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ORIGINAL ARTICLE

Study of transcranial therapy 904 nm in experimental model of stroke

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Abstract

Cerebrovascular accidents (CVAs), commonly known as strokes, can damage the brain through vascular injuries caused by either blood vessel blockages (ischemic stroke) or ruptures (hemorrhagic stroke) which disrupt regular brain blood supply and can cause severe damage to the individual. The objective of the present study was to evaluate the effects of photobiomodulation with a light-emitting diode (LED) device (904 nm, 110 mW, 7 J/cm^2) on neurogenesis, muscle resistance, and motor behavior in animals submitted to an experimental model of hemiplegia. The sample consisted of 30 Wistar rats, divided into two groups: control group (GC) and 904-nm LED-treated group (TG). All animals underwent stereotactic surgery for electrode implant and subsequent electrolytic injury to induce an ischemic stroke. TG was subjected to daily LED irradiation (904 nm, 110 mW, 7 J/cm^2) for 63 s. Suspension test results indicate an improvement of TG muscle resistance when compared with baseline evaluation (BLT); a reduction in open-field freezing time and the number of fecal bolus pellets suggest diminished anxiety induced by 904-nm LED treatment on treatment days 7 and 21 (TG7 and TG21) compared with the baseline results; and lastly, histological analysis showed important signs of neurogenesis in TG in comparison to CG, especially on treatment days 7 and 21 (TG7 and TG21). In conclusion, the present study suggests that 904-nm LED irradiation may beneficially affect neurogenesis, muscle resistance, and animal motor behavior following ischemic CVA.

Keywords Cerebrovascular accident · Stroke · 904-nm light-emitting diode · Neurogenesis · Muscle resistance · Motor behavior

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Introduction

Cerebrovascular accident (CVA) is a type of vascular injury that occurs in the brain and can cause damage through disruption of blood supply. Depending on severity or damage extension, CVAs are very challenging to treat. During the past 40 years, CVAs have been ranked as one of the main causes of death or long-term disability, especially in areas with an elevated population of individuals over 65 years of age, where CVAs may generate great economic and social impact [1, 2].

Ischemic CVAs are the most common variation. These are induced by cerebrovascular occlusions causing irreversible tissue infarction and abnormal cell death, which lead to various motor and cognitive impairments, compromising bodily functions and daily life activities. Treatment is generally focused on improving strength and functionality and in minimizing cognitive impairment. Among the various rehabilitation modalities available, light-emitting diode (LED) therapy has been suggested to be an effective treatment option [2–4].

In fact, LED photobiomodulation has shown promising results in the treatment of several diseases and neurological disorders, including CVAs. On a cellular level, LED photobiomodulation—as other forms of low-level light therapy (LLLT)—has been shown to increase adenosine triphosphate (ATP) production, modulate reactive oxygen species, activate replication of mitochondrial DNA, increase early-response genes and expression of growth factors, induce synapses, and stimulate cell proliferation, and in animal studies, LED photobiomodulation has induced positive results upon neurogenesis [5–7].

Neurogenesis is the biological process that involves coordinated proliferation, differentiation, and migration of cells to form neural brain tissue, and may be involved in the targeting and repair of damaged tissue following ischemic CVAs [8–11].

In this context, the objective of the present study was to evaluate the effects of photobiomodulation with a light-emitting diode (LED) device (904 nm, 110 mW, 7 J/cm^2) on neurogenesis, muscle resistance, and motor behavior in Wistar rats submitted to an ischemic CVA protocol.

Methodology

Sample

Ethical approval

All experiments were carried out in accordance with the Ethics Committee on the Use of Animals (CEUA) of the Central Western State University (UNICENTRO)—research protocol number 034/2017.

