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Ocimum basilicum L. (Lamiaceae) leaves aqueous extract improve learning and memory in the monosodium glutamate-induced neurotoxicity model of Alzheimer's disease through attenuating brain oxidative damage in experimental mice

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Abstract

Ethnopharmacological relevance: *Ocimum basilicum* L. (Lamiaceae) is a medicinal plant known in Cameroon for the treatment of many ailments like anxiolytic, antispasmodic and mental disorders.

Aim of the study: The aim of this study is to evaluate the neuroprotective effects of *Ocimum basilicum*.

Materials and methods: T-maze test and open field test and the assay of oxidative stress parameters were used for detecting its capacity to protect neurons against excitotoxicity induced by monosodium glutamate.

Results: It was found that the *Ocimum basilicum* significantly increased the time spent in the preferred arm of the T-maze as well as the number of entries in the same arm. The first time arm choice latency decreased significantly in the animals treated by the compared to those treated with glutamate monosodium. In the open field test, it was noted an increase in the time spent in the center and the number of lines crossed. Results shows that the of *Ocimum basilicum* significantly reversed the oxidative damage induced by monosodium Glutamate by reducing the levels of MDA and increasing the concentration of CAT, GSH, SOD compared to the negative glutamate monosodium and distilled water treated groups respectively [$F(5, 15) = 13.51$; $P < 0.001$]; [$F(5, 15) = 12.42$; $P < 0.001$]; [$F(6, 24) = 79.74$; $P < 0.001$] and [$F(5, 15) = 18.27$; $P < 0.001$].

Conclusion: These results suggest that *Ocimum basilicum* possess neuroprotectives properties in mice that might involve an action on antioxidant defense system in the central nervous system

Keywords: Glutamate monosodium; *Ocimum basilicum*; Neuroprotection; Oxidative stress; Alzheimer disease

1. Introduction

Glutamate, present in nearly two-thirds of brain synapses, is the main excitatory neurotransmitter [1]. Because of its status as the preferred neurotransmitter of the pyramidal neurons of the neocortex and the hippocampus, it is strongly involved in higher brain functions, particularly memory. It is also involved in the excitotoxicity process present in the pathogenesis of many neurological diseases such as cerebral ischemia, epilepsy and Alzheimer's disease (AD). The molecular mechanisms underlying the pathophysiology of AD remain poorly elucidated to date. However, research in both animals and humans suggests that a major factor in the onset of AD is the loss of cholinergic neurons [1, 2]. Various

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sources indicate that several other metabolic pathways are involved in the pathophysiology of AD, including dysfunction of ß-amyloproteine metabolism, abnormalities in adrenergic, serotoninergic and dopaminergic transmission, and the possible involvement of mediators of inflammation and oxidative stress [3, 4, 1]. The etiological role of oxidative stress in cell death mechanisms during neurodegenerative diseases has been discussed for several years. According to World Health Organization [5], Alzheimer's disease is the most common form of dementia and is thought to account for 60-70% of cases.

There is currently no treatment for neurodegenerative diseases. It is therefore urgent to develop new treatments capable of countering or even stopping the progression of these diseases. The treatments given to patients treat the symptoms or try to slow down the progression of the disease but the real causes are not treated. In addition, current medications often have undesirable side effects, which must also be taken into account. However, a number of phytomedicines have been shown to be effective free radical scavengers, which may reduce reactive oxygen species and are beneficial for neurodegenerative diseases. Current research has explored traditional medicine to protect cells from oxidative damage [6]. The genus *Ocimum*, widely used in cooking, native to central Africa and widespread throughout the world, belongs to the family Lamiaceae [7]. The species *basilicum*, traditionally, it is used in phytotherapy for several of its virtues. Indeed it is used as a tonic, stimulant, and carminative, stomachic, antispasmodic, antiviral and deworming, especially against stomach cramps, diarrhea, constipation, angina, cough, kidney dysfunction, bronchitis, lung diseases, rheumatism, inflammation, headaches, and hypertension and against nervous system disorders [8]. In tea, the leaves were recommended against nausea, flatulence and dysentery [8]. However, there have been no studies concerning the effects of this plant on glutamate-induced neurodegenerative disorders. In this study, we aimed to investigate the neuroprotective effect of *Ocimum basilicum* and the underlying mechanism by which it protects against glutamate-mediated neurotoxicity.

