey AN. Beneficial effects of fruit extracts on neuronal function and behavior in a rodent model of accelerated aging. Neurobiol Aging. 2007;28(8):1187-1194.
- 32. Haleng J, Pincemal J, Defraigne JO, Charlier C, Chappelle JP. Le stress oxydant, Rev Med Leige. 2007;62(10):628-638.
- Silman I, and Sussman JL. AChE: classical and non classical functions and pharmacology. Curr opin pharmacol. 2005;5:293-302.
- 34. Kouémou NE, Taiwe GS, Moto FC, Pale S, Ngoupaye GT, Njapdounke JSK, Nkantchoua GCN, Pahaye DB, and Ngo Bum E. Nootropic and neuroprotective effects of Dichrocephala integrifolia on scopolamine mouse modele of Alzeimer's Disease. Front. Pharmacol. 2017;8:847.
- Kim SC, Adesogan AT, Arthington J. Optimizing nitrogen utilization in growing steers fed forage diets supplemented with dried citrus pulp. J. Anim. Sci. 2007; 85(10):2548-2555
- Nagata S, Nagase H, Kawane KN, Mukae H., and Fukuyama. Degradation of chromosomal DNA during apoptosis. Cell Death Differ. 2003;10:108-116.
- Rajamurugan R, Deepa V, Sivashanmugam M, Raghavan CM. Phytochemistry, antioxidant and antibacterial activities of medicinal plants: a comparative Study. Int. J. Curr. Res. Rev. 2013;5:8-19.

HOW TO CITE THIS ARTICLE

Beppe GJ, Barga PB, Atsamo DA, Fedaski CR, Folefack AI, Alla-Doum NG, Gamo FZ, Dongmo AB. Memory-Enhancing and Antioxidant Activities of the Hydroethanolic Leave Extract of *Piliostigma reticulatum* (Fabaceae) in the Monosodium Glutamate-Induced Alzheimer's Disease Model. J Phytopharmacol 2023; 12(1):1-10. doi: 10.31254/phyto.2023.12101

Creative Commons (CC) License-

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. (http://creativecommons.org/licenses/by/4.0/).