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MINI REVIEW: NEUROPROTECTION OF NANOMATERIALS CONTAINING ANTIOXIDANTS IN CEREBRAL ISCHEMIA MODELS

MINI REVISÃO: NEUROPROTEÇÃO DE NANOMATERIAIS CONTENDENDO ANTIOXIDANTES EM MODELOS DE ISQUEMIA CEREBRAL

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ABSTRACT

Stroke, especially ischemic stroke, is a major public health problem worldwide, however, currently there are few treatments available for this disease. Antioxidants have been the target of searches for new therapies, but many of their properties hinder pharmacological use, such as low bioavailability and autoxidation. Nanotechnology can be used to improve these properties and deliver the compounds to the brain. Thus, this integrative review analyzed studies that have used nanostructures containing actives with antioxidant properties with protective effects against the damage followed cerebral ischemia. The results indicated that all the nanostructured actives used had efficient neuroprotective action and antioxidant potential, both in vitro and in vivo, which was demonstrated by the increase in the levels of antioxidant enzyme activities, decrease on lipid peroxidation, and reduction on cellular apoptosis and tissue infarction. Several studies also showed additional neurobehavioral improvement in rodents. Therefore, the nanostructuring of active compounds with antioxidant properties can be a good adjuvant in the treatment of the ischemic stroke, as it can effectively enhance the bioavailability of these compounds and provide better access to the damaged region to induce protection to the brain.

Keywords: Ischemic stroke; oxidative stress; nanotechnology; brain health.

RESUMO

O acidente vascular cerebral, especialmente o isquêmico, é um grande problema de saúde pública mundial, porém, atualmente, existem poucos tratamentos disponíveis para essa doença. Os antioxidantes têm sido alvo de buscas por novas terapias, mas muitas de suas propriedades dificultam o uso farmacológico, como baixa biodisponibilidade e autoxidação. A nanotecnologia pode ser usada para melhorar essas propriedades e levar os compostos ao cérebro. Assim, esta revisão integrativa analisou estudos que utilizaram nanoestruturas contendo ativos com propriedades antioxidantes com efeitos protetores contra os danos decorrentes da isquemia cerebral. Os resultados indicaram que todos os ativos nanoestruturados utilizados apresentaram eficiente ação neuroprotetora e potencial antioxidante, tanto in vitro quanto in vivo, o que foi demonstrado pelo aumento dos níveis de atividades de enzimas antioxidantes, diminuição da peroxidação lipídica e redução da apoptose celular e infarto tecidual. Vários estudos também mostraram melhora neurocomportamental

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adicional em roedores. Portanto, a nanoestruturação de compostos ativos com propriedades antioxidantes pode ser um bom adjuvante no tratamento do acidente vascular cerebral isquêmico, pois pode efetivamente aumentar a biodisponibilidade desses compostos e fornecer melhor acesso à região danificada para induzir proteção ao cérebro.

Palavras-chave: *Acidente vascular cerebral isquêmico; estresse oxidativo; nanotecnologia; saúde cerebral.*

INTRODUCTION

Stroke occurs when supplying vessel has blood reduced by narrowing of the artery or rupture, causing blockage of blood flow or hemorrhage to the brain. Ischemic strokes, accounting for more than 85% of cases, result from a blood occlusion in a cerebral vessel, leading to severe physical and cognitive deficit with a high mortality (Feigin *et al.*, 2022). The World Health Organization highlights that stroke is the costliest long-term disabling condition for both the state and the individual in adult life worldwide. Annually, the expectation is that approximately 15 million people worldwide suffer stroke, with 5 million facing mortality and another 5 million left permanently disabled, placing a burden on family and community (World Health Organization, 2024. During COVID-19 pandemic, approximately 1.4% of patients hospitalized with COVID-19 infection suffered stroke (Martin *et al.*, 2024).

Ischemic stroke presents limited treatment options, with thrombolysis considered the gold standard. However, currently, less than 5% of patients affected by this disease receive this treatment (Carradori *et al.*, 2016). Furthermore, the available drugs exhibit limited efficiency and have short therapeutic windows (Hassanzadeh *et al.*, 2017). Therefore, the quest for new strategies in the treatment and prevention of ischemic stroke is imperative, and antioxidants have emerged as a primary focus in these endeavors. The ischemic damage generates energy failure, initiating an excitotoxicity process through the massive release of excitatory neurotransmitters such as glutamate. This process promotes depolarization of the plasma membrane, leading to an influx of calcium ion (Ca^{2+}) and subsequently increasing levels of oxidative stress, apoptosis, and neuronal death (Ayuso; Montaner, 2015). Oxidative stress is widely recognized in the literature for its generalized role in brain damage associated with central nervous system (CNS) diseases, including the ischemic process. Disturbances in cell homeostasis can result in toxicity due to the excessive production of reactive oxygen species (ROS) and free radicals, causing damage to all cellular components, including proteins, lipids, and DNA (Li *et al.*, 2019).

Antioxidants have demonstrated significant potential in the treatment and prevention of ischemic stroke (Li *et al.*, 2019). However, many of these antioxidants possess properties that impede their use as pharmacological strategies, including low water solubility, metabolism in peripheral organs, low bioavailability, and susceptibility to autoxidation. Moreover, when addressing CNS disorders, the blood-brain barrier poses a significant challenge due to its selectivity (Kantawala *et al.*, 2024).

Consequently, nanoparticles are considered among the most promising and versatile drug delivery systems for applications in challenging and inaccessible regions, such as the brain. They have the capability to protect therapeutic agents and efficiently deliver them to damaged areas (Teleanu *et al*., 2019). Considering these facts and the importance of advancing knowledge in this field for the scientific community, this integrative review aims to examine the neuroprotective effects of nanostructured actives with antioxidant properties against damage resulting from cerebral ischemia.

MATERIALS AND METHODS

The present study is an integrative literature review, which summarizes studies have been evaluated different methodological approaches. The synthesis and analysis of the data were carried out to develop a more comprehensive explanation of a specific phenomenon based on the findings of the studies, with theoretical and/or interventionist purposes. Six steps were taken to establish this review, according to Souza *et al.* (2010): (1) elaboration of the guiding question, (2) search or sampling in the literature, (3) data collection, (4) critical analysis of the included studies, (5) discussion of the results and (6) presentation of the integrative review.

