Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i3.3365

Research Article

Astaxanthin Nanoemulsion Pre-Supplementation Mitigates Ischaemic Stroke Injury by Enhancing Neuroprotection and Reducing Infarcted Volume Area

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ABSTRACT

Ischaemic stroke is the third leading neurological disease that causes death around the world. One of the approaches to reduce severity after an ischaemic stroke is by preparing the brain for neuroprotection. Astaxanthin is a natural product produced by microalgae, *Haematococcus pluvialis,* against a harsh environment for its survival. It is a well-known carotenoid recognized for its anti-inflammatory and anti-apoptotic benefits. The advancements in nanomedicine of drug design and delivery systems suggest that formulating astaxanthin into a nanoemulsion could enhance its ability to penetrate the BBB and provide neuroprotection, including for ischaemic stroke injury. Rats were assigned to six groups: sham control (SCG), stroke (SG), stroke pretreated with palm olein (SOG), stroke pretreated with astaxanthin extract (SXG), stroke pretreated with astaxanthin macroemulsion (SMG), and stroke pretreated with astaxanthin nanoemulsion (SNG). Supplementation was given orally for 7 days before and once 3 hr after permanent middle cerebral artery occlusion (pMCAO). All groups were subjected to pMCAO for stroke induction except SCG, which received sham surgery. After twenty-four hr, the rats were tested for neurological tests and sacrificed for infarct volume and neuronal markers injuries quantification. This study demonstrated that pre-supplementation with astaxanthin nanoemulsion was able to minimize brain injury caused by ischemic stroke, as evidenced by a low modified neurological severity score, reduced percentage of failures between grids in the grid walking test, and increased latency on the rotarod in the rotarod test. Furthermore, pre- and post-supplementation of astaxanthin nanoemulsion demonstrated a reduction in neuronal cell death as shown by TTC staining and a reduction in neuro-ischemic markers, evidenced by a low level of S100β and NSE in the plasma. In summary, our findings suggest that astaxanthin nanoemulsion could be a promising approach in mitigating the effects of ischemic stroke.

Key words: Astaxanthin, blood-brain barrier, ischemic stroke, neuroprotection, nanomedicine

INTRODUCTION

Stroke is a leading global cause of disability and death, significantly impacting quality of life (Kuriakose & Xiao, 2020). Stroke has been affecting about 13.7 million people, with 5.5 million deaths worldwide in 2016 (Saini *et al*., 2021). It is classified into two types: ischemic and haemorrhagic (Chang, 2020). Ischemic stroke raises more concern as more ischemic stroke cases (85%) were reported compared to haemorrhagic (15%) (Abdu *et al*., 2021). Ischemic stroke occurs due to a blockage in a cerebral artery, impairing blood flow and resulting in brain deterioration, inflammation, and apoptosis (Salaudeen *et al*., 2024). Permanent middle cerebral artery occlusion (pMCAO) is one of the techniques to induce ischemic stroke by permanently blocking the middle cerebral artery in a rat model (Zeng *et al*., 2023). Following the neuroprotective window, brain cells either die (ischemic core) or are injured (penumbra) (Sodaei & Shahmaei, 2020). The penumbra has become the therapeutic target for stroke management and therapies (Yang & Liu, 2021). In recent years, more research has been shifted to neuroprotection in stroke studies to increase the percentage in protecting the brain from injuries, extension of therapeutic windows, and reduce functional loss after a stroke attack (Paul & Candelario-Jalil, 2021). Neuroprotection is a term used to describe the preservation, recovery, and regeneration of the nervous system (Haupt *et al*., 2023). More therapeutic agents, including natural products, were investigated for their wide range of pharmacological potential that might act on certain proteins, receptors, and kinases (Xie *et al*., 2021).