Sample

The sample consisted of 30 *Rattus norvegicus*, Wistar strain, weighing approximately 200 g purchased from the Londrina State University (UEL) vivarium. Experimental procedures were performed in the UNICENTRO Laboratory of Neuroanatomy and Neurophysiology. The animals were kept in groups of 5 in acrylic boxes (41 × 34 × 16 cm) with carbon steel wire mesh covers and a polypropylene water fountain (700 ml). The animals had free access to water and food (ad libitum) which was restricted prior to surgery, i.e., no food for 8 h and no water for 4 h. The animals were kept at a 12-h light-dark cycle (lights on from 7 am to 7 pm), and room temperature was kept by an 7000BTUs sprint air conditioning unit at 23 ± 1 °C.

Experimental groups

The 30 animals selected for the study were divided into two groups:

(1) Control group (CG) with 15 animals equally divided into three subgroups: control group 3 days (GC3), with euthanasia on the fourth day; control group 7 days (GC7), with euthanasia on the eighth day; and control group 21 days (GC21), with euthanasia on the twenty-second day. CG animals were not submitted to LED irradiation.
(2) 904-nm LED-treated group (TG) with 15 animals equally divided into three subgroups: 904-nm LED-treated group 3 days (TG3), with euthanasia on the fourth day; 904-nm LED-treated group 7 days (TG7), with euthanasia on the eighth day; and 904-nm LED-treated group 21 days (TG21), with euthanasia on the twenty-second day.

Baseline evaluation results were conducted prior to ischemic CVA protocol and identified in the graphs as LBC (for baseline control group) and LBT (for baseline treatment group).

Suspension test

The suspension test was performed in an illuminated environment, free of noise and with temperature controlled at 23 ± 1 °C. The test consists of manually suspending the animals by the front legs to a bar. The evaluation was repeated three times during baseline and before euthanasia, and the mean total suspension time is considered indicative of motor function coordination and muscle resistance [12].

Open-field assessment protocol

The test was performed in an illuminated environment, free of noise and with temperature controlled at 23 ± 1 °C. During this test each animal was placed in the center of the open-field apparatus and recorded with a Logitech® 3MP Webcam camcorder for a period of 5 min. Total "freezing" time (the amount of time the animal remains immobile) and the number of fecal bolus pellets were registered by an evaluator blinded to group assignment. The open-field apparatus was cleaned with alcohol and let to dry between each evaluation [13, 14].

Surgical procedure

The animals were anesthetized intraperitoneally (i.p.) with a pre-mixed solution of 80 mg/kg ketamine hydrochloride (ketamine, 10-ml bottle) to 15 mg/kg xylazine hydrochloride (Dopaser, 10-ml bottle) ratio, then were taken to a stereotaxic apparatus (model David Kopf, USA) and had their heads fixed by the external auditory canal and upper incisors. Next, part of the tissue covering the skull was removed with scissors, and after finding the Bregma reference point, with the lambdoid and bregmatic sutures in the same horizontal plane, an electrode was implanted in the internal capsule by following the stereotactic coordinates AP = − 1.72 mm, ML = − 3.4 mm, and DV = 4.4 mm as previously described by Paxinos and Watson [15]. After implantation, the electrodes were fixed in the calvaria with an autopolymerizable acrylic prosthesis, using the VIPIFLASH Autopolymerizable Acrylic Resin Kit®. The animals were rested for 5 days and then were anesthetized again and taken to the stereotaxic apparatus where they received 20 mA of electric current with the DC POWER SUPPLY MPS-3005 device for 45 s. The electrodes were made with enamel wire number 34 with approximately 10 mm in length.

Procedures for analgesia

The animals were individually administered v.o. tramadol by gavage (2 mg/kg diluted in 0.2 ml of saline) every 12 h for a period of 7 days. The animals were kept under observation for 12 h to look for signs of any intercurrence.

LED treatment

The LED device used was made of PVC material (polyvinyl chloride). The device consisted of seven LEDs (RL5-09030, Super Bright LEDs) each with a 5-mm-diameter encapsulation and geometrically positioned in such a way that all light produced was focused onto a single area. The equipment consisted of LEDs that emitted 904-nm infrared light. The device's total irradiance was 110 mW (assessed with the Thor Labs Power and Energy Meter Console PM100D).