This review used articles indexed in the following databases: US National Library of Medicine National Institute Health (PubMed) and Latin American and Caribbean Literature in Health Sciences (LILACS), searching for articles published until 2024. Descriptors used in the research were: ((ischemia and brain) or cerebral ischemia or stroke) and nano and antioxidants. In this review, two authors (AER and BSL) independently used the descriptors mentioned above and did the search and selection of articles. Subsequently, the abstract from the remaining articles were evaluated to identify the eligible articles. In case of doubts about the exclusion of a study, it was included for full text evaluation. Full article reading and analysis have defined studies inclusion in, and exclusion from, the current review. In case of disagreement, a third independent author (CRB) would decide whether the article would be included or not.

SELECTION AND EXCLUSION CRITERIA

The included articles were those that: were original and written in English, used brain ischemia models, either *in vitro* and/or *in vivo*, that evaluated the neuroprotective potential of nanoformulations containing antioxidant compounds with properties against cellular damage from cerebral ischemia and oxidative stress, and that were published until 2024. The excluded articles were those that: used another type of ischemia as a model (e.g muscle ischemia), other than that induced in brain tissue/cell; did not use the nanostructured active compound; the nanoparticle had an average size above 500 nm; the particle size was not described; used substances with exclusive scavenging properties for metals.

RESULTS

In a total of 141 articles were found on both platforms, which 16 were reviews and 11 were duplicates, so 27 were excluded. Of the remaining articles, through the analysis of titles and abstracts, 30 were selected to read the full text, and the other articles were excluded because they used a different type of ischemia in their methodology, other than cerebral, or they did not use nanoformulations containing actives with antioxidant effect. From the complete reading of the 30 articles, 13 were selected to be reviewed in this study, which are described in table 1. The other 17 articles were excluded from the review because: structure was not nanoparticle (Zhang *et al.*, 2018), it has scavenging properties for metals (Bao *et al.*, 2018; Chonpathompikunlert *et al.*, 2012; Ganesana *et al.*, 2012; Hosoo *et al.*, 2017; Li *et al.*, 2019; Liu *et al.*, 2017), does not have a bioactive compound (Jiang *et al.*, 2016; Petro *et al.*, 2016; Yun *et al.*, 2013) and does not have an antioxidant test on the tissue or cell subjected to ischemia (Ahmad *et al.*, 2016; Fabian *et al.*, 2018; Gao *et al.*, 2018; Min *et al.*, 2018) (Table 1).

Table 1 - Research design and main results of each study analyzed.

Reference	Study	Metodology*	Main results
Ahmad *et al.*, 2013	A comparative study of Poly(N-isopropylacrylamide) nanoparticles of curcumin, demethoxycurcumin, and bisdemethoxycurcumin and their effects on oxidative stress markers in experimental stroke.	1) Nanocapsule 2) Curcumin, demethoxycurcumin and bisdesmethoxycurcumin 3) Occlusion of the middle cerebral artery in rats	The nanocapsule with curcumin increased the activity of the enzymes GPx, GR, SOD and catalase, decreased lipid peroxidation and neurological damage, proving to be more potent in preventing injuries resulting from cerebral ischemia in comparison with the other nanoparticles loaded with curcuminoids.
Kakkar *et al.*, 2013	Curcumin loaded solid lipid nanoparticles: An efficient formulation approach for cerebral ischemic reperfusion injury in rats.	1) Solid lipid nanoparticles 2) Curcumin 3) Occlusion of bilateral common carotid arteries in rats	The nanoparticle showed a protective role against cerebral ischemia by increasing the activity of antioxidant enzymes (GSH, SOD and catalase) and decreasing the activity of acetylcholinesterase, lipid peroxidation and nitric oxide levels, in addition to the neurological improvement of rats.
Ding *et al.*, 2016	Enhanced Neuroprotection of Acetyl-11-Keto-β-Boswellic Acid (AKBA)-Loaded O-Carboxymethyl Chitosan Nanoparticles Through Antioxidant and Anti-Inflammatory Pathways.	1) Nanocapsule 2) Acetyl-11-keto-β-boswellic acid 3) *In vitro*: oxygen-glucose deprivation in primary cortical neurons -*In vivo*: middle cerebral artery occlusion in rats	*In vitro*, there was an increase in the viability of primary neuronal cells and a reduction in LDH release. *In vivo*, there was an increase in SOD and GPx activity, in addition to a reduction in the volume of infarction and neurological deficit, demonstrating the neuroprotective effect of nanocapsules.
Galho *et al.*, 2016	Protective role of free and quercetin-loaded nanoemulsion against damage induced by intracerebral haemorrhage in rats.	1) Nanoemulsion 2) Quercetin 3) Intracerebral hemorrhage induced by type VII collagenase in rats	The nanoemulsion containing quercetin was able to reduce hematoma size and levels of lipid peroxidation, preserve GST activity, increase GSH content and total antioxidant capacity.