Carotenoids are naturally occurring compounds known for their anti-inflammatory and anti-apoptotic properties (Nabi *et al*., 2020; Terao, 2023). Astaxanthin, a xanthophyll carotenoid, is found in plants, crustaceans, and microalgae (Donoso *et al*., 2021; Meléndez-Martínez *et al*., 2022). Haematococcus pluvialis is one of the microalgae that naturally produce astaxanthin under harsh conditions such as high temperature and high salinity conditions (Ambati *et al*., 2014; Lafarga *et al*., 2021). Astaxanthin has been known to be high in antioxidant, anti-apoptotic, and anti-inflammatory properties (Si & Zhu, 2022; Nishida *et al*.,

Article History
Accepted: 4 August 2025
First version online: 30 September 2025
Cite This Article:
Mohd Shafie, A.S., Kamarudin, S.N., Meor Mohd Affandi, M.M.R. & Siran, R. 2025. Astaxanthin nanoemulsion pre-supplementation mitigates ischaemic stroke injury by enhancing neuroprotection and reducing infarcted volume area. Malaysian Applied Biology, 54(3): 110-119. https://doi.org/10.55230/mabjournal.v54i3.3365

2023). Plus, more scientific research has been done to investigate the astaxanthin therapeutic effect on human health (Nishida *et al*., 2023). The consumption of astaxanthin has shown health benefits in many diseases and conditions, including ocular, cardiovascular, cancer, and diabetes (Giannaccare *et al*., 2020; Kato *et al*., 2020; Landon *et al*., 2020; Sun *et al*., 2020). Despite its potential, research on astaxanthin's neuroprotective effects is limited due to the blood-brain barrier (BBB), which restricts molecules penetration (Wu *et al*., 2023). BBB functions as a protective layers that protect the brain from foreign molecules that might cause contamination and affect the homeostasis that minimizes the chances of the therapeutic agents entering the brain (Kadry *et al*., 2020). With the advancement in drug design and delivery, formulating astaxanthin into nanoemulsion may help overcome these barriers (Cai *et al*., 2021). Astaxanthin nanoemulsion contains fine particles less than 200 nm that might improve its bioavailability and penetration against the BBB to exert its therapeutic potential (Smejkal *et al*., 2021). Furthermore, a recent study showed astaxanthin's presence in the cortex, hippocampus, and cerebellum of rats' brains after 28 days of daily pre-supplementation with astaxanthin nanoemulsion, suggesting its neuroprotective potential in stroke-affected rats (Wan Chik *et al*., 2022). Thus, our study was designed to investigate the neuroprotective potential of astaxanthin nanoemulsion against ischemic stroke rats via pMCAO in neurolobehaviour tests, infarcted area measurements, and level of neuronal injuries.

MATERIALS AND METHODS

Animals

Forty-two male Sprague-Dawley rats (10-12 weeks old, 200-250 g) were sourced from the Laboratory Animal Facility and Management of UiTM Puncak Alam (Selangor, Malaysia) and housed individually under controlled conditions (23 ± 2°C) with ad libitum access to food and water. This study was approved by the Animal Ethics Committee of Universiti Teknologi MARA (UiTM CARE: 398/2023).

Astaxanthin extract, macroemulsion, and nanoemulsion formulation

Astaxanthin was obtained from Fuji Chemical Industries (Nakaniikawa, Toyama, Japan) in oil form (AstaReal L10), containing 10% w/w astaxanthin extracted from Haematococcus pluvialis. The astaxanthin extract, macroemulsion, and nanoemulsion were formulated with the same composition: 2% w/w astaxanthin, 2.4% w/w Tween 80, 1.6% w/w lecithin, 20% w/w palm olein, and 80% w/w purified water. The astaxanthin extract was mixed using a spatula; meanwhile, astaxanthin macroemulsion and nanoemulsion were mixed using a high-speed homogenizer (Silverstone, England) at 9000 rpm for 5 min. Only the mixture of astaxanthin nanoemulsion was further homogenized with high pressure homogenizer (Nano DEBEE, USA) at 20000 psi for 6 cycles to form the nanoemulsion (Meor Mohd Affandi *et al*., 2012). Then, the particle size of each mixture was measured using Zetasizer (Malvern Instruments, Worcestershire, UK). Only particles smaller than 200nm were accepted as nanoparticles. .