2. Material and methods

2.1. Plant material

The leaves of *Ocimum basilicum* used for the study were collected between March 2018 and April 2018, in Ombessa, area of the Mbam & Inoubou division, Center Region of Cameroon. The area of study did not involve endangered or protected species. The collected species was identified by at National Herbarium of Yaoundé (Cameroon), where a voucher was deposited at number 17300/SFR Cam.

2.2. Preparation of *Ocimum basilicum* aqueous extracts

The leaves of *Ocimum basilicum* were dried, pulverized, and the obtained powder (16 g) was were boiled in 250 ml of distilled water for 20 min and the supernatant was filtered using Whatman No 1 filter paper. The resulting filtrate was then administered orally to mice in a volume of 10 mL/kg. The s of *Ocimum basilicum* were prepared daily according to Traditional Healers's instructions.

2.3. Animals

Thirty adult male Swiss mice weighting 24 – 32 g were obtained from the National Veterinary Laboratory, Garoua, Cameroon, and used throughout these experiments. They were housed in standard plexiglas cages with food and water *ad libitum*. The animal house was maintained constantly at 25°C on a 12 h light-dark cycle. The protocols were performed in concordance with the International Guide for the Care and Use of Laboratory Animal (National Institute of Health; publication No. 85-23, revised 1996) and the Cameroon National Ethical Committee, Yaoundé (No. FW-IRB00001954). In addition, the protocols for pharmacological studies were also realised in compliance with the recommendations provided in the Animal Research: Reporting of *In Vivo* Experiment (ARRIVE) guidelines published online in PLOS Biology [9], and the general guidelines for experimental research and screening of traditional medicine as promulgated by WHO [10].

2.4. Behavioural testing

2.4.1. T-maze test

The T-maze test was developed by Blodgett and Mc Cutchen [11] and is used to evaluate the spontaneous alternation behavior of animals. The device used is a T-shaped maze with three branches, consisting of a starting compartment, a central lane, and two opposite arrival compartments. The start compartment (30 cm long x 10 cm wide x 20 cm high) and the arrival compartments (30 cm long x 10 cm wide x 20 cm high) are separated from the central aisle by sliding

doors that can be operated manually [12]. This test consists of 3 phases: habituation (day 8 of treatment), acquisition (day 9 of treatment) and retention (day 10 of treatment); each phase marked by a parameter measurement.

2.4.2. Evaluation of locomotion, exploratory behavior and emotional reactivity in the open field test

The open field used in these experiments consist of a wooden square box (40 × 40 × 45 cm), and the floor of this apparatus was divided into 16 smaller squares (10 × 10 cm) of equal dimensions [13]. Several groups of six mice each were given orally different doses of *Ocimum basilicum* (and mg/kg ;) and glutamate monosodium for test groups, vitamin C and glutamate monosodium (mg/kg; positive control group) or distilled water (10 mL/kg; normal group). One-hour post-treatment animals were placed individually in the centre of the open field, and they could explore their experimental environment for 5 minutes duration. The number of crossing (number of square floor units entered) and centre time (time spent by the animal at the center of the device) were recorded for each animal [14]. At the end of behavioural evaluations, all the animals were euthanized by inhalation of high concentration of compressed carbon dioxide (CO_2) gas in cylinders, and the whole brain was collected for biochemical analyses

2.4.3. Biochemical estimation

Activity of catalase, and superoxide dismutase; the concentration of malondialdehyde and reduce glutathione in the brain homogenate was quantified as described previously with slight modification. Brains were removed, washed in 0.9% NaCl for assessment of oxidative stress marker levels. To each brain sample was added 500 µL of Tris-HCl buffer (50 mM; KCL 150 mM; pH 7.4). After crushing in a Potter, the mixture was introduced into a labeled tube and centrifuged at 40000 rpm for 15 minutes. The supernatant was then pipetted and introduced into a new tube for assays of brain catalase (CAT), reduce glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD). Briefly, SOD activity was assessed according to Misra and Fridovich [15] GSH level was measured according to the method described by Ellman [16] and MDA concentration was determined according to the method described by Wilbur and collaborators [17].

2.5. Statistical analysis

Data are shown as means ± Standard Error of the Mean (S.E.M.) for each animal. Statistical analysis of significance was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests.