Irradiation was punctually applied with the 904-nm LEDs in contact with the frontal region of the brain for 63 s yielding a dose of 7 J/cm^2 [16], with the objective of obtaining irradiation intensities useful for clinical applications in phototherapy.

Euthanasia

For this procedure, the animals were carefully separated to minimize stress. First 80 mg/kg of ketamine and 15 mg/kg of xylazine were administered intraperitoneally. After anesthesia was confirmed, 175 mg/kg of thiopental was administered intraperitoneally.

Histological analysis

The laminae in H&E (hematoxylin and eosin) staining were analyzed in the neurophysiology laboratory, part of the Department of Physical Therapy (DEFISIO) of the Universidade Estadual do Centro-Oeste (UNICENTRO). Ten images of each slide were captured in the × 4 and × 40 objectives and analyzed in the MIPro Standard v1.1 software for *windows*.

Statistical analysis

The GraphPad Prism 5.01 program was used for the statistical analyses. The Shapiro-Wilk test was applied to verify the normality of the sample. The data was analyzed using ANOVA parametric test followed by Tukey's multiple comparison test. Differences with a value of $p < 0.05$ were considered significant.

Results

Suspension test analysis

Figure 1 shows the means and standard deviation in groups 3, 7, and 21 days for the suspension test. Groups 3D and 7D are not statistically different from each other. In group 21D graph, there was significance between TG21 and LBC21 and between TG21 and GC21. It can be observed in all graphs, LED treatment improves suspension times in comparison with LBT, evidencing an improvement of the functionality and muscle resistance.

Open-field test

Results depicted in Fig. 2 demonstrate that there were no statistically significant results with freezing behavior in the open-field test, although LED treatment decreased total freezing time in TG21 group.

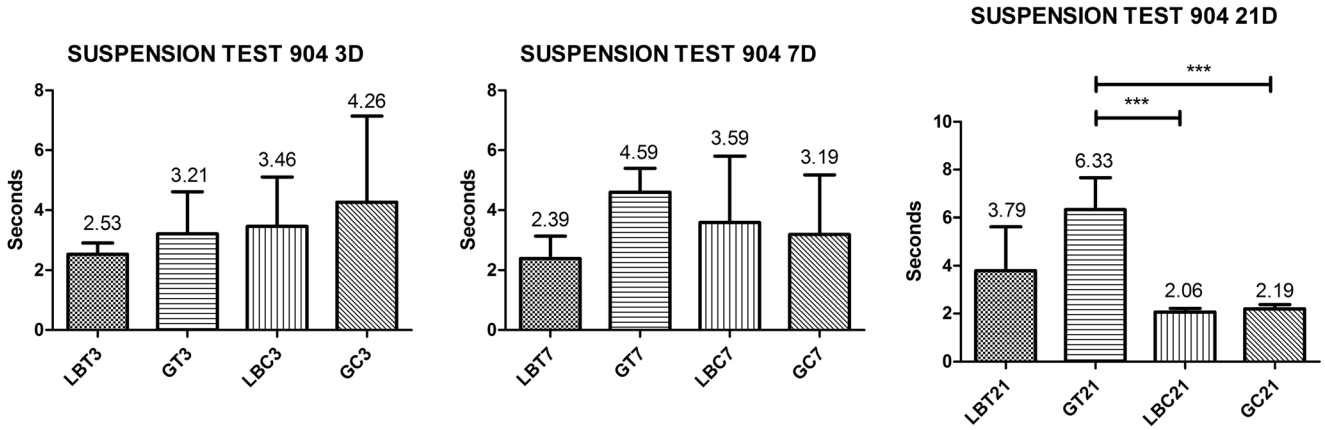

Fig. 1 Representation of means and standard deviation of the suspension test in groups 3, 7, and 21 days; mean values were acquired by measuring each animal three times. ***$p < 0.0001$. The means of the TG group in days 7 and 21 were statistically significant

Figure 3 shows the means and standard deviations of fecal bolus pellet count in the open-field test. Statistically significant differences can be seen between TG7 and LBC7 and with GC7 ($p < 0.001$) and also between LBT21 and LBC21 ($p < 0.01$). There was also a decrease of the means in TG7.