Hassanzadeh *et al.*, 2017	Application of nanostructured lipid carriers: the prolonged protective effects for sesamol in *in vitro* and *in vivo* models of ischemic stroke via activation of PI3K signalling pathway.	1) Nanoemulsion 2) Sesamol 3) *In vitro*: oxygen-glucose deprivation in PC12 cells -*In vivo*: modified four vessel occlusion in rats	In vitro, the sesamol-containing nanoemulsion prevented cell death and increased LDH. In vivo, there was a neurobehavioral improvement in the animals and a decrease in the infarcted area. In both models there was a decrease in MDA, an increase in GSH and in the activity of the enzymes SOD and catalase.
Ahmad *et al.*, 2017	The effect of safranal loaded mucoadhesive nanoemulsion on oxidative stress markers in cerebral ischemia.	1) Mucoadhesive nanoemulsion 2) Safranal 3) Occlusion of the middle cerebral artery in rats	Safranal nanoemulsion showed neuroprotective potential through significant improvement in the activity of antioxidant enzymes such as SOD, GPx, GR and catalase. There was also a decrease in the level of lipid peroxidation and in the volume of tissue infarction and neurobehavioral improvement in the animals.
Ghosh *et al.*, 2017	Triphenyl phosphonium coated nano-quercetin for oral delivery: Neuroprotective effects in attenuating age related global moderate cerebral ischemia reperfusion injury in rats.	1) Nanocapsule 2) Quercetin 3) Bilateral occlusion of the common carotid artery in young and old rats.	Controlled mitochondrial delivery of nanoencapsulated quercetin resulted in improvement in oxidative stress by reducing ROS and increasing the antioxidant activity of the enzymes SOD and catalase. There was a decrease in lipid peroxidation and protection against cell loss.
Zhao *et al.*, 2018	Nano-Liposomes of Lycopene Reduces Ischemic Brain Damage in Rodents by Regulating Iron Metabolism.	1) Nanoliposomes 2) Lycopene 3) Transient occlusion of the middle cerebral artery in rats	The liposome containing lycopene increased the levels of GSH and activity of the antioxidant enzymes SOD and catalase. It also regulated iron metabolism, providing neuronal protection against ischemic injury. In addition, it reduced lipid peroxidation, cerebral infarction and levels of apoptosis.
Yuan *et al.*, 2018	Tanshinol borneol ester on nanostructured lipid carriers has longer brain and systemic effector retention and better antioxidant activity in vivo.	1) Nanoemulsion 2) Borneol tanshinol ester 3) Bilateral occlusion of the carotid artery in rats	The nanostructures reduced levels of lipid peroxidation and increased levels of SOD and GSH in the brain, reducing oxidative stress and showing antioxidant effect.
Moghaddam *et al.*, 2020	Silymarin-loaded chitosan nanoparticles shows anti-oxidative and anti-inflammatory effects on global cerebral ischemia/reperfusion model.	1) Nanocapsule 2) Silymarin 3) Bilateral common carotid artery occlusion	Nanoparticles pretreatment ameliorated depressive-like behaviors and infarct volume; decreased the levels of MDA, IL-6 and TNF-α and increased the activities of SOD, catalase and GSH levels in damaged brain.
Azadi *et al.*, 2021	Micelles containing berberine improved the compound's therapeutic efficacy and action on the secretion of inflammatory cytokines in cerebral ischemia	1) Nanomicelles 2) Berberine 3) Bilateral Common Carotid Artery Occlusion in rats	The nanomicelles showed a significant decrease in the levels of TNF-α, IL-1ß and MDA compared to the stroke group.
Du *et al.*, 2023	Polymeric nanodots with iron-gallic acid as neuroprotective antioxidant for ischemic stroke therapy, guided by PET/MR imaging	1) Nanodots 2) Iron-gallic acid 3) Middle cerebral artery occlusion in rats	Nanodots protected cell viability after hydrogen peroxide treatment and improved recovery of neurological damage after occlusion model, observed by PET/MR imaging.
Wang *et al.*, 2023	Lamellar double hydroxide nanosheets containing gadolinium, atorvastatin and ferritin for magnetic resonance imaging and ischemia-reperfusion therapy simultaneously	1) Nanosheets 2) gadolinium/artovastatin/ferritin 3) Middle cerebral artery occlusion in mice	Nanomaterial showed high antioxidant effect and reduced reperfusion-induced apoptosis, decreasing the infarct area and neurological deficit score, as well as an excellent performance in magnetic resonance imaging

*1) type of formulation; 2) active; 3) ischemia model.

A study carried out on rats (male, 250-300g) used solid lipid nanoparticles (average size of 134.6 nm) containing curcumin (a substance with antioxidant properties found in powder extracted from turmeric) in order to evaluate the neuroprotection of these nanostructures. The global cerebral ischemia model was used, through occlusion of the bilateral common carotid arteries, which occurred for 10 minutes, followed by 72 hours of reperfusion. The nanoformulations were administered orally (doses of 25 mg/kg and 50 mg/kg), and the rats were pre-treated for 5 days before ischemia and 3 days after. The study obtained as a result a significant improvement in the cognition and neurological score of the rats in relation to the control group. In the biochemical tests that used brain tissue, there was also a significant increase in the levels of glutathione (GSH) and in the activity of the enzymes superoxide dismutase (SOD), catalase and enzymes of the mitochondrial complex (Complex I, II, III and IV), reaching basal levels. Also, there was a reduction in the levels of lipid peroxidation, nitrite and in the activity of acetylcholinesterase, which indicates recovery of acetylcholine levels induced by the treatment. In addition, the results indicated an improvement in brain bioavailability after oral and intravenous administration of the nanostructures. Free curcumin did not have a significant neuroprotective effect in the tests performed, which demonstrates the efficiency of the nanoformulation (Kakkar *et al.*, 2013).

Similar results were found in the study that used nanocapsules of curcumin, demethoxycurcumin and bisdesmethoxycurcumin, in which the rats (300-400g, 16 weeks old) were exposed to ischemia for 2 hours, followed by 22 hours of reperfusion by the middle cerebral artery occlusion model. The nanocapsules were administered intranasally as a pre-treatment (100 µg/kg) 1 hour before occlusion. Results revealed that the three formulations with curcuminoids were effective in reducing oxidative stress, however the nanoformulation containing curcumin (mean size of 92.46 nm) was more active than the other two formulations in the treatment of cerebral ischemia, increasing the activity of glutathione peroxidase (GPx), glutathione reductase (GR), SOD, catalase and decreasing lipid peroxidation in the brain tissue of animals. In addition, the nanocapsules were also effective in improving neurological damage after ischemia (Ahmad *et al.*, 2013).

Another study investigated *in vivo* and *in vitro* the neuroprotective effect of nanocapsules of acetyl-11-keto-β-boswellic acid (main active constituent of Boswellia serrata resin). In vitro, in a primary culture of cortical neurons with an oxygen-glucose deprivation model, the cells were exposed to ischemia/reperfusion for 60 minutes, followed by incubation with the treatments for 24 hours. In vivo, rats (adults, men, 192-228 g) with a model of ischemia/reperfusion by occlusion of the middle cerebral artery were used and treatments were administered intravenously (dose of 10 mg/kg) 1 hour after reperfusion (Ding *et al.*, 2016).