P values less than 0.05 were considered as significant

3. Results

3.1. Effects of *Ocimum basilicum* on behaviour alteration in the T-maze test

3.1.1. Effects of Ocimum basilicum on the latency of arm choice in the T-maze

Glutamate monosodium administrations significantly increase latency during habituation, acquisition and retention trials. Daily administration of *Ocimum basilicum* for 14 days significantly reduced the latency of arm choice as compared to the control animals treated only by monosodium glutamate [F ((5, 24) = 48.77; P <0.001)]. This time decrease from 16.60±2.07 and 21.20 ± 2.86, corresponding to a decrease rate of 67.47 % and 74.52%, respectively for distilled-water and glutamate monosodium treated mice to 5.40±1.14 for plant at the dose 129 mg/kg treated mice. Remarkably, Vitamin C administered at a dose of 100 mg/kg, significantly decreased the latency time from 21.20 ± 2.86 in the glutamate monosodium-treated group to 10.00 ± 1.58 (p<0.001), a decrease of 52.83, to vitamin C treated mice (Fig 1). In the results of *post hoc* analysis, it was also found that, like vitamin C, *Ocimum basilicum* extract significantly reduced the latency time both in the habituation phase and in the retention comparatively to distilled water-treated group and to glutamate monosodium treated group (Fig 1).

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, significantly different compared to distilled water treated group, $^{\alpha}P<0.05$, $^{\beta}P<0.01$, $^{\gamma}P<0.001$, significantly different compared to the significantly different compared to glutamate monosodium treated group. DW, distilled water; Ob64, 129, 259 mg/kg *Ocimum basilicum*; Vit C, vitamin C; MSG, monosodium glutamate.

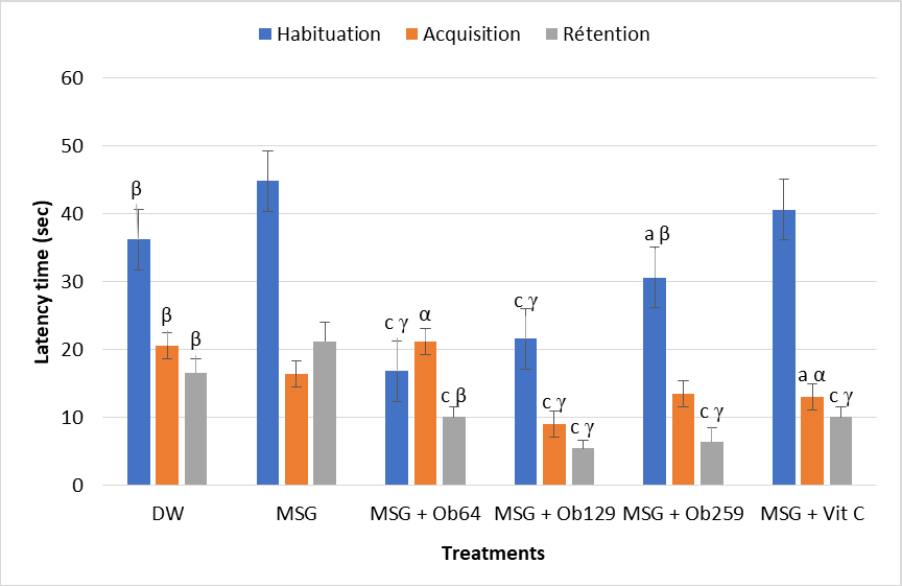

Figure 1 Effects of *Ocimum basilicum* on the latency of arm choice in the T-maze

3.1.2. Effects of Ocimum basilicum on the time spent in the preferred arm in the T-maze

As in latency of arm choice, glutamate monosodium administrations significantly reduce latency during habituation, acquisition and retention trials. Daily administration of *Ocimum basilicum* for 14 days significantly increase the time spent in preferred arm as compared to the control animals treated only by monosodium glutamate [(F (27, 112) = 15; P <0,001)]. This time increase from 100.80±7.22 (17.51% increase rate) and 83.00 ± 2.76 (32.07% increase rate) respectively for distilled-water and glutamate monosodium treated mice to 122.20±5.71 for plant at the dose 129 mg/kg treated mice Remarkably, Vitamin C administered at a dose of 100 mg/kg, significantly increased the time spent in preferred arm from 83.00 ± 2.76 in the glutamate monosodium-treated group to 107. 20 ± 4.31 (p<0.001) to vitamin C treated mice (Fig 2). In the results of *post hoc* analysis, it was also found that, like vitamin C, *Ocimum basilicum* extract significantly improved the time spent in preferred arm both in the habituation phase and in the retention comparatively to distilled water-treated group and to glutamate monosodium treated group (Fig 2)