Experimental grouping

Forty-two male SD rats were randomly divided into six groups (n=7); sham control (SCG), stroke (SG), stroke pretreated with palm olein (SOG), stroke pretreated with astaxanthin extract (SXG), stroke pretreated with astaxanthin macro-emulsion (SMG), and stroke pretreated with astaxanthin nano-emulsion (SNG). The treatment groups received their respective formulation (1280 mg/kg body weight) once daily for 7 days before and 3 hr after stroke induction. SOG, SXG, SMG, and SNG were performed pMCAO for stroke induction at day 8, except for SCG, which only performed sham surgery.

Ischemic stroke induction

Ischemic stroke was induced on day 8 using the permanent middle cerebral artery occlusion (pMCAO) method as follows (Kamarudin *et al*., 2020). Rats were anesthetized with a mixture of xylazine (1.5 mL of 100 mg/mL) and ketamine (10 mL of 100 mg/mL) given intraperitoneally (0.1 mL/100 g body weight). The right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed. The CCA and ECA were permanently ligated, and a 4.0 fine MCAO suture (Doccol, USA) was inserted through a small arteriotomy in the CCA through ICA to induce ischemia until resistance was felt at the middle cerebral artery (MCA). Then, the opening of the arteriotomy in the CCA was permanently ligated using 5.0 silk suture before the incision area was stitched with 3.0 silk suture. Although no strict time limit was imposed during the pMCAO, all surgeries were completed within 15 min to minimize physiological stress and variability. Postoperatively, rats were placed in individual cages at 37°C until recovery, then moved to normal cages with ad libitum access to food and water.

Neurological test

The neurological tests (Modified Neurological test, Grid Walking test, and Rotarod test) were performed after 24 hr of induction of ischemic stroke (Kamarudin *et al*., 2020).

Modified Neurological Severity Score (mNSS)

The mNSS test was used to evaluate the severity of the stroke in rats in motor, sensory, and reflex impairments (Ruan & Yao, 2020). The scoring marks started with 0 (no stroke), 1-6 (mild injury), 7-12 (moderate injury), and 13-18 (severe injury) (Kamarudin *et al*., 2020).

Grid walking test

The Grid Walking test assessed sensory and motor impairments by placing each rat on an elevated, square-shaped grid (Shi *et al*., 2021). Foot slips were recorded when a rat's paw missed a rung, fell between rungs, or slipped off with motor impairment expressed as the percentage of foot slips relative to total footsteps (Kamarudin *et al*., 2020). Performance was recorded for 5 min using a video recorder (Handycam DCR-SX22E, Sony) and analysed by a blinded observer.

Rotarod test

The rotarod test evaluated coordination and motor movement (Eltokhi *et al*., 2021). Rats underwent three days of training, performing three trials each day. On test day, each rat was placed on a moving rotarod at 16 rpm for 200 sec, with three consecutive trials and 30 min rest between trials (Kamarudin *et al*., 2020). Results were expressed as average latency in sec and recorded for blinded analysis.

Infarction area measurement

After the neurological tests, rats were euthanized by terminal cardiac puncture after anaesthesia with a mixture of xylazine and ketamine, and brain and blood samples were collected for analysis (Kamarudin *et al*., 2020). Blood was collected from the left ventricle using a 5 mL syringe with an 18-gauge needle. The rat was then decapitated using a guillotine (NEMI Scientific Inc., USA). The brain was rinsed and chilled in ice-cold saline for 2 min before being cut into five slices (+5, +3, +1, -1, and -3 mm anterior-posterior from bregma) using an acrylic brain matrix (Ted Pella Inc, USA) and sterilized disposable blades. The brain slices were stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC) solution (Merck, USA) for 30 min at 37°C in the dark (L. Li *et al*., 2018). Images were captured using a digital camera (Lumic DMC-S5, Panasonic) and analysed using ImageJ software (National Institute of Health, USA). The total infarct volume (mm^3) was calculated by multiplying the infarcted area (mm^2) by the 2 mm thickness. An indirect formula was used to calculate the infract volume (mm^3) (Kamarudin *et al*., 2020):

RI (infarct volume in the right hemisphere) = LT (Total volume in the left hemisphere on the same brain slice) – RN (Non-infarcted area in the right hemisphere on the same brain slice)

Assessment of S100β and NSE

Blood samples were allowed to clot for 1 hr at room temperature and centrifuged at 1000 x g for 20 min to obtain serum (Kamarudin *et al*., 2020). S100 calcium-binding protein β (S100β) and neuron-specific enolase (NSE), used to evaluate cerebral tissue damage after stroke (Khandare *et al*., 2022), were measured by ELISA kits (Elabscience, USA) using a MultiskanTM microplate reader (Thermo Fisher Inc., USA) at 450 nm following the manufacturer's instructions.