Histological analysis

Figure 4 a and b show histological results of TG3 (a) and GC3 (b) treated groups at × 4 and × 40. In TG3 (a), a significant number of fibrous astrocytes (indicated by 1) were observed; in GC3 (b), the number of astrocytes was reduced, implying lower tissue support and nutrition in GC3 (b); oligodendrocytes (signaled by 2) were more abundant in TG3 (a) than in GC3 (b); both in TG3 (a) and in GC3 (b), there was a relevant cellular activity of pyramidal motor neurons which are the main excitatory neurons of the cerebral cortex (signaled by 3); in GC3 (b), the cellular distribution of pyramidal neurons was in a more dispersed form, and in TG3 (a), cells were more close together, showing a higher concentration of nuclei per unit area which denotes a response of the tissue to anoxia; in relation to the distribution of sensory neurons (signaled by 4), TG3 (a) and GC3 (b) present a considerable distribution of this cellular type throughout the cerebral parenchyma; microglia (signaled by 5), an evidence of necrosis or inflammatory process due to its phagocytic activity, were abundant in both groups; the outbreaks of edema identified by lighter areas (marked by 6) are more noticeable in GC3 (b) than in TG3 (a); in the analysis of the cerebral parenchyma, GC3 (b) was more hypodense than TG3 (THE). Signs of neurogenesis were identified in both groups, but in TG3 (a), these signs of greater density of the cerebral parenchyma were more evident.

Figure 4 c and d show histological results of TG7 (c) and GC7 (d) at × 4 and × 40. In TG7 (c), an increase in astrocytes was observed (signaled by 1); in GC7 (d), the number of astrocytes was reduced, implying lower tissue support and nutrition in GC7 (c); oligodendrocytes (signaled by 2) are more abundant in TG7 (c) than in GC7 (d); the number of pyramidal motor neurons (signaled by 3) and sensory neurons (signaled by 4) is considerably greater in TG7 (c) than in GC7 (d); microglia (signaled by 5) was more abundant in GC7 (d) than in TG7 (c); the outbreaks of edema identified by lighter areas (marked by 6) are more intense in GC7 (d) than in TG7 (c); the cerebral parenchyma was less dense in GC7 (d) than in TG7 (c). Signs of neurogenesis were considerably more evident in TG7 (c).

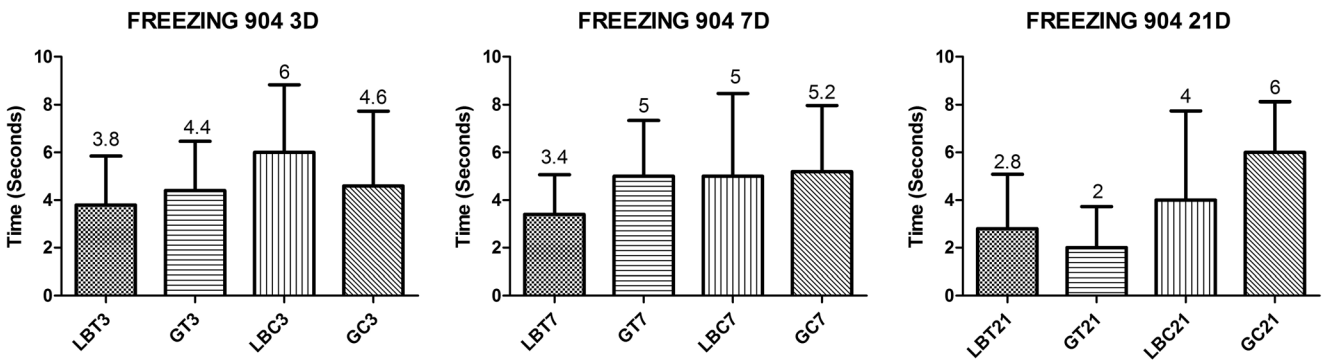

Fig. 2 Representation of the means and standard deviation in the open-field test—freezing variables (TG3, TG7, and TG21 groups with their respective baseline evaluations). There were no statistically significant differences