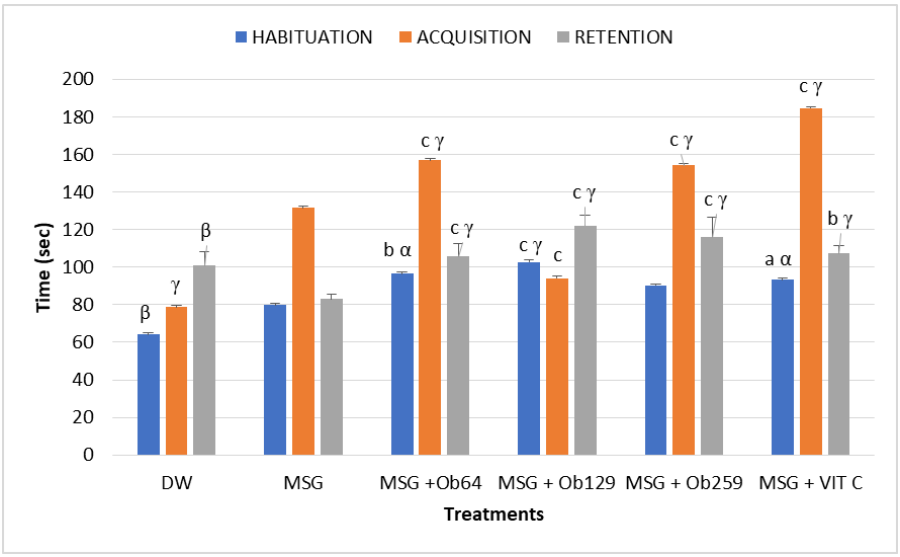

Figure 2 Effects of *Ocimum basilicum* on the time spent in the preferred arm in the T-maze

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, significantly different compared to distilled water treated group, $^{\alpha}P<0.05$, $^{\beta}P<0.01$, $^{\gamma}P<0.001$, significantly different compared to the significantly different compared to glutamate

monosodium treated group. DW, distilled water; Ob64, 129, 259 mg/kg *Ocimum basilicum*; Vit C, vitamin C; MSG, monosodium glutamate.

3.1.3. Effects of Ocimum basilicum on the number of entries in the preferred arm in the T-maze

Results show that, like vitamin C, *Ocimum basilicum* aqueous extracts significantly induced a significant increase in the number of entries into preferred arms [(F (27, 112) = 15; P <0,001)] from 6.80 ± 1.92(49.25%) and 5.80 ± 0.84 (56.71%) in the distilled water-treated group and monosodium glutamate treated group respectively to 13.40±1.52 (p<0.001) in the test group treated with the doses of 259 mg/kg *Ocimum basilicum* aqueous extracts. As the aqueous extracts, vitamin C, the positive control induced a significant increase in the number of entries in the preferred arms (p<0.001) (Figure 3)

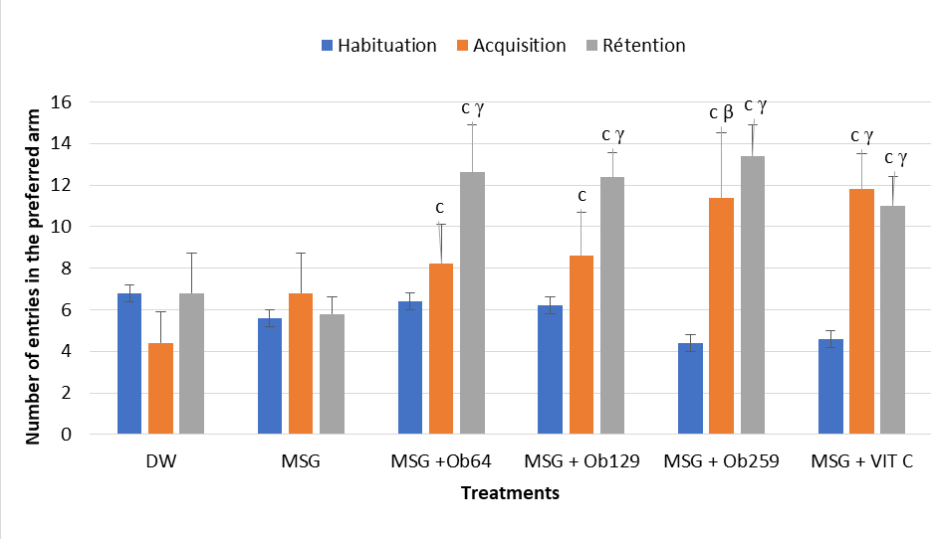

Figure 3 Effects of *Ocimum basilicum* on the number of entries in the preferred arm in the T-maze

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, ^{c}P<0.001, significantly different compared to distilled water treated group, $^{\beta}$P<0.01, $^{\gamma}$P<0.001, significantly different compared to glutamate monosodium treated group. DW, distilled water; Ob64, 129, 259 mg/kg *Ocimum basilicum*; Vit C, vitamin C; MSG, monosodium glutamate.

3.2. Effects of *Ocimum basilicum* aqueous extracts on exploratory behavior and locomotion in the open field test

As shown in figure 4, glutamate monosodium alter behavior in the open field. However, oral administration of *Ocimum basilicum* aqueous extracts produced a significant effect on mice spontaneous locomotors activities. One way ANOVA revealed a significant changes in the number of crossing [F (4, 25) = 17.47, P<0.001] (Fig. 4A) and the centre time [F (4, 25) = 22.73, P<0.001] (Fig. 4B) of the animals in the open field test. Similarly, vitamin C administration significantly ameliorates the exploratory activity of the animals in the open field test. The number of crossing was increased (p<0.001) from 66.60±8.08 for the MSG treated group to 99.20±5.54 (48.95%) and 91.60 ±13.76 (37.54%) respectively by the doses 259 mg/kg of the plant and vitamin C

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, ^{c}P<0.001, significantly different compared to distilled water treated group, $^{\gamma}$P<0.001, significantly different compared to glutamate monosodium treated group. DW, distilled water; Ob64, 129, 259 mg/kg *Ocimum basilicum*; Vit C, vitamin C; MSG, monosodium glutamate.

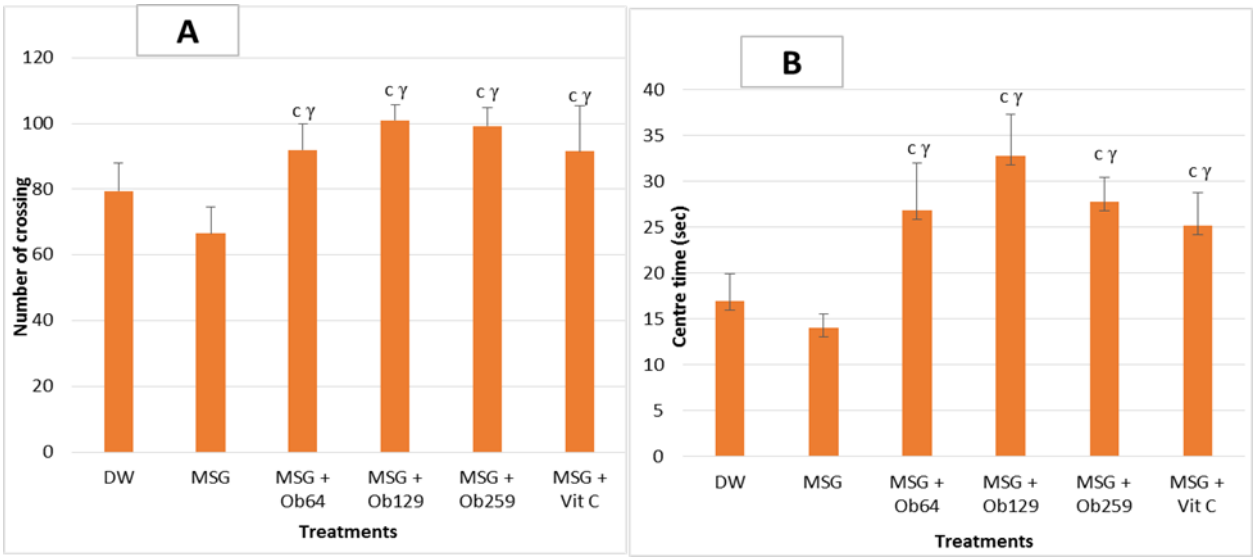

Figure 4 Effects of *Ocimum basilicum* on the number of crossing (A) and centre time (B) in the open field

3.3. Effect of *Ocimum basilicum* aqueous extracts on endogenous antioxidant pathway in neuroprotective properties

The neuroprotective effects of *Ocimum basilicum* aqueous extract administered at the animals influence endogenous levels of malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) in the brain of experimental animals as shown in Table 1. *Post hoc* analysis revealed a significant change of MDA level in the brain of glutamate monosodium treated mice as compared to distilled water treated group ($p<0.001$). This number varies from 0.87 ± 0.01 for distilled water-treated group to 1.57 ± 0.05 for glutamate monosodium-treated group. *Ocimum basilicum* aqueous extract treatment significant decrease the level of MDA in the brain of the treated mice [$F(5, 15) = 13.51$; $P < 0.001$]. As the plant, vitamin C treatment interestingly reverses the effects of monosodium glutamate.

Captivatingly, Table 2 shows that *Ocimum basilicum* aqueous extract treatment blocked the decrease catalase activity induced by glutamate monosodium. Analysis revealed a significant differences of *Ocimum basilicum* aqueous extract treatment [$F(5, 15) = 12.42$; $P < 0.001$], and glutamate monosodium treated group. As the plant, vitamin C treatment interestingly reverses the effects of monosodium glutamate.

In addition, as shown in Table 1, the one-way ANOVA revealed a main effect of *Ocimum basilicum* aqueous extract treatment [$F(6, 24) = 79.74$; $P < 0.001$] and glutamate monosodium treated group on reduced glutathione level in the brain of mice. Indeed, the glutathione level varies from 0.99±0.01 for monosodium treated group to 2.05±0.2 ($p<0.001$) for 259 mg/kg *Ocimum basilicum* aqueous extract treated group. As the plant, vitamin C treatment interestingly reverses the effects of monosodium glutamate.

Table 1 Effects of *Ocimum basilicum* on antioxidant enzymes and oxidative stress markers in the brain of glutamate monosodium treated mice

Treatments	MDA (µmol/g)	CAT (mmol H_2O_2/min/mg)	GSH (µmol/g)	SOD (U/min/mg)
DW	0.87±0.01$^{\gamma}$	86.98 ± 7.2$^{\gamma}$	1.86±0.10$^{\gamma}$	2.23±0.24$^{\alpha}$
MSG	1.57±0.05	47.98± 16.1	0.99 ± 0.01	1.31±2.63
MSG +Ob64	0.94±0.02$^{\beta}$	90.07± 1.9$^{\beta}$	1.56 ± 0.05$^{\gamma}$	2.36±0.38$^{\alpha}$
MSG + Ob129	0.90±0.02$^{\gamma}$	95.98 ± 3.3$^{\gamma}$	1.99± 0.08$^{\gamma}$	2.71±0.26$^{a\gamma}$
MSG + Ob 259	0.86±0.01$^{\gamma}$	95.80 ±1 2.0$^{\gamma}$	2.05±0. 20$^{\gamma}$	3.28±0.14$^{b\gamma}$
MSG + Vit C	0.84±0.02$^{\gamma}$	90.25 ± 12.5$^{\gamma}$	1.95 ± 0.13$^{\gamma}$	3.33±0.50$^{b\gamma}$

The superoxide dismutase activity is also affected by monosodium glutamate administration. However, *Ocimum basilicum* aqueous extract treatment significantly [F (5, 15) = 18.27; P < 0.001] reverse this effect. The activity of this enzyme varies from 1.48 ±0.33 for monosodium treated group to 3.28±0.14 ($p<0.001$) for 259 mg/kg *Ocimum basilicum* aqueous extract treated group. As the plant, vitamin C treatment reverses the effects of monosodium glutamate.

Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by one-way ANOVA, followed by Tukey's (HSD) multiple comparison test, $^{a}p<0.05$, $^{b}p<0.01$, significantly different compared to distilled water treated group, $^{\alpha}p<0.05$, $^{\beta}P<0.01$, $^{\gamma}P<0.001$, significantly different compared to glutamate monosodium treated group. DW, distilled water; Ob64, 129, 259 mg/kg *Ocimum basilicum*; Vit C, vitamin C; MSG, monosodium glutamate.

4. Discussion

The T-maze test aimed at evaluating memory functions is based on spatio-temporal learning tasks: ability to familiarize oneself with an environment, to orient oneself and to perform different memory tasks [18]. It is observed in mice treated with monosodium glutamate a cognitive deficit, mice show progressive learning impairment. A T-maze test is an advance a cognitive tasks sensitive to frontostriatal and hippocampal function [19]. *Ocimum basilicum* co-administered with monosodium glutamate treated mice showed a decrease in the latency to enter the preferred arm. The decrease in latency time indicates an improvement in memory [20]. The significant increase in the number of entries and time spent in the preferred arm reflects memory function enhancement [21, 22]. These results show the antagonistic effects of *Ocimum basilicum* to the deleterious action of glutamate, which could be due to the presence of certain compounds in the plant that would possess neuroprotective properties. Moreover, the increase in the number of entries, the time spent in the preferred arm, and the decrease in the number of visits to the discriminated arm suggest an increase in exploration and therefore in memory faculties [22].

Open field test was cast-off to evaluate individual-specific behavior responses when introduced on a novel environment [23]. This test is performed a prior to any drug treatment, with the objective of perceiving individual partiality in the animal behavior that could affect cognitive performance. Locomotors activities can influence exploratory drive [24] and therefore, it is required to assure that, freely of housing cage, group and/or individual differences, in each series the control animals and the test animals would not display significant differences on mean velocity and distance covered. In etude, open field test is used to assess exploration behavior and locomotors activity in mice in response to a novel environment [25]. As in T-maze, monosodium treated mice presented altered behavior. In plant extract treated mice, an increase in the number of lines crossed and time spent in the centre of the experimental dispositive were observed. These results indicate the increase in the level of exploration, locomotor activity and improved retention. According to these results, which are in agreement with those of Botton and collaborators [25], it can suggested that the of *Ocimum basilicum* have memory improvements properties; which would be mediated by the regulation of glutamate neurotransmitter level in cerebral cortex and hippocampus [25].

Oxidative stress is a typical feature in a number of neurodegenerative conditions [26]. The brain is particularly susceptible to oxidative stress damage because of its high rate of high content of polyunsaturated fatty acids, oxidative metabolic activity, moderately low antioxidant capability, and the abundance of redox-active transition metal ions and nonreplicating nature of its neuronal cells [27]. Brain aging is a factor that leads to the gradual loss of memory and learning disability [28]. Reactive oxygen species and oxidative stress are associated with brain aging observed in various neurovegetative diseases such as Alzheimer's disease. In order to scavenge reactive oxygen species, different protection systems are present in the brain, such as non-enzymatic (glutathione and uric acid), enzymatic (superoxide dismutase, glutathione peroxidase and catalase) and dietary antioxidants. If reactive oxygen species are not effectively removed, they can cause peroxidation of proteins, cell membrane phospholipids and DNA [29]. Hence, antioxidants are the first line of defense against free radical injury and are critical for keeping health. Due to this, antioxidant supplements and antioxidants enclosing diet are being recognized as an important means of improving free radical protection [30].

In the current study, treatment with *Ocimum basilicum* significantly attenuated the glutamate induced excitation and oxidative stress in mice. Co-administration of *Ocimum basilicum* to and monosodium glutamate to animals showed significantly increased levels of GSH, CAT, SOD and decreased levels of lipid peroxidation when compared with monosodium glutamate only treated mice, these results corroborate the earlier results indicating antioxidant property of *Ocimum basilicum* [31]. Therefore, these results indicate use of *Ocimum basilicum* leaves may protect the body from free radical injury. Moreover, knowing that a decrease in the level of anxiety improves memory capacities and that a reduction of stress is favorable for a decent functioning of the memory [21], we can conclude that *Ocimum basilicum* leaves would inhibit the anxiolytic effects while optimizing learning and memorizing.

5. Conclusion

In conclusion, results provide the evidence that *Ocimum basilicum* aqueous extract exerts neuroprotective property in mice. It also increased the brain reduce glutathione concentration and attenuated the malondialdehyde concentration. It is also noticed that the plant aqueous extract increased the activities of catalase and superoxide dismutase. These findings demonstrate that *Ocimum basilicum* improve learning and memory by protecting brain against toxic excitation induced by glutamate. These might involve by an action on endogenous antioxidants system.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

The protocols were performed in concordance with the International Guide for the Care and Use of Laboratory Animal (National Institute of Health; publication No. 85-23, revised 1996) and the Cameroon National Ethical Committee, Yaoundé (No. FW-IRB00001954).

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