Statistical analysis

Data were analysed using GraphPad Prism version 10.2.3 for Windows (GraphPad Software, CA). The Kruskal–Wallis test followed by the Mann-Whitney test was used for mNSS test data, while one-way ANOVA followed by Tukey's post hoc test was used for all other tests. Differences at $p<0.05$ were considered statistically significant.

RESULTS

Determination of particle size

The particle sizes of astaxanthin extract, astaxanthin macroemulsion, and astaxanthin nanoemulsion were 728.5 nm, 190.5 nm, and 132.6 nm, respectively, as shown in Supplementary Materials 1,2, and 3. Although astaxanthin macroemulsion produced particles with a size less than 200 nm but it has three different peaks of 202.8 nm (peak 1), 4185 nm (peak 2), and 44.88 nm (peak 3), making it unsuitable to be classified as nanoparticles due to its broad size distribution and heterogeneity. In contrast, astaxanthin nanoemulsion only exhibits a single peak at 152.3 nm, indicating uniform particle size and meeting the classification as a nanoparticle system.

Neurological test

Neurological tests of mNSS, grid walking, and rotarod test were measured to evaluate the motor function and coordination after pMCAO in rats (Ruan & Yao, 2020). SCG rats have 0 score in the stroke test, 0% grid slipping, and completed 200 sec on the rotarod as the rats are normal and healthy. The stroke score was significantly reduced in SNG compared to all other stroke groups ($p<0.001$). The percentage of grid slipping in SMG was significantly reduced compared to SG ($p<0.01$), SOG ($p<0.05$), and SXG ($p<0.05$), but significantly higher compared to SCG ($p<0.001$) and SNG ($p<0.01$). SNG recorded the lowest percentage of slipping, significantly lower than SG ($p<0.001$), SOG ($p<0.001$), SXG ($p<0.001$), and SMG ($p<0.01$). The rotarod test showed similar trends. The latency on the rotarod of SMG was significantly longer than SG ($p<0.01$), SOG ($p<0.01$), and SXG ($p<0.01$), but significantly shorter than SCG ($p<0.001$) and SNG ($p<0.001$). Interestingly, SNG showed no significant difference from SCG in the rotarod test. SNG had the longest latency on the rotarod, significantly different from all other stroke groups, SG, SOG, SXG, and SMG ($p<0.001$) as shown in Figure 1. The results of three neurological tests showed that supplementation of astaxanthin nanoemulsion in SNG showed better motor function and coordination compared to all other treatment groups.

Infarction volume

Infarction volume was measured by TTC staining analysis of brain slices. The red part of the brain indicated the healthy brain cells; meanwhile, the pink area indicated the penumbra area (reversible injury area), and the white part indicated the ischemic core (irreversible injury area) of the brain after pMCAO (Kamarudin *et al*, 2020). SCG showed no infarcted area in the brain slices, as the rat is normal and healthy without any pMCAO induction. Meanwhile, the infarcted area of SG, SOG, and SXG was significantly higher compared to SCG ($p<0.001$), SMG ($p<0.001$), and SNG ($p<0.001$). The infarcted area of SMG was significantly reduced compared to SG, SOG, and SXG ($p<0.001$) but significantly higher compared to SCG ($p<0.001$) and SNG ($p<0.001$). SNG showed significantly the smallest infarction volume compared to all stroke groups, including SMG, but significantly bigger infarction volume compared to the sham group, SNG as shown in Figure 2(A) and Figure 2(B). This result showed that supplementation of SNG was able to reduce the infarction volume better than other treatment groups, and to reduce the loss of brain cells involved in motor functions and coordination.