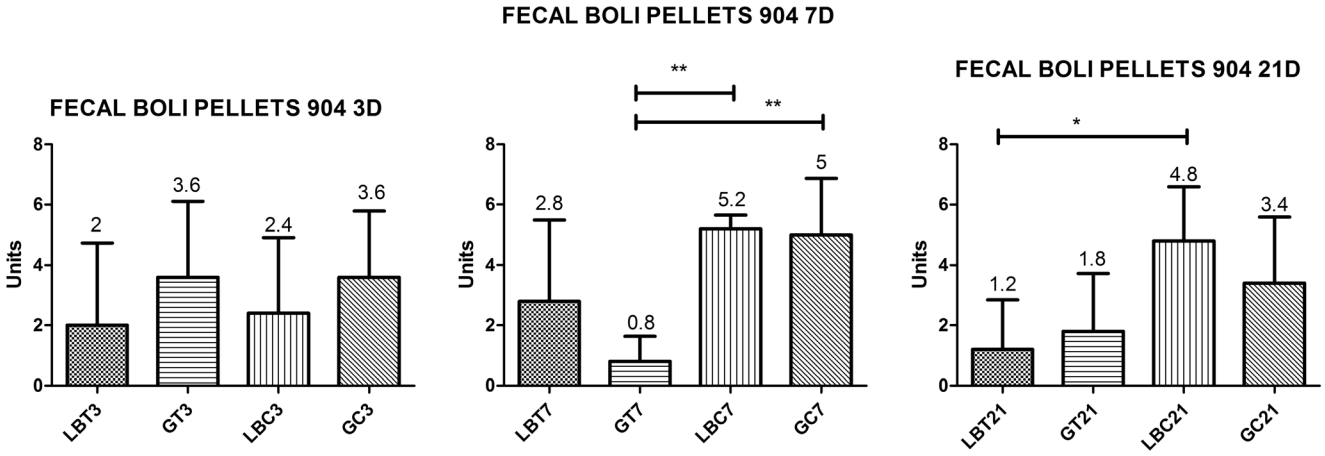

Fig. 3 Representation of means and standard deviations of fecal bolus pellet count in the open-field test. *$p < 0.01$; **$p < 0.001$

Figure 4 e and f show histological results of TG21 (e) and GC21 (f) at × 4 and × 40. In TG21 (e), astrocytes (signaled by 1) are more evident than in GC21 (f), which was reduced; oligodendrocytes (signaled by 2) are in greater amounts in TG21 (e) than in GC21 (f); pyramidal motor neurons (signaled by 3) and sensory neurons (signaled by 4) are in greater quantity in TG21 (e) than in GC21 (f); microglia (signaled by 5) is in greater quantity in GC21 (f) than in TG21 (e); cerebral parenchyma in GC21 (f) is more dense than in TG21 (e). Signs of neurogenesis were more noticeable in TG21 (e) than in GC21 (f).

Discussion

Surprisingly, there have not yet been many trials that looked at LED photobiomodulation on CVA or brain injury [17]; therefore, the present study investigated LED's effects on three different stages following CVA: acute (3 days), subacute (7 days), and chronic (21 days).