Hassanzadeh *et al.* (2017) investigated the neuroprotective effect of sesamol nanoemulsions (phenolic compound, main constituent of sesame seed oil) in brain ischemia models *in vitro* and *in vivo*, through oxygen-glucose deprivation in PC12 cell line derived from pheochromocytoma and oc-

clusion of four vessels in rats (males, 300-350g), respectively. The cells were exposed to oxygen-glucose deprivation for 1 and 8 hours, followed by reperfusion for 24 hours, and treatment (doses of 20, 40, 80 and 100 μM) was carried out for 48 hours, starting 24 hours before the ischemia. In the *in vivo* model, nanoemulsions (mean size of 92.3 nm) were administered (doses of 5, 10, 20 and 25 mg/kg), via intravenous injections, four days before, immediately after the onset of ischemia (10 minutes) and during the reperfusion period for 72 hours. The nanoemulsion containing sesamol was able to prevent cell death and increased LDH after 1 and 8 hours of ischemia in the *in vitro* model, while free sesamol was efficient only after 1 hour. In the *in vivo* model, nanoemulsion improved neurobehavioral deficits and reduced the infarcted area in the animals' brains. In both models of ischemia, there was a decrease in malondialdehyde (MDA) and an increase in GSH levels and in the activity of the antioxidant enzymes SOD and catalase, being that in the *in vivo* model, these tests were performed with the animals' hippocampus tissue.

Another study carried out in rats used the ischemia model of middle cerebral artery occlusion to assess the neuroprotective potential of safranal mucoadhesive nanoemulsions (lipophilic organic compound isolated from Crocus sativus (saffron)) administered nasally. The rats (8-10 weeks old, 300-400g) were pretreated for 21 days with the nanoemulsions (10 mg/kg dose) and subjected to 2 hours of ischemia, followed by 22 hours of reperfusion. The nanoemulsions (mean size of 89.64 nm) containing safranal revealed a neuroprotective potential in conditions where oxidative stress is the source of cellular damage as results showed a neurobehavioral improvement, a reduction in the infarct volume and lipid peroxidation, and increased activity of GR, GPx, catalase and SOD in the brain tissue of animals. In addition, the nanostructures containing the active showed better neuroprotective results when compared to the free active (Ahmad *et al.*, 2017).

The study by Galho *et al.* (2016) compared the neuroprotective effects of a nanoemulsion loaded with quercetin (polyphenolic bioflavonoid widely found in the diet) and the free form of the active in an VII collagenase-induced intracerebral hemorrhage ischemia model in Wistar rats (males, 250-300g). The treatments (30 mg/kg) were administered by a single intraperitoneal injection 2 hours after surgery. Quercetin showed antioxidant activity in both formulations (free form and loaded nanoemulsions), but the incorporation in nanoemulsions (average size of 19.25 nm) increased its antioxidant effect, which was reflected in the improvement of motor skills and in decreasing the extent of the hematoma. Nanoemulsions also led to an increase in the activity of glutathione S-transferase (GST) in relation to the ischemic group, however the increase did not reach the level of control. Furthermore, there was an increase in the GSH content and antioxidant capacity of the brain tissue against peroxyl radicals, in addition to a decrease in the levels of lipid peroxidation.

Similar results were found by Ghosh *et al.* (2017), using quercetin in the form of a nanocapsule, in a model of cerebral ischemia induced by bilateral occlusion of the common carotid artery, followed by 30 minutes of reperfusion. The rats (male) were divided into two groups: 2 months old and 20

months old, treated orally (2 mg/kg), 24 hours before ischemia. In addition, nanocapsules containing quercetin (average size of 42 nm) were specific for mitochondria, using triphenylphosphoric cations as one of the components of the matrix. Controlled mitochondrial delivery of quercetin preserved mitochondrial structural and functional integrity by capturing reactive oxygen species, modulating apoptotic cell death in young and elderly rats. When compared to the free quercetin, nanoemulsions significantly improved the neuroprotective potential of the active, protecting the brain against the formation of induced edema, providing complete protection against cell loss and induced neurodegeneration and increasing levels of GSH and activity of the mitochondrial enzymes SOD and catalase in the brain tissue of animals.

Nanoliposomes containing lycopene (6 mg; acyclic open chain unsaturated carotenoid found in many fruits and vegetables) were administered in male rats for 14 days, intragastrically, followed by exposure to 60 minutes of ischemia through transient occlusion of the middle cerebral artery and 7 days of reperfusion. The study aimed to investigate the neuroprotective potential of liposomes and their effects on iron regulatory proteins. The results show that the liposomes containing lycopene (nanoparticle sizes between 58-105 nm) reduced cerebral infarction, improved the neurological deficits of rats and reduced levels of oxidative and nitrosative stress by increasing GSH levels and the activity of the enzymes SOD and catalase in the animals' cortical tissue. Nanoliposomes also reduced lipid peroxidation, inflammatory response, suppressed apoptosis and modulated iron metabolism, normalizing its levels. In addition, the results also showed that encapsulation in nanoliposomes significantly improved the effectiveness of lycopene by providing neuronal protection against ischemic injury, when compared to the free active (Zhao *et al.*, 2018).

Another study verified the antioxidant potential of nanoemulsions containing tanshinol ester borneol (a hybrid of danshensu and borneol that has anti-ischemic activity in animals) in an ischemia/reperfusion model through bilateral occlusion of the carotid artery in mice, which occurred during 20 minutes. The treatments (doses of 6 or 12 mg/kg) were administered immediately after reperfusion by an intravenous injection. Treatment with nanoemulsions (average size of 181.3 nm) achieved antioxidant activity by reducing the levels of lipid peroxidation, increasing the levels of SOD and GSH in brain tissue, and, when compared to the free form, improved therapeutic effects of the active (Yuan *et al.*, 2018).

Moghaddam *et al.* (2020) utilized silymarin-loaded chitosan nanoparticles to investigate its anti-oxidative and anti-inflammatory effects on a rat model of global cerebral ischemia/reperfusion. Male Wistar rats (8 weeks, 250-300 g) were randomly distributed into four groups (control, ischemia, active and nanoparticles) and oral administration was conducted 14 days prior to bilateral common carotid artery occlusion. Depressive-like behaviors, infarct volume, oxidative stress markers and inflammatory factors were assessed after induction of ischemia/reperfusion. The nanoparticles pretreatment significantly ameliorated depressive-like behaviors and infarct volume after surgery. It also significantly decreased the levels of MDA, expression of IL-6 and TNF-α, and significantly increased

the activities SOD, catalase, GPx, glutathione reductase (GRx), and glutathione (GSH) levels in the brain. Therefore, a pretreatment with silymarin nanoparticles effectively prevents oxidative and inflammatory damage caused by ischemia/reperfusion in the brain.