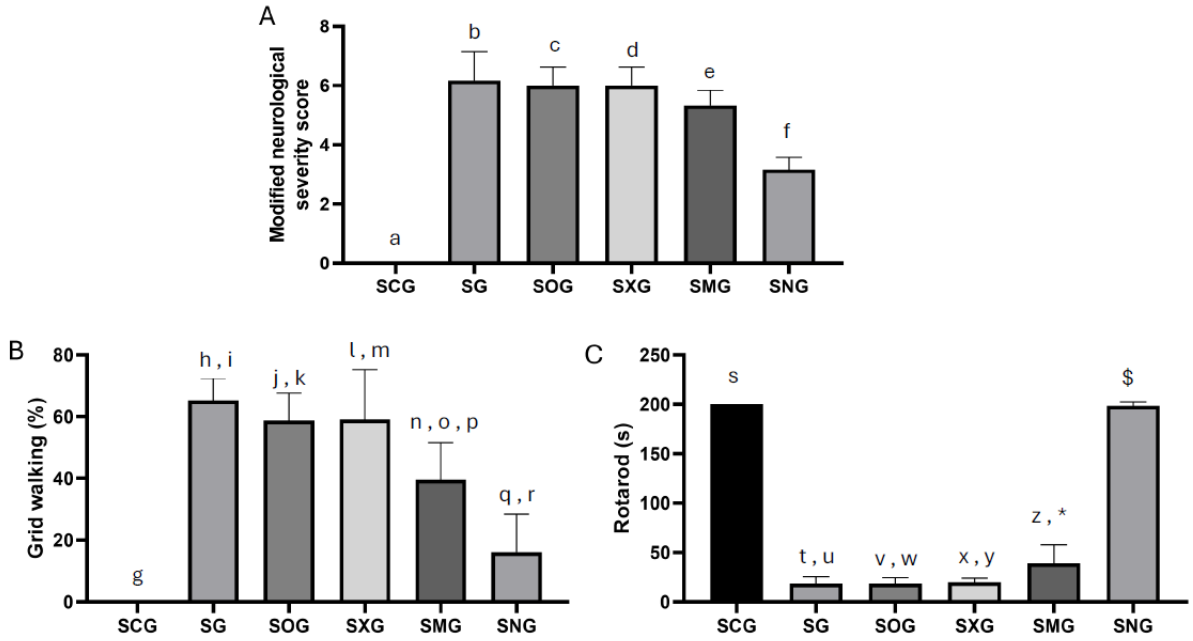

Fig.1. (A) The modified neurological severity score (mNSS). The data showed was mean ± S.E.M. (n=7) [a]p<0.001 statistical comparison between SCG with all groups. [b]p<0.001 statistical comparison between SG with SCG and SNG. [c]p<0.001 statistical comparison between SOG with SG and SNG. [d]p<0.001 statistical comparison between SXG with SG and SNG. [e]p<0.001 statistical comparison between SMG with SG and SNG. [f]p<0.001 statistical comparison between SNG with all groups. **(B)** The percentage of grid falling in the grid test. The data showed was mean ± S.E.M. (n=7) [g]p<0.001 statistical comparison between SCG with SG, SOG, SXG, and SMG. [h]p<0.001 statistical comparison between SG with SCG and SNG. [i]p<0.01 statistical comparison between SG with SMG. [j]p<0.001 statistical comparison between SOG with SG and SNG. [k]p<0.05 statistical comparison between SOG with SMG. [l]p<0.001 statistical comparison between SXG with SG and SNG. [m]p<0.05 statistical comparison between SXG with SMG. [n]p<0.001 statistical comparison between SMG with SG. [o]p<0.01 statistical comparison between SMG with SG and SNG. [p]p<0.05 statistical comparison between SMG with SOG and SXG. [q]p<0.001 statistical comparison between SNG with SG, SOG, and SXG. [r]p<0.01 statistical comparison between SNG with SMG. **(C)** The latency on the rotarod in the rotarod test. The data showed was mean ± S.E.M. (n=7) [s]p<0.001 statistical comparison between SCG with SG, SOG, SXG, and SMG. