

Ocimum gratissimum* leaves aqueous extract can minimize middle cerebral artery occlusion and reperfusion-induced injury in rat's brain*Abstract**

Background and Aim: *Ocimum gratissimum* is widely used in traditional medicine for its good antioxidant and anti-inflammatory activity. Stroke is an important factor of disability and death worldwide causing brain injury through oxidative stress and inflammation. The present study aimed to evaluate the neuroprotective effect of *Ocimum gratissimum* on middle cerebral artery occlusion and reperfusion-induced brain injury in rat.

Experimental Procedure: The rats (48) were divided into 8 groups of 6 animals each and treated as followed: 3 groups receiving distilled water (10 mL/kg); one piracetam (250 mg/kg) and 4 groups receiving the plant extract (30, 60, 120 and 240 mg/kg) for 3 days. On the fourth day, ischemia by middle cerebral artery occlusion and reperfusion (MCAO) surgery was performed on the groups receiving piracetam and extract. As for the 3 groups receiving distilled water, one was not operated; one was operated without MCAO (sham operated) while the last one was operated with MCAO. The temperature, neurological function through behavioral tests, heat sensibility, and open arena tests were assessed. Inflammatory and oxidative stress markers were analyzed in the region of the brain affected by MCAO.

Results and Conclusion: Three days pretreatment with the plant extract prevented the drop of temperature and heat sensibility, reduced the neurological score and associated anxiety-like behavior. The extract also prevented inflammation and oxidative stress induced by MCAO in

the brain. All these observations suggest that *Ocimum gratissimum* by its antioxidant and anti-inflammatory potencies can protect the brain against ischemic stroke-induced brain injury.

Keywords: *Ocimum gratissimum*, stroke, anti-inflammatory, antioxidant.

Abbreviations:

BBB: blood-brain barrier

ROS: oxygen radical species

MCAO: middle cerebral artery occlusion

NC: normal control

NegC: negative control

Ip: intraperitoneal

1. Introduction

Cerebral ischemia, among the most important causes of disability and death worldwide, is increasing sharply [1]. “The brain is protected from systemic toxins under normal physiological conditions by the blood-brain barrier (BBB). During cerebral ischemia, oxygen radical species (ROS) production and action lead to the breaking down of BBB with associated infiltration of inflammatory mediators” [2]. Oxidative stress and inflammation play an important role in cell death during ischemia and the close relationship between the two phenomena is now well described [3]. It is well known that in human, damages following stroke occur within minutes, and patients who survived are disabled for their daily work because of paralysis, impairment of memory, thinking, talking and moving. Therefore, preventive treatment for patients with stroke risk factors such as diabetes and hypertension is of interest. In recent years, interest in medicinal plants as a potential source in treatment of

ischemia–reperfusion has increased [4]. Many medicinal plants have proved their effectiveness to protect the brain against ischemic injuries, most of them because of their antioxidant and/or anti-inflammatory activity [4,5]. “Classes of drug under investigation for the treatment of acute ischemic stroke include those that promote early cerebral reperfusion, neuroprotective agents, and drugs to reduce cerebral edema. Early thrombolytic therapy remains under scrutiny because it has a better outcome, but also associated with an increase in mortality or morbidity. Benefit from antithrombotic therapy also remains unproven, although one recent pilot study with low-molecular-weight heparin reported improved outcome” [4,5]. “Of all drugs tested, piracetam showed a promised activity on acute ischemic models in rats. Indeed, piracetam has neuroprotective and antithrombotic effects that may help to reduce death and disability in people with acute stroke. The exact mechanism of action of piracetam is not known and several different effects have been described: a neuroprotective effect and antithrombotic effect (improvement of microcirculation, decrease of platelet aggregation)” [4,5].

Ocimum gratissimum used in the present study is an aromatic perennial herb of 1-3 meters tall, stem erect widely used throughout the western part of Africa as a febrifuge, antimalarial and anticonvulsant in traditional medicine. The plant is known for its good antioxidant and anti-inflammatory activity [6,7]. “The leaves are rich in essential oil where thirty-seven compounds were identified, with eugenol (55.6%) as the major component” [8]. Indeed, eugenol has proved strong antioxidant and anti-inflammatory activities in models of inflammation and oxidative stress [9]. Moreover, the ethanolic extract from the leaves of *Ocimum gratissimum* had proved neuroprotective activity [10]. The present study was therefore carried out to assess the neuroprotective effects of *Ocimum gratissimum* leaves aqueous extract on ischemia by middle cerebral artery occlusion (MCAO) and reperfusion injuries in rat and explore some mechanism of action.

2. MATERIALS AND METHODS

2.1 Plant collection and extraction

The whole plant of *Ocimum gratissimum* has been harvested in 2018 at Fouban (west region of Cameroun) and identified at the National Herbarium of Cameroon where a voucher specimen number 5817/SRFCAM was deposited. The leaves were collected, washed with tap water, dry in an oven at 45°C, and ground. The powder (40 g) was mixed with 400 mL of distilled water, boiled for 20 min, and cold down. The mixture was then filtered with Whatman paper number 3 and, after evaporation in an oven (45°C), 3.32 g of extract were obtained, yield 8.3%. Phytochemical analysis of the extract was performed. The chemical classes compounds as alkaloids, flavonoids, saponins, polyphenols, tannins, and triterpenes were assessed according to the procedure described by Odebiyi and Sofowora [11]

Animals were male Wistar rats, 6 to 8 weeks old, weighting between 120 - 140 g. Rats were raised in the animal house of the laboratory of Animal Physiology in plastic cages (5 rats per cage) under standard light (12-hour day/night natural cycle) and temperature ($25 \pm 2^\circ$ C) with free access to a standard animal diet and tap water.

2.2 Animals grouping, treatment and surgical procedure

The rats (48) were divided into 8 groups of 6 animals each and treated as followed: three groups receiving distilled water (10 mL/kg), one group receiving piracetam (250 mg/kg), and four groups receiving the plant extract (30, 60, 120 and 240 mg/kg, respectively from primary studies) daily at the same time during 3 days. On the fourth day, ischemia by middle cerebral artery occlusion (MCAO) surgery was performed on the groups receiving piracetam (Pi) and extract (E30, E60, E120, E240). As for the 3 groups receiving distilled water, one was not operated (NC), one was operated without MCAO (sham) while the last one was operated with MCAO (NegC). Focal ischemia was performed according to a method previously described

[12]. Briefly, animals were anesthetized with 20 mg/kg diazepam intraperitoneal (ip) followed by 70 mg/kg ketamine (ip). A midline incision was made in the neck region and the left carotid artery was exposed; care being taken to preserve the nerve. The carotid artery was ligated without tiding at two points from its first junction: toward the head (distal) and the heart (proximal) using cotton thread (6-0; Docol Corp, Redlands, CA, USA). A 3-0 silicone-coated nylon suture was introduced through the external carotid artery stump. The occlusion was advanced into the internal carotid artery 20-22 mm beyond the carotid bifurcation until mild resistance indicated that the tip was lodged in the anterior cerebral artery and blocked the blood flow to the middle cerebral artery. The distal ligature was then tied to maintain the nylon in place. Reperfusion was started by withdrawing the suture after 90 minutes of ischemia. Core temperature was measure three times: before surgery, just (90 minutes from MCAO) after and 24 hours after ischemia induction and reperfusion with a medicinal electric thermometer introduced in the rectum of the rat.

2.3 Evaluation of neurological functions

Immediately after reperfusion, the wound was closed with resorveable traid and treated with penicillin pomade. Twelve hours after, a variety of motor, sensory, reflex and balance responses were evaluated according to the global neurological scoring scale [13]. Briefly, animals underwent a variety of tests.

2.3.1. Spontaneous Activity

“The animal was observed for 5 minutes in its normal environment (cage). The rat’s activity was assessed by its ability to approach all four walls of the cage. Scores indicate the following: 3, rat moved around, explored the environment, and approached at least three walls of the cage; 2, slightly affected rat moved about in the cage but did not approach all sides and hesitated to move, although it eventually reached at least one upper rim of the cage; 1,

severely affected rat did not rise up at all and barely moved in the cage; and 0, rat did not move at all” [28].

2.3.2. Symmetry in the Movement of Four Limbs

“The rat was held in the air by the tail to observe symmetry in the movement of the four limbs. Scores indicate the following: 3, all four limbs extended symmetrically; 2, limbs on left side extended less or more slowly than those on the right; 1, limbs on left side showed minimal movement; and 0, forelimb on left side did not move at all” [28].

2.3.3. Forepaw Outstretching

“The rat was brought up to the edge of the table and made to walk on forelimbs while being held by the tail. Symmetry in the outstretching of both forelimbs was observed while the rat reached the table and the hindlimbs were kept in the air. Scores indicate the following: 3, both forelimbs were outstretched, and the rat walked symmetrically on forepaws; 2, left side outstretched less than the right, and forepaw walking was impaired; 1, left forelimb moved minimally; and 0, left forelimb did not move” [28].

2.3.4. Climbing

“The rat was placed on the wall of a wire cage. Normally the rat uses all four limbs to climb up the wall. When the rat was removed from the wire cage by pulling it off by the tail, the strength of attachment was noted. Scores indicate the following: 3, rat climbed easily and gripped tightly to the wire; 2, left side was impaired while climbing or did not grip as hard as the right side; and 1, rat failed to climb or tended to circle instead of climbing” [28].

2.3.5. Body Proprioception

The rat was touched with a blunt stick on each side of the body, and the reaction to the stimulus was observed. Scores indicate the following: 3, rat reacted by turning head and was equally startled by the stimulus on both sides; 2, rat reacted slowly to stimulus on left side; and 1, rat did not respond to the stimulus placed on the left side.

2.3.6. Response to Vibrissae Touch

“A blunt stick was brushed against the vibrissae on each side; the stick was moved toward the whiskers from the rear of the animal to avoid entering the visual fields. Scores indicate the following: 3, rat reacted by turning head or was equally startled by the stimulus on both sides; 2, rat reacted slowly to stimulus on left side; and 1, rat did not respond to stimulus on the left side” [28].

The score given to each rat at the completion of the evaluation is the summation of all six individual test scores that was then divided to 6. The minimum neurological score was 2 and the maximum was 5.

The next day, the open field test was performed [14] on each animal individually; making sure that the arena was cleaned with ethanol 70° after each passage. The last test was the pain sensibility evaluation [15]. Briefly, the tail of each animal was introduced into hot water ($55 \pm 0.5^\circ\text{C}$) and the time before removing the tail was recorded with a chronometer. The maximal time was fixed at 30 seconds to prevent cell destruction [16]

2.4 Evaluation of some biochemical parameters

After neurological function evaluation, animals were sacrificed under anesthesia, blood was collected into dry *Eppendorf* tubes; and brains were removed, rinsed in ice-cold 0.9% sodium chloride solution, blotted with filter paper weighed and homogenized in Tris (HCl 50 mM; KCl 150 mM; pH 7.4) buffer to give 20% homogenates. The homogenates were centrifuged at

10,000 rpm for 15 minutes at 4°C and stored at -20°C. Biochemical parameters assessment was performed using commercial diagnostic kits (Quantikine, France) for some inflammatory markers (IL1- β , TNF- α , IL-6, INF- γ). Reduced Glutathione (GSH) and the end product of lipid peroxidation, Malone Dialdeide (MDA) were determined [17, 18].

2.5 Statistical analysis

Results are expressed as the mean \pm standard error on the mean (SEM). The difference between the groups was compared using one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test. A value of $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Phytochemical screening

The phytochemical screening of *Ocimum gratissimum* aqueous extract revealed the presence of all the groups of compounds tested, namely alkaloids, flavonoids, saponins, polyphenols, tannins and triterpenes.

3.2 Effect of *Ocimum gratissimum* aqueous extract on the neurological score

Figure 1 represents the effects of *O. gratissimum* aqueous extract on the neurological score of rats 12 hours after MCAO ischemia and reperfusion. Brain ischemia led to a significant decrease of 46.66% ($p < 0.001$) in the neurological score of MCAO ischemic rats pretreated with distilled water as compared to both normal and sham-operated rats. The pretreatment with the extract (120 mg/kg and 240 mg/kg) as well as piracetam significantly prevented that decrease. The neurological scores of these 3 groups of animals were by 56.25 % ($p < 0.01$) for both groups of extract and by 62.5 % ($p < 0.001$) with piracetam higher as compared to MCAO ischemic rats without pretreatment.

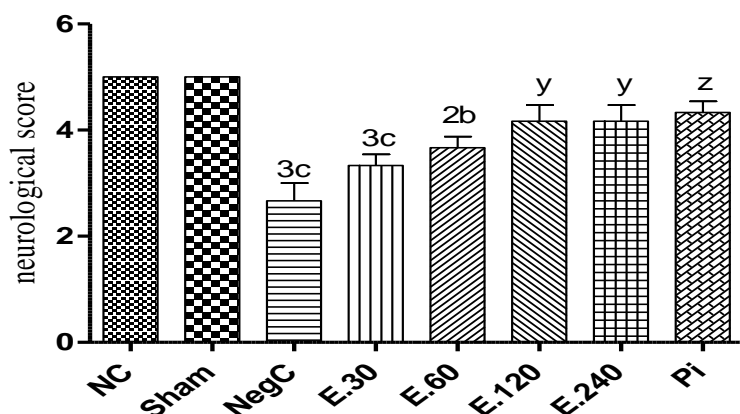


Figure 1: Effects of aqueous leaf extract of *Ocimum gratissimum* on the neurological score in focal ischemia-reperfusion rats.

Each bar represents the mean \pm SEM of the group, n = 6. ²p < 0.01 and ³p < 0.001 significant difference compared to the normal control; ^bp < 0.01 and ^cp < 0.001 significant difference compared to sham; ^yp < 0.01 and ^zp < 0.001 significant difference compared to the negative control. NC: Normal control; negC: Negative control; Pi: Positive control treated with piracetam (250 mg / kg); E.30-E.240: Test groups treated at doses of 30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg/kg of *O. gratissimum* extract; i: ischemia; sham: animals operated without MCAO ischemia.