The use of photobiomodulation with low-level light-emitting diodes (LEDs) has greatly expanded in recent years as the therapy has demonstrated promising results on various neurological diseases, including CVA, apart from the fact that the

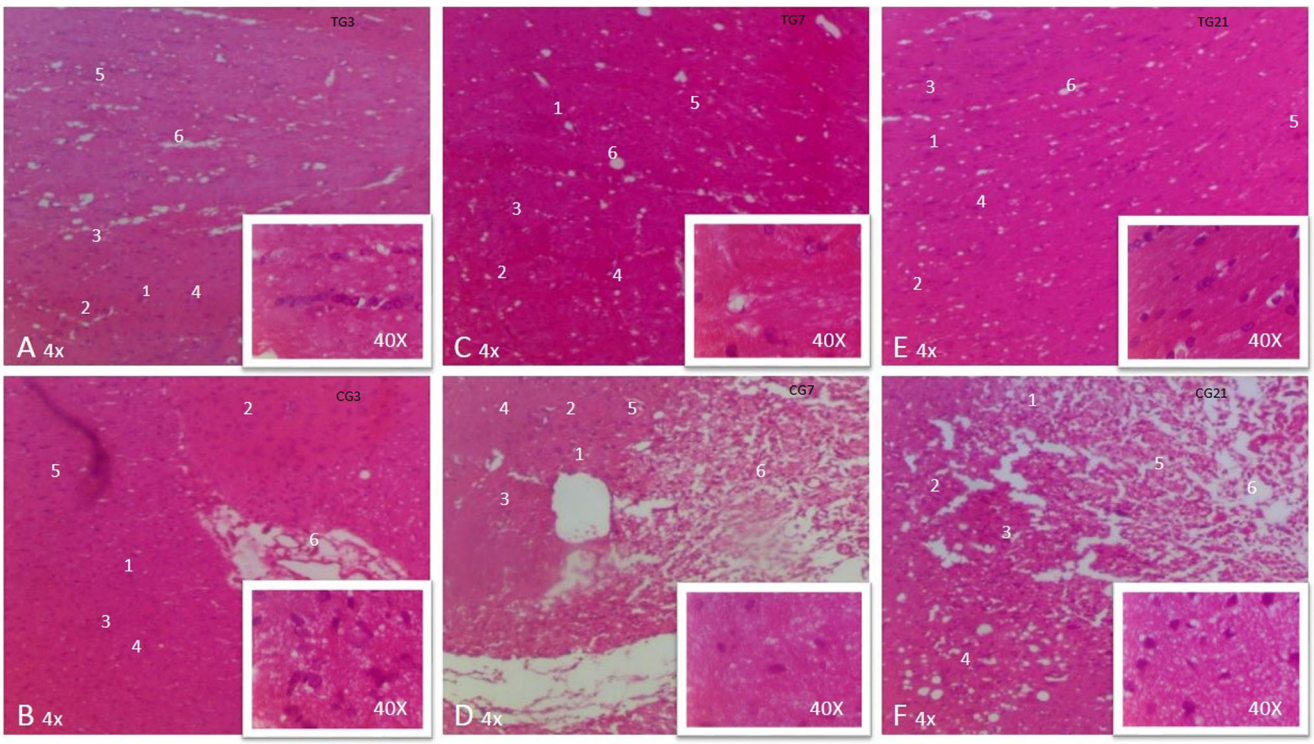

Fig. 4 Histological evaluation: **a** 904-nm LED-treated group (TG) and control (GC) group; **b** treated group—3 days (TG3), control group—3 days (GC3); **c** treated group—7 days (TG7); **d** control group—7 days (GC7); **e** treated group—21 days (TG21); and **f** control group—21 days (GC21)

devices are generally portable, safe, and easy to use [4]. Most importantly, Henderson and Morries [18] determined that photobiomodulation in the range of 810 to 980 nm has a penetration of up to 3 cm, effectively infiltrating through the scalp and skull to act directly upon the brain tissue, which makes infrared LED photobiomodulation a viable option for the treatment of neurological injuries.

In the present study, 904-nm LED photobiomodulation improved mean suspension time (on days 7 and 21) indicating an increase in muscle resistance in the suspension test which is a physical functional improvement that could be explained by increased energy metabolism and elevated intracellular ATP availability induced by the treatment. Hamblin (2016) describes that photobiomodulation acts upon the enzyme cytochrome C oxidase (CCO—mitochondrial respiratory chain complex IV) which is responsible for the final reduction of oxygen in the water using the electrons generated by glucose metabolism. The theory is that the activity of the CCO enzyme can be inhibited by nitric oxide (ON) especially in hypoxic or damaged cells. This inhibitory NO can be dissociated by light photons that are absorbed by the CCO. When NO is dissociated, the mitochondrial membrane potential is increased; consequently, more oxygen is consumed, more glucose is metabolized, and more ATP is produced by the mitochondria, thus increasing metabolism and therefore improving muscle resistance due to increased ATP availability [19].

In the study by Vahid-Ansari et al. [20] when studying post-CVA depression in mice with unilateral medial prefrontal injury due to endothelin-1 (ET-1) injection, behavioral tests such as the open field, the elevated plus maze, the light-dark, and the food restriction test indicated a 50% increase in immobility and significant depressive and anxiety symptoms compared with the control groups within 6 weeks after CVA. In the present study, corroborating Vahid-Ansari et al.'s [20] findings, it was observed that following CVA, the animals present behavioral changes commonly described in animal models as depression and anxiety-like. In the open-field test, freezing behavior increased in groups 3 and 7 days, whereas in group 21 days, freezing behavior decreased, although not statistically significant, suggesting that 904-nm LED treatment effectively influenced the animals' behavior. Concomitantly, the total number of fecal bolus pellets also decreased.

Lee and collaborators [4] used 20-min LED photobiomodulation twice daily for 3 days beginning treatment 4 h post-ischemia. LED group showed significantly better results in neurological function based on the neurological test score. Additionally, LED significantly reduced neuroinflammatory responses, including neutrophil infiltration and microglial activation in the ischemic cortex. The results obtained herein are in accordance with Lee and collaborators' study [4], as LED photobiomodulation decreased microglia activation which may indicate a decrease in necrosis and inflammation, as these cells have phagocytic activity. Additionally, LED treatment also increased fibrous astrocyte activity. As astrocytes are abundant in the white substance, their increase may indicate neural protection, support, and tissue nutrition. Oligodendrocytes were also increased as a result of the treatment. These cells are responsible for the formation and maintenance of the myelin sheaths of the axons, generating a greater speed in the action potential of the nervous connections. Lastly, LED irradiation increased the activity of pyramidal neurons, which are the main excitatory neurons of the cerebral cortex responsible for motor and sensory functions.

It has been previously shown that photobiomodulation can promote neuroprotection and prevent neuronal death after brain injury such as traumatic brain injury [21]. In a study by Lee et al. [22] with an experimental model of ischemic CVA, 610-nm (orange) LED treatment (irradiance of 1.7 mW/cm^2, with a dose of 2.0 J/cm^2, twice daily for 2 days) induced significant histological changes 24 h after the injury, i.e., reduction of neural cell necrosis and decreased edema which suggests that photobiomodulation may suppress neuroinflammation. In the present study, LED irradiation following ischemic injury reduced tissue necrosis, edema, and neuronal inflammatory cell activity in all treatment groups, therefore corroborating LED effectiveness upon neuroinflammation [22, 21].

Conclusion

In conclusion, the present study suggests that 904-nm LED irradiation may beneficially affect neurogenesis, reduce edema and density of the cerebral parenchyma, increase muscle resistance and animal motor behavior, and positively influence fear and anxiety following ischemic CVA.

Given the relevance of CVA, continued research with LED photobiomodulation, especially in a clinical setting, could prove very rewarding. One of the limitations of the present study is the fact that only one wavelength was assessed; it is quite possible that other wavelengths or combinations of different wavelengths may be even more beneficial and/or induce different results.

Compliance with ethical standards

All experiments were carried out in accordance with the Ethics Committee on the Use of Animals (CEUA) of the Central Western State University (UNICENTRO)—research protocol number 034/2017.

Conflict of interest The authors declare that there is no conflict of interest.

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