Micelles containing berberine, a plant alkaloid with anti-inflammatory and antioxidant effects, but with low oral bioavailability, were used to improve the compound's therapeutic efficacy and action on the secretion of inflammatory cytokines in another cerebral ischemia study. The micelles were smaller than 20 nm in size, with PDI, zeta potential and encapsulation efficiency of 0.227, - 22 mV and 81%, respectively. In the *in vivo* model, Wistar rats (200-220 g, 8-10 weeks) were pretreated with free active (100 mg/kg) and berberine micelles (25, 50, 75, 100 mg/kg) for 14 days. On the last day, stroke induction was performed by bilateral common carotid artery occlusion and, afterwards, the levels of cytokines and MDA were measured in the brain supernatant. Both free active and micelles (100, 75, 50 mg/kg) treated groups showed a significant decrease in the levels of TNF-α, IL-1ß and MDA compared to the stroke group, demonstrating the anti-inflammatory and antioxidant properties in ischemia, which were even more accentuated in the animals treated with the nanoformulation (Azadi *et al*., 2021).

Du *et al.* (2023) tested polymeric nanodots (Fe-GA CPNs) with iron-gallic acid as neuroprotective antioxidant for ischemic stroke therapy, guided by Positron Emission Tomography and Magnetic Resonance Imaging (PET/MRI). *In vitro* experiments revealed that the nanodots protected cell viability after hydrogen peroxide (H_2O_2) treatment. Following the middle cerebral artery occlusion model in adult Sprague-Dawley male rats (280-300 g), neurological damage observed by PET/MR imaging revealed improved recovery after treatment with Fe-GA CPNs. Furthermore, immunohistochemical staining indicated that the nanodots inhibited apoptosis through restoration of protein kinase B (Akt), while western blot and immunofluorescence indicated activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1). Therefore, Fe-GA CPNs exhibited robust antioxidant and neuroprotective potential in the treatment of ischemic stroke through recovery of redox homeostasis and activation of Akt and Nrf2/HO-1 pathway.

Lastly, a study by Wang and collaborators (2023) used a neuroprotective agent (AFGd-LDH) composed of lamellar double hydroxide nanosheets containing gadolinium (as a nanocarrier/magnetic resonance contrast agent), atorvastatin (neuroprotective substance) and ferritin (as a transport agent across the blood-brain barrier). In addition to an excellent biocompatibility and blood-brain barrier transit properties, the nanosheets had about 90% intracellular ROS scavenging efficiency, higher effect than cerium oxide (CeO_2) (50%, a known ROS scavenger) and edaravone (52%, a neuroprotective substance). Results of the ischemia-reperfusion model in mice showed that the nanomaterial drastically reduced reperfusion-induced apoptosis, decreasing the infarct area by 67% and the animals' neurological deficit score from 3.2 to 0.9. In addition to the physiological effects, the nanomaterial also had excellent performance in magnetic resonance imaging, thus allowing imaging of the site and ischemia-reperfusion therapy simultaneously.

DISCUSSION

Stroke is a major public health problem, being one of the biggest causes of death worldwide and having a huge financial impact on the healthcare of many countries. Taking this into account, numerous studies have been dedicated to seeking new therapies for the prevention and treatment of stroke, and antioxidants have been one of the major protagonists of these researches (Li *et al*., 2019). In this integrative review, it was possible to observe that the several nanostructured actives with antioxidant properties used in the studies showed great neuroprotective potential in the *in vitro* and *in vivo* models, when used as a treatment against ischemia damage, with promising results to be deepened for future pharmacological strategies. In addition, the active substances, when nanostructured, caused a significant increase in the non-enzymatic antioxidant levels (GSH) and enzymatic activity (LDH, GPx, GR, SOD, catalase) when compared with the free forms, showing the protection efficiency and controlled release of the nanomaterials (Ahmad *et al*., 2017; Moghaddam *et al.* 2020; Yuan *et al*., 2018; Zhao *et al*., 2018).

Studies with nanomaterials for brain delivery are increasing exponentially, due to the possibility of multifunctionalization of these materials, coupled with their ability to carry drug payloads, included blood-brain barrier-impermeant drugs. Providing surface multifunctionalization may promote targeting of the blood-brain barrier or the enhancement of its crossing. Even more, nanostructuring can confer actives with features such as high chemical and biological stability, feasibility of incorporating both hydrophilic and hydrophobic substances, and the ability to be administered by a variety of routes (Masserini, 2013). In ischemic stroke, specifically delivering therapeutics to the injured brain is essential for the success of the treatment. Nanomaterials can target the brain and deliver a wide range of drugs safely, increasing permeation across the blood-brain barrier, with improved bioaviability (Dong *et al*., 2020).

In all articles selected for this review, nanotechnology was used to improve the characteristics of the compounds, in addition to improving pharmacological delivery due to the ability of nanostructures to cross the blood-brain barrier (Alajangi *et al*., 2022). Curcumin, acetyl-11-keto-β-boswelic acid, quercetin, tanshinol borneol ester and safranal have low bioavailability due to their low solubility in water, while sesamol and lycopene have low bioavailability due to its low stability and susceptibility to oxidation and isomerization (Ahmad *et al*., 2013; Ding *et al*., 2016; Galho *et al*., 2016; Hassanzadeh *et al*., 2017; Yuan *et al*., 2018). In spite of this, all these actives were nanostructured with the objective of increasing their absorption and bioavailability through the protection of the compounds, and improving pharmacological delivery to the brain. It was noteworthy the diverse types of nanoformulations observed in this review, such as nanocapsules, solid lipid nanoparticles, nanoemulsions, micelles, nanodots and nanosheets. Nanoparticles seemed to be the most popular system studied, possibly due to their biocompatibility and biodegradability profile into the brain, intrinsic

ability to cross the blood brain barrier because of their dimensions, preferably bellow 300 nm, and opportunity for production with desired reproducibility (Gugleva; Andonova, 2023).

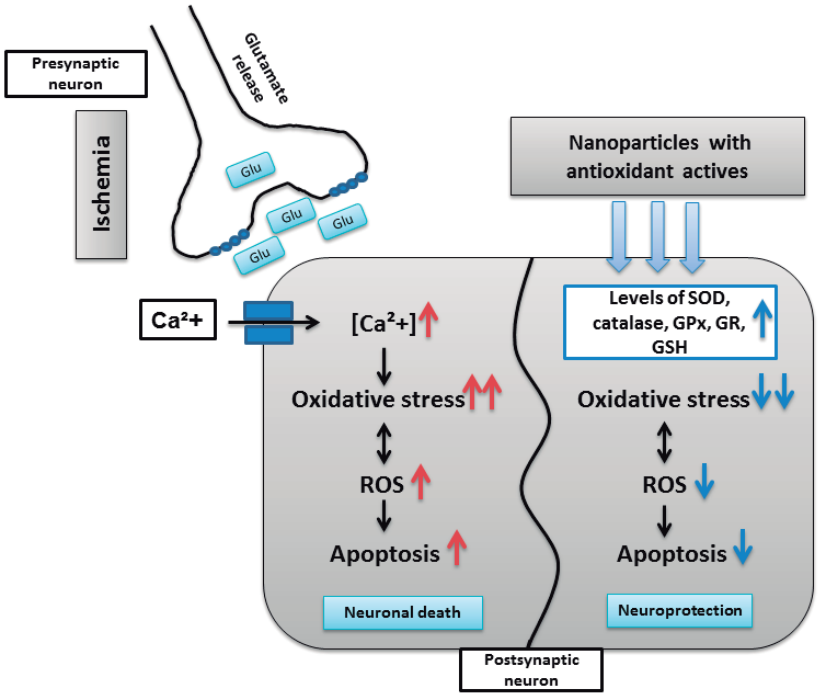
The route of administration has also been used as a pharmacological delivery strategy in some studies. For example, the safranal nanoemulsions were administered intranasally, since the compound is degraded in the gastrointestinal tract (Ahmad *et al.*, 2017). This same route of administration was used in the study with nanocapsules containing curcuminoids, demonstrating that the intranasal route is a good strategy for better pharmacological delivery to the brain, reducing unwanted systemic effects and improving bioavailability (Ahmad *et al.*, 2013). One of the studies that used quercetin also used the administration route as a strategy, using the intraperitoneal route, taking into account that quercetin has low oral bioavailability (Ghosh *et al.*, 2017).

Studies that used actives with scavenging properties were excluded from this review, however, for the purposes of discussion, it is interesting to analyze that many of these compounds have also shown great neuroprotective potential. An example is the study that used polyoxometalate nanoclusters against damage from cerebral ischemia, and obtained significant results, with the improvement in oxidative stress levels through the elimination of ROS, effective inhibition of apoptosis and recovery of neuronal function in adult rats (Li *et al.*, 2019). Another example is the study that evaluated the antioxidant activity of cerium nanoparticles containing the chelator edaravone in a model of cerebral ischemia, and obtained results of reduced infarct volume and oxidative stress in the damaged brain (Bao *et al.*, 2018).

There was also studies that used nanoparticulate antioxidant enzymes and evaluated their neuroprotective effects. These articles were excluded because they did not contain a bioactive compound added to a nanoformulation, however, it is important to report that the two articles that used the nanostructured SOD enzyme found evidence of neuroprotection. The articles obtained results of protection of primary neurons *in vitro* against damage resulting from oxygen-glucose deprivation, with induction of limitation of apoptosis extension, in addition to significant reduction in cerebral infarction levels, inflammatory markers and better *in vivo* behavior in adult mice (Jiang *et al.*, 2016; Yun *et al.*, 2013).

The brain is highly vulnerable to oxidative damage, due to factors such as decreased antioxidant defenses in relation to other tissues, limited regenerative capacity, excitotoxicity, high concentrations of polyunsaturated fatty acids prone to peroxidation, and high calcium concentration (Patel, 2016). The process that results in cerebral ischemia has several mechanisms that include excitotoxicity by excitatory neurotransmitters such as glutamate, due to reductions in oxygen rates, and excessive influx of Ca^{2+} into the cell (Figure 1).

Figure 1- Simplified scheme of the mechanisms of ischemia in cell damage and the effect of nanoparticles containing antioxidant actives as neuroprotective agents.



Glu: Glucose; ca2+: calcium ion; ROS: reactive oxygen species; SOD: superoxide dismutase; GPx: glutathione peroxidase, GR: Glutathione reductase, GSH: glutathione. Source: Author's production.

The excess of intracellular Ca² + can cause several responses that lead to cell death, such as the induction of oxidative stress, release of reactive oxygen and nitrogen species, oxidation of macromolecules (proteins, lipids and DNA), mitochondrial dysfunction (alteration of the potential for membrane and pore formation) and activation of pro-apoptotic and pro-inflammatory cell signaling pathways (Heinrich, 2016). All works analyzed in this review used the measurement of antioxidant enzymes in brain tissue such as SOD, catalase, GR, LDH and GPx to obtain results of improvement in oxidative stress levels, thus demonstrating the neuroprotective effect of nanostructures. All studies obtained improvements in the activity of antioxidant enzymes. In addition, several articles also observed decreased levels of lipid peroxidation, a well-known parameter to assess oxidative stress.

With ischemic damage, levels of neuronal death from apoptosis and necrosis are high, leading to severe, often persistent cognitive and behavioral deficits (Pluta; Januszewski; Czuczwar, 2021). The results of improvement in behavioral parameters, brought by some studies that carried out these evaluations in animals, can be directly related to the modulation of the neurotransmitter system (ex. glutamate and acetylcholine), of the pro-death or pro-survival signaling pathways, and antioxidant pathways through nanostructured actives. This modulation by the active was presented in all studies, which, in one way or another, reduces the levels of reactive oxygen and nitrogen species, reduces oxidative stress, and, consequently, reduces neuronal death, conferring the observed neuroprotection and the behavior recovery.

The efficiency of nanostructures was also analyzed in two studies through pharmacokinetic evaluations. Due to the slow release of nanoparticles, these articles obtained evidence of an increase in the concentration of compounds in plasma, as well as an increase in circulatory half-life and longer retention periods in the brain of rats (Ding *et al.* 2016; Yuan *et al.* 2018).

CONCLUSION

In view of this evidence, it is possible to conclude that nanostructures containing active ingredients with antioxidant properties, such as those presented in this work, have significant neuroprotective potential. They effectively reduced oxidative stress caused by cerebral ischemic damage in all the analyzed studies, as demonstrated by positive results in several tests. While acknowledging the need for further research on the actives, this integrative review suggests that when nanostructured, these compounds hold significant potential to become part of future pharmacological strategies. Nanostructuring enhances the bioavailability of actives and provides better access to the brain, enabling them to effectively counteract the damage caused by cerebral ischemia.

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REFERENCES

AHMAD, N.; AHMAD, R.; ABBAS NAQVI, A.; *et al.* The effect of safranal loaded mucoadhesive nanoemulsion on oxidative stress markers in cerebral ischemia. **Artificial Cells, Nanomedicine, and Biotechnology**, v. 45, n. 4, p. 775-787, 2017.

AHMAD, N.; AHMAD, R.; ALAM, M. A.; *et al.* Quantification and evaluation of thymoquinone loaded mucoadhesive nanoemulsion for treatment of cerebral ischemia. **International Journal of Biological Macromolecules**, v. 88, p. 320-332, 2016.

AHMAD, N.; UMAR, S.; ASHAFAQ, M.; *et al.* A comparative study of PNIPAM nanoparticles of curcumin, demethoxycurcumin, and bisdemethoxycurcumin and their effects on oxidative stress markers in experimental stroke. **Protoplasma**, v. 250, n. 6, p. 1327-1338, 2013.

ALAJANGI, H. K. *et al.* Blood-brain barrier: emerging trends on transport models and new-age strategies for therapeutics intervention against neurological disorders. **Molecular Brain**, v. 15, n. 1, 2022.

AYUSO, M. I.; MONTANER, J. Advanced neuroprotection for brain ischemia: an alternative approach to minimize stroke damage. **Expert Opinion on Investigational Drugs**, v. 24, n. 9, p. 1137-1142, 2015.

AZADI, R.; MOUSAVI, S. E.; KAZEMI, N. M.; *et al.* Anti-inflammatory efficacy of Berberine Nanomicelle for improvement of cerebral ischemia: formulation, characterization and evaluation in bilateral common carotid artery occlusion rat model. **BMC Pharmacology and Toxicology**, v. 22, n. 1, p. 1-10, 2021.

BAO, Q.; HU, P.; XU, Y.; *et al.* Simultaneous Blood-Brain Barrier Crossing and Protection for Stroke Treatment Based on Edaravone-Loaded Ceria Nanoparticles. **ACS Nano**, v. 12, n. 7, p. 6794-6805, 2018.

CARRADORI, D. *et al.* Application of Nanomedicine to the CNS Diseases. **International Review of Neurobiology**, p. 73-113, 2016.

CHONPATHOMPIKUNLERT, P.; FAN, C.-H.; OZAKI, Y.; *et al.* Redox nanoparticle treatment protects against neurological deficit in focused ultrasound-induced intracerebral hemorrhage. **Nanomedicine**, v. 7, n. 7, p. 1029-1043, 2012.

DING, Y.; QIAO, Y.; WANG, M.; *et al.* Enhanced Neuroprotection of Acetyl-11-Keto-β-Boswellic Acid (AKBA)-Loaded O-Carboxymethyl Chitosan Nanoparticles Through Antioxidant and Anti--Inflammatory Pathways. **Molecular Neurobiology**, v. 53, n. 6, p. 3842-3853, 2016.

DONG, X. *et al.* Nanomedicine for Ischemic Stroke. **International Journal of Molecular Sciences**, v. 21, n. 20, p. 7600, 2020.

DU, Y.; HUO, Y.; YANG, Q.; *et al.* Ultrasmall iron-gallic acid coordination polymer nanodots with antioxidative neuroprotection for PET/MR imaging-guided ischemia stroke therapy. **Exploration**, v. 3, n. 1, 2023.

FABIAN, R. H.; DERRY, P. J.; REA, H. C.; *et al.* Efficacy of Novel Carbon Nanoparticle Antioxidant Therapy in a Severe Model of Reversible Middle Cerebral Artery Stroke in Acutely Hyperglycemic Rats. **Frontiers in Neurology**, v. 9, n. april, 2018.

FEIGIN, V. L.; BRAININ, M.; NORRVING, B.; *et al.* World Stroke Organization (WSO): Global Stroke Fact Sheet 2022. **International Journal of Stroke**, v. 17, n. 1, p. 18-29, 2022.

GALHO, A. R.; CORDEIRO, M. F.; RIBEIRO, S. A.; *et al.* Protective role of free and quercetin-loaded nanoemulsion against damage induced by intracerebral haemorrhage in rats. **Nanotechnology**, v. 27, n. 17, p. 175101-175114, 2016.

GANESANA, M.; ERLICHMAN, J. S.; ANDREESCU, S. Real-time monitoring of superoxide accumulation and antioxidant activity in a brain slice model using an electrochemical cytochrome c biosensor. **Free Radical Biology and Medicine**, v. 53, n. 12, p. 2240-2249, 2012.

GAO, Y.; CHEN, X.; LIU, H. A facile approach for synthesis of nano-CeO2 particles loaded co-polymer matrix and their colossal role for blood-brain barrier permeability in Cerebral Ischemia. **Journal of Photochemistry and Photobiology B: Biology**, v. 187, p. 184-189, 2018.

GHOSH, S.; SARKAR, S.; CHOUDHURY, S. T.; GHOSH, T.; DAS, N. Triphenyl phosphonium coated nano-quercetin for oral delivery: Neuroprotective effects in attenuating age related global moderate cerebral ischemia reperfusion injury in rats. **Nanomedicine: Nanotechnology, Biology and Medicine**, v. 13, n. 8, p. 2439-2450, 2017.

GUGLEVA, V.; ANDONOVA, V. Drug delivery to the brain - lipid nanoparticles-based approach. **Pharmacia**, v. 70, n. 1, p. 113-120, 2023.

HASSANZADEH, P.; ATYABI, F.; DINARVAND, R.; *et al.* Application of nanostructured lipid carriers: the prolonged protective effects for sesamol in in vitro and in vivo models of ischemic stroke via activation of PI3K signalling pathway. **DARU Journal of Pharmaceutical Sciences**, v. 25, n. 1, p. 25, 2017.

HEINRICH, I. A. Padronização da técnica de cultura organotípica de hipocampo de rato para avaliação do efeito neuroprotetor da lectina canavalia brasiliensis (conbr) em um modelo in vitro de isquemia cerebral. **Dissertação (mestrado) - Universidade Federal de Santa Catarina, Centro de Ciências Biológicas, Programa de Pós-Graduação em Neurociências**, 2016.

HOSOO, H.; MARUSHIMA, A.; NAGASAKI, Y.; *et al.* Neurovascular Unit Protection From Cerebral Ischemia-Reperfusion Injury by Radical-Containing Nanoparticles in Mice. **Stroke**, v. 48, n. 8, p. 2238-2247, 2017.

JIANG, Y.; AROUNLEUT, P.; RHEINER, S.; *et al.* SOD1 nanozyme with reduced toxicity and MPS accumulation. **Journal of Controlled Release**, v. 231, p. 38-49, 2016.

KAKKAR, V.; MUPPU, S. K.; CHOPRA, K.; *et al.* Curcumin loaded solid lipid nanoparticles: An efficient formulation approach for cerebral ischemic reperfusion injury in rats. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 85, n. 3, p. 339-345, 2013.

KANTAWALA *et al.* Revolutionizing neurotherapeutics: blood-brain barrier-on-a-chip technologies for precise drug delivery. **Annals of medicine and surgery**, v. 86, n. 5, p. 2794-2804, 2024.

LI, S.; JIANG, D.; EHLERDING, E. B.; *et al.* Intrathecal Administration of Nanoclusters for Protecting Neurons against Oxidative Stress in Cerebral Ischemia/Reperfusion Injury. **ACS Nano**, v. 13, n. 11, p. 13382-13389, 2019.

LIU, Y.; AI, K.; JI, X.; *et al.* Comprehensive Insights into the Multi-Antioxidative Mechanisms of Melanin Nanoparticles and Their Application To Protect Brain from Injury in Ischemic Stroke. **Journal of the American Chemical Society**, v. 139, n. 2, p. 856-862, 2017.

MARTIN, S. S.; ADAY, A. W.; ALMARZOOQ, Z. I.; *et al.* 2024 Heart Disease and Stroke Statistics: A Report of US and Global Data From the American Heart Association. **Circulation**, 2024.

MASSERINI, M. Nanoparticles for Brain Drug Delivery. **ISRN Biochemistry**, v. 2013, p. 1-18, 2013.

MIN, S. K.; KWON, Y. S.; CHO, M. K.; SHIN, H. S. Nasal Delivery of Antioxidants by Cholesterol--incorporated Liposomes Extends the Neuroprotective Time Window in Cerebral Ischemia. **Current Pharmaceutical Design**, v. 23, n. 40, p. 6223-6230, 2018.

MOGHADDAM, A. H.; MOKHTARI SANGDEHI, S. R.; RANJBAR, M.; HASANTABAR, V. Preventive effect of silymarin-loaded chitosan nanoparticles against global cerebral ischemia/reperfusion injury in rats. **European Journal of Pharmacology**, v. 877, p. 173066, 2020.

PATEL, M. Targeting Oxidative Stress in Central Nervous System Disorders. **Trends in Pharmacological Sciences**, v. 37, n. 9, p. 768-778, 2016.

PETRO, M.; JAFFER, H.; YANG, J.; *et al.* Tissue plasminogen activator followed by antioxidant--loaded nanoparticle delivery promotes activation/mobilization of progenitor cells in infarcted rat brain. **Biomaterials**, v. 81, p. 169-180, 2016.

PLUTA, R.; JANUSZEWSKI, S.; CZUCZWAR, S. J. Neuroinflammation in Post-Ischemic Neurodegeneration of the Brain: Friend, Foe, or Both? **International Journal of Molecular Sciences**, v. 22, n. 9, p. 4405, 2021.

SOUZA, M. T. DE; SILVA, M. D. DA; CARVALHO, R. DE. Integrative review: what is it? How to do it? **Einstein (São Paulo)**, v. 8, n. 1, p. 102-106, 2010.

TELEANU, D. *et al.* Nanomaterials for Drug Delivery to the Central Nervous System. **Nanomaterials**, v. 9, n. 3, p. 371, 2019.

WANG, L.; ZHANG, B.; YANG, X.; *et al.* Targeted alleviation of ischemic stroke reperfusion via atorvastatin-ferritin Gd-layered double hydroxide. **Bioactive Materials**, v. 20, p. 126-136, 2023.

WORLD HEALTH ORGANIZATION. WHO EMRO | Stroke, Cerebrovascular accident | Health topics. 2024. Available in: https://www.emro.who.int/health-topics/stroke-cerebrovascular-accident/index.html. Access: 29/05/2024.

YUAN, X.; FEI, F.; SUN, H.; *et al.* Tanshinol borneol ester on nanostructured lipid carriers has longer brain and systemic effector retention and better antioxidant activity in vivo. **International Journal of Nanomedicine**, v. 13, p. 2265-2274, 2018.

YUN, X.; MAXIMOV, V. D.; YU, J.; *et al.* Nanoparticles for Targeted Delivery of Antioxidant Enzymes to the Brain after Cerebral Ischemia and Reperfusion Injury. **Journal of Cerebral Blood Flow & Metabolism**, v. 33, n. 4, p. 583-592, 2013.

ZHANG, T.; LI, C.; JIA, J.; *et al.* Combination Therapy with LXW7 and Ceria Nanoparticles Protects against Acute Cerebral Ischemia/Reperfusion Injury in Rats. **Current Medical Science**, v. 38, n. 1, p. 144-152, 2018.

ZHAO, Y.; XIN, Z.; LI, N.; *et al.* Nano-liposomes of lycopene reduces ischemic brain damage in rodents by regulating iron metabolism. **Free Radical Biology and Medicine**, v. 124, p. 1-11, 2018.