3.3 Effect of *Ocimum gratissimum* on the thermoregulation

Figure 2 represents the variation of the temperature before the surgery, 90 minutes and 24 hours after MCAO ischemia and reperfusion. The MCAO ischemia resulted in a drop of temperature of 7.73 % (p < 0,001) and 7.41 % (p < 0,001) respectively 90 minutes and 24 hours after, as compared to the temperature before ischemia. The pretreatment with *O. gratissimum* aqueous extract (30 mg/kg) did not prevent the drop of the temperature due to MCAO ischemia 90 minutes after reperfusion, and even 24 hours later, the temperature remained significantly low as compared the initial temperature (before MCAO). Nonetheless,

from the dose of 60 mg/kg upward, the pretreatment with the plant extract completely prevented the drop of the temperature induced by MCAO ischemia, and even increased the temperature 24 hours after reperfusion at the dose of 24 mg/kg by 3.04% as compared with the initial temperature. Piracetam acted almost the same and increased the temperature 24 hours after ischemia by 3.16%.

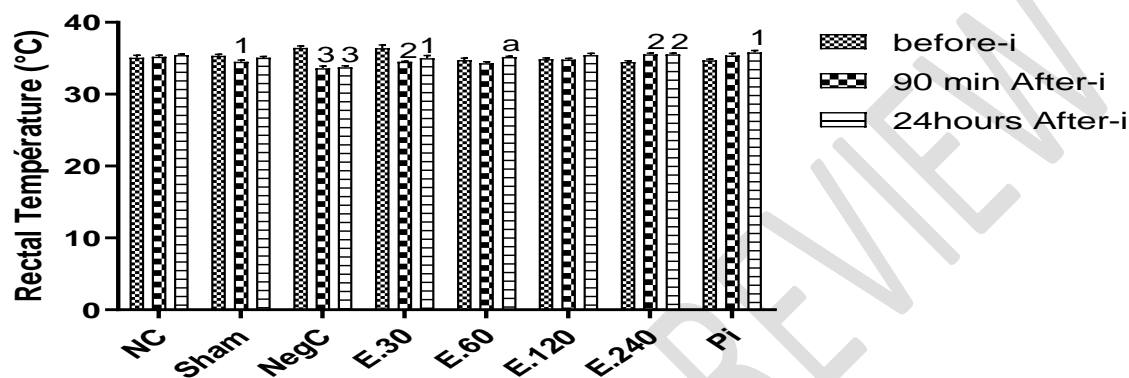


Figure 2: Effects of aqueous leaf extract of *O. gratissimum* on body temperature of focal ischemia-reperfusion rats.

Each bar represents the mean \pm SEM of the group, n = 6. ¹p <0.05; ²p <0.01 and ³p <0.001 significant difference compared to animals before induction of ischemia (before-i); ^ap <0.05 significant difference compared to animals 90 minutes after ischemia; NC: Normal control; negC: Negative control; Pi: Positive control treated with piracetam (250 mg/kg); E.30-E.240: Test groups treated at doses of 30 mg/kg, 60 mg/kg, 120 mg/ kg and 240 mg / kg of *O. gratissimum* extract; i: ischemia; sham: animals operated without MCAO ischemia.

3.4 Effect of *Ocimum gratissimum* on heat sensibility

The effect of *O. gratissimum* extract on heat sensibility measured with the time spends before removing the tail from hot water is illustrated in figure 3. On animals with MCAO ischemia pretreated with distilled water the time was significantly increased by 188.23 % (p < 0.001) as

compared to normal control. With the extract (30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg/kg) pretreatment, that time was significantly decreased, and the values were respectively of 28.57 % ($p < 0.001$), of 30.61 % ($p < 0.001$), of 42.85 % ($p < 0.001$) and of 53.06% ($p < 0.001$) lower as compared to MCAO ischemia pretreated with distilled water. The decrease induced by the piracetam was of 59.18 % ($p < 0.001$) as compared with MCAO ischemia pretreated with distilled water.

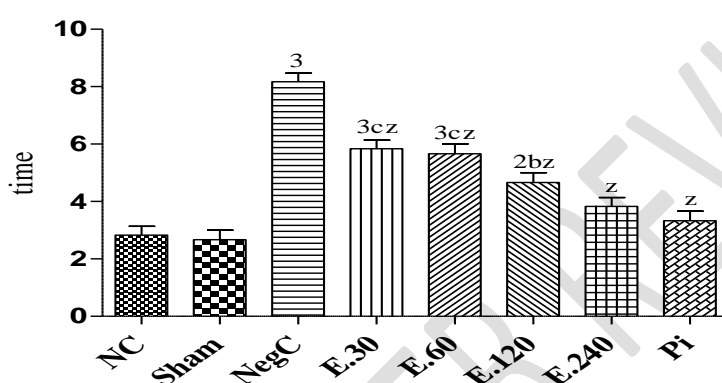


Figure 3: Effects of aqueous leaf extract of *O. gratissimum* on heat sensitivity in focal ischemia-reperfusion rats.

Each bar represents the mean \pm ESM of the group, $n = 6$. ² $p < 0.01$ and ³ $p < 0.001$ significant difference compared to the normal control; ^b $p < 0.01$ and ^c $p < 0.001$ significant difference compared to sham; ^z $p < 0.001$ significant difference compared to the negative control. NC: Normal control; negC: Negative control; Pi: Positive control treated with piracetam (250 mg/kg); E.30-E.240: Test groups treated at doses of 30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg / kg of *O. gratissimum* extract; i: ischemia; sham: animals operated without MCAO ischemia.

3.5 Effect of *Ocimum gratissimum* on the open field arena parameters

The effect of *O. gratissimum* on open field arena parameters is illustrated in figure 4. The MCAO ischemia induced a significant decrease of 50.98 % ($p < 0.001$) on crossing as compared to normal control group (fig. 4A). The pretreatment with the plant extract (30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg/kg) or piracetam significantly prevented that decrease. The values were increased by 84 % ($p < 0.001$), by 176 % ($p < 0.001$), by 244 % ($p < 0.001$), by 352 % ($p < 0.001$) and by 336 % ($p < 0.001$) respectively with the extract (30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg/kg) or piracetam pretreatment as compared with MCAO ischemia pretreated with distilled water group.

The number of grooming was significantly decreased by 60 % ($p < 0.01$) in MCAO ischemia pretreated with distilled water group (fig 4B). The pretreatment with the plant extract (240 mg/kg) or piracetam prevented the decrease by 6.25 % ($p < 0.05$) and by 17.64 % ($p < 0.01$) in the number of grooming respectively as compared to MCAO ischemia pretreated with distilled water group.

The time spent in the center was significantly reduced by 76.08 % ($p < 0.001$) in MCAO ischemia pretreated with distilled water group as compared with normal control group. The pretreatment with the plant extract (60 mg/kg, 120 mg/kg and 240 mg/kg) prevented that reduction of that time. The values were by 231.81 % ($p < 0.001$), by 322.72 % ($p < 0.001$), by 313.63 % ($p < 0.001$) and by 345.45 % ($p < 0.001$) respectively greater than those in MCAO i schemia pretreated with distilled water group (fig.4C).

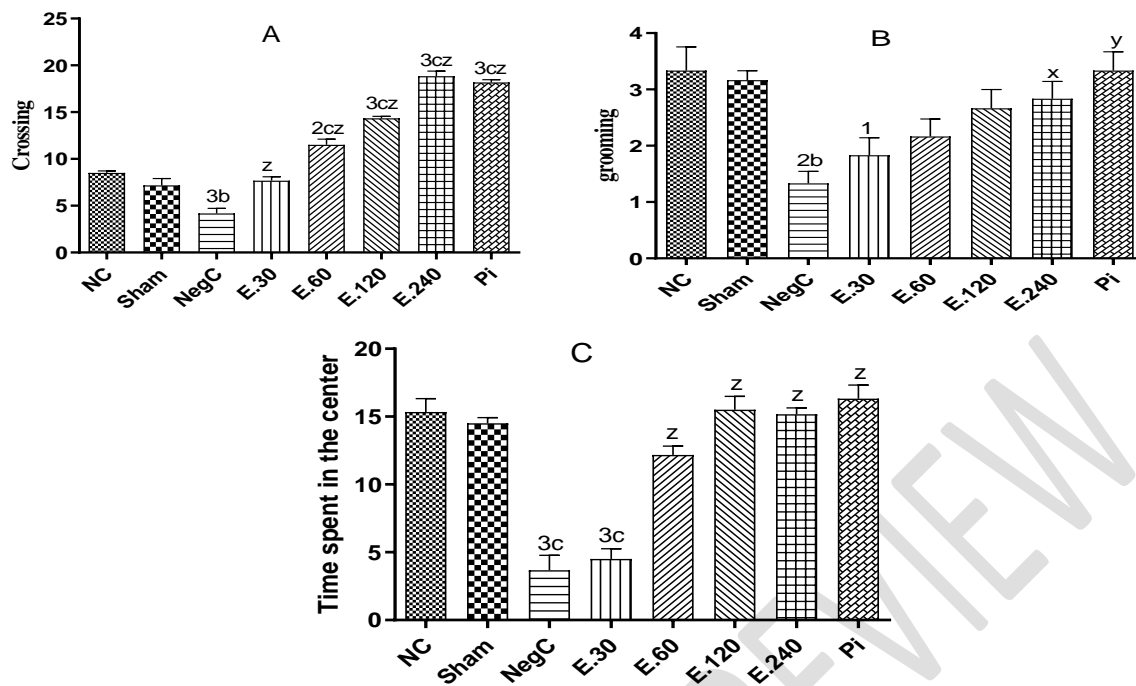


Figure 4: Effects of the aqueous leaf extract of *Ocimum gratissimum* on crossing (A), grooming (B) and the time spent in the center (C).

Each bar represents the mean \pm SEM of the group, $n = 6$. ¹ $p < 0.05$, ² $p < 0.01$ and ³ $p < 0.001$ compared to the normal control; ^b $p < 0.01$ and ^c $p < 0.001$ significant difference compared to sham; ^x $p < 0.05$, ^y $p < 0.01$ and ^z $p < 0.001$ significant difference compared to the Negative control. NC: Normal control; negC: Negative control; Pi: Positive control treated with piracetam (250 mg / kg); E.30-E.240: Test groups treated at doses of 30 mg / kg, 60 mg / kg, 120 mg / kg and 240 mg / kg of *O. gratissimum* extract; i: ischemia; sham: animals operated without MCAO ischemia.

3.6 Effect of *Ocimum gratissimum* aqueous extract on oxidative stress and inflammatory markers in brain

The effect of *O. gratissimum* aqueous extract on oxidative status in the brain of the rats after MCAO ischemia and reperfusion is summarized in Table 1. The two tested markers were malondialdehyde (MDA) and reduced glutathione (GSH). It is shown a significant increase of 140.74% ($p < 0.001$) in the among of MDA in the brain of MCAO ischemia pretreated with distilled water group as compared to normal rats. The pretreatment with the extract (120 mg/kg and 240 mg/kg) or piracetam significantly prevented that increase. The values were of 34.17 %, of 51.98 % and 56.41 % ($p < 0.001$) respectively lower as compared with the MCAO ischemia pretreated with distilled water group.

The among of GSH was significantly reduced by 43.29 % ($p < 0.001$) in MCAO ischemia pretreated with distilled water group as compared to normal control. The pretreatment the extract with (30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg/kg) or piracetam prevented that decrease. The values were by 23.37 % ($p < 0.01$), by 39.23 % ($p < 0.001$), by 41.35 % ($p < 0.001$) and by 73.51 % ($p < 0.001$) respectively with the extract and by 70.67 % ($p < 0.001$) with piracetam higher as compared with the MCAO ischemia pretreated with distilled water group.

Table 2 represents the effects of *O. gratissimum* on some inflammatory markers in the brain of rats after MCAO ischemia and reperfusion. It is shown MCAO ischemia and reperfusion led to an increase of 73.28 % ($p < 0.001$) in tumor necrosis factor-alpha (TNF- α) as compared to normal control group. The pretreatment with the plant extract (30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg/kg) or piracetam significantly ($p < 0,001$) prevented that increase. The values were of 13.98 %, of 30.11 %, of 41.10 %, of 41.57 % and of 40.43 %, respectively greater than those of the MCAO ischemia pretreated with distilled water group.

The amount of interferon-gamma (INF- γ) was also increased by 92.54 % ($p < 0.001$) in the brain after MCAO ischemia and reperfusion as compared to normal rats. The

pretreatment with the plant extract (30 mg/kg, 60 mg/kg, 120 mg/kg and 240 mg/kg) or piracetam significantly ($p < 0.001$) prevented that increase. The values were of 10.92 %, of 15.34 %, of 34.65 %, of 46.26 % and of 45.76 %, respectively higher than those of MCAO ischemia pretreated with distilled water group.

The amount of interleukin 1-beta (IL1- β) was significantly ($p < 0.001$) increased by 137.96 % in MCAO ischemia pretreated with distilled water group as compared to normal control group. The pretreatment with the plant extract 30 mg/kg, 60 mg/kg, 120 mg/kg, 240 mg/k or piracetam prevented significantly ($p < 0.001$) that increase. The values were by 15.71 %, by 46.34 %, by 51.49 %, by 56.49 % and by 55.69 %, respectively greater as compared to MCAO ischemia pretreated with distilled water group.

The amount of interleukine-6 (IL-6) was significantly ($p < 0.001$) increased of 5.90 % after MCAO ischemia and reperfusion as compared to normal rats. The pretreatment with plant extract (30 mg/kg, 60 mg/kg, 120 mg/kg, and 240 mg/kg) or piracetam significantly prevented that increase. The values were of 19.35 % ($p < 0.05$), 24.19 % ($p < 0.01$), 26.42 % ($p < 0.01$), 28.31 % ($p < 0.001$) and 28.38 % ($p < 0.001$) respectively higher than those of MCAO ischemia pretreated with distilled water group.

Table 1: Effects of aqueous extract of *Ocimum gratissimum* on oxidative stress markers in focal ischemia-reperfusion rats

Parameters	MDA (nmol/g of tissue)	GSH (mol/g of tissue)
NC	148.50±0.39	207.50±1.83
Sham	154.50±0.53	208.50±6.33
NegC	357.50±0.52 ³	117.66±6.33 ³
E.30	358.33±0.41 ^{3c}	145.16±11.77 ^{3cy}
E.60	331.83±0.69 ^{3c}	163.83±8.16 ^{3cz}
E.120	235.33±0.63 ^{3cz}	166.33±20.22 ^{3cz}
E.240	171.66±0.61 ^z	204.16±5.83 ^z
Pi	155.83±0.49 ^z	200.83±3.94 ^z

Each value represents the mean ± ESM of the group, n = 6.3p <0.001 significant difference compared to the normal control; cp <0.001 significant difference compared to sham; yp <0.01 and zp <0.001 significant difference compared to the sick control. NC: Normal control; negC: Negative control; Pi: Positive control treated with piracetam (250 mg / kg); E.30-E.240: Test

groups treated at doses of 30 mg / kg, 60 mg / kg, 120 mg / kg and 240 mg / kg of *O. gratissimum* extract; i: ischemia; sham: animals operated without MCAO ischemia.

Table 2: Effects of aqueous extract of *Ocimum gratissimum* on markers of cerebral inflammation in rats undergoing focal ischemia-reperfusion

Parameters	TNF- α (pg/mg)	INFY (pg/mg)	IL1- β (pg/mg)	IL-6 (pg/mg)
NC	202.16 \pm 1.88	187.83 \pm 2.77	175.16 \pm 6.55	344.83 \pm 9.88
Sham	202.16 \pm 1.88	191.83 \pm 3.88	174.33 \pm 3.44	369.83 \pm 17.44
NegC	350.33 \pm 4.66 ³	361.66 \pm 9.44 ³	416.83 \pm 6.16 ³	485.66 \pm 6.11 ³
E.30	301.33 \pm 7.66 ^{3cz}	322.16 \pm 10.22 ^{3cz}	351.33 \pm 2.66 ^{3cz}	391.66 \pm 88.55 ^x
E.60	244.83 \pm 13.61 ^{3cz}	306.16 \pm 1.88 ^{3cz}	223.66 \pm 15.88 ^{3cz}	368.16 \pm 7.83 ^y
E.120	206.33 \pm 2.77 ^z	236.33 \pm 4.66 ^{3cz}	202.16 \pm 7.16 ^{3cz}	357.33 \pm 9.66 ^y
E.240	204.66 \pm 3.44 ^z	194.33 \pm 7.33 ^z	181.33 \pm 5.66 ^z	348.16 \pm 5.55 ^z
Pi	208.66 \pm 4.22 ^z	196.16 \pm 5.77 ^z	184.66 \pm 4.66 ^z	347.83 \pm 8.88 ^z

Each value represents the mean \pm ESM of the group, n = 6. 3p <0.001 significant difference compared to the normal control; cp <0.001 significant difference compared to sham; xp <0.05, yp <0.01 and zp <0.001 significant difference compared to the sick control. NC: Normal control; negC: Negative control; Pi: Positive control treated with piracetam (250 mg / kg); E.30-E.240: Test groups treated at doses of 30 mg / kg, 60 mg / kg, 120 mg / kg and 240

mg / kg of *O. gratissimum* extract; i: ischemia; sham: animals operated without MCAO ischemia.

4. DISCUSSION

Motor, sensory, reflex and balance responses deficit were observed on the rats of the present study after MCAO and reperfusion. These impairments are among those observed in ischemic stroke. This resulted in the present study to a significant reduction in the neurological score as well as the heat sensibility after MCAO ischemic stroke. All this was associated with anxiety that was manifested with the decrease of the time spent in the center, the crossing and the grooming; as compared to the normal rats. The significant reduction of all these impairments with the pretreatment with *O. gratissimum* aqueous extract or piracetam suggests a neuroprotective activity of the plant extract. Similar observations were made with *Thymus vulgaris* ethanolic extract on injury induced by transient global cerebral ischemia and reperfusion in rat [19]. This finding confirms the neuroprotective activity of *Ocimum gratissimum* formally evaluated [20] with ethanolic extract from the leaves against focal ischemia and reperfusion-induced cerebral injury. The neuroprotective activity of *O. gratissimum* ethanolic extract from the leaves was also evaluated [10] on monosodium glutamate-induced oxidative stress in developing wistar rat cerebellum. Nonetheless, aqueous extract is the one most used by the local population and the anti-inflammatory as well as the antioxidant activity of that extract is not yet completely explored. In the present study, however, to elucidate the probable mechanism of action of the *O. gratissimum* aqueous extract, both anti-inflammatory and antioxidant activity were investigated.

The results of the present study revealed that the oxidative stress markers parameters, malondialdehyde (MDA) was significantly increased and glutathione (GSH) was reduced on ischemic rats as compared to normal rats. It is well established that oxidative stress is among

the first and fundamental mechanisms of cell damage following cerebral ischemia [3]. “During brain ischemia/reperfusion, multiple detrimental processes take place, including overproduction of oxidants, inactivation of detoxification systems, and consumption of antioxidants” [21]. “These changes disrupt the normal antioxidative defense ability of brain tissue” [21]. “The pretreatment with the plant extract prevented the increase of MDA (one of the final products of lipid peroxidation) observed in ischemic rats. Lipid peroxides derived from polyunsaturated fatty acids are unstable and decompose to form a complex series of compounds such as MDA. Cerebral ischemia can cause a significant amount of MDA formation in the ischemic hemisphere” [21]. The fact that from the dose of 120 mg/kg the plant extract has significantly prevented that increase suggests that its antioxidant activity may pass through lipid peroxidation inhibition. Similar results were obtained [22] with *Lavandula officinalis* ethanolic extract on blood-brain barrier permeability in a rat stroke model. The increase in MDA following cerebral ischemia/reperfusion was prevented with *Antiaris africana* leaf extract [5]. “The prevention of the decrease in the amount of GSH by MCAO ischemia with the plant extract pretreatment may give insight and then corroborate the reports from several studies on ROS generation as a cellular event that leads to oxidative neuronal damage in MCAO induced cerebral ischemia” [5,10,19]. It can then be suggested that, in addition of inhibiting lipid peroxidation, *O. gratissimum* exerts its antioxidant activity by inhibiting GSH depletion. This could be either by direct scavenging of ROS and/or GSH production stimulation. The scavenging potential of extract has even been reported [7]. As already suggested [20] the neuroprotective potential of *O. gratissimum* in cerebral ischemia through its antioxidant activity is mediated by its bioactive phytochemicals such as flavonoids, alkaloids and polyphenols found in the extract and known for their antioxidant activity.

“After an ischemic insult, inflammatory mediators in the ischemic brain are upregulated from resident brain cells and infiltrating immune cells, which play a complex role in the pathophysiology of cerebral ischemia” [23]. As expected in the present study, MCAO ischemia and reperfusion increased all the inflammatory markers investigated, confirming the stroke. The TNF- α expression is initially increased the first hours (1 to 3h) after the ischemic onset [24] and induces apoptosis by activating sequential caspases [25]. IL6 are key contributors to ischemic brain injury and seem to be a robust early marker for outcome in acute ischemic stroke [26]. “Some data show that IL-6 leads to an excessive inflammatory response, which might increase injury due to stroke” [23]. From the smaller dose (30 mg/kg), the aqueous extract of *O. gratissimum* significantly prevented the increase of all these markers, confirming the strong anti-inflammatory potency of the plant [6]. According to these authors, *O. gratissimum* was more efficient at reducing membrane destabilization than indomethacin in the membrane stability assay. It can, therefore, be suggested that the neuroprotective activity of the aqueous extract of *O. gratissimum* passes through its anti-inflammatory activity. Many studies have linked the anti-inflammatory activity of plant extract and their neuroprotective potential [4]. It is therefore obvious according to the results of the present and previous studies that *O. gratissimum* has a high neuroprotective potential due to its antioxidant and anti-inflammatory activities. These activities are linked to its huge amount of identified bioactive compounds, with eugenol (55.6%) as the major one [8]. Eugenol is known for its antioxidant potential by its ability to sequester free radicals in the DPPH assay, as well as to inhibit reactive oxygen species, H₂O₂ and NO [9]. Furthermore, eugenol possesses good anti-inflammatory potential [27]. It can, therefore, be one of the major components involved in the antioxidant and anti-inflammatory activities of *O. gratissimum* neuroprotective effect.

Conclusion

This study showed that *O. gratissimum* aqueous extract prevented the drop of temperature as well as the neurological score. The extract therefore exerts a neuroprotective effect through its anti-inflammatory and antioxidative activities in the brain, that in turn prevent the anxiety induced by stroke. All these observations suggest that *O. gratissimum* by its antioxidant and anti-inflammatory potencies can protect the brain against stroke-induced injuries. This extract could be used as adjunct to treat individuals having stroke risk factors or neurological insults.

Ethical Approval

Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethical Committee (Reg. N°FWAIRD 0001954).

Data availability

The data can be available upon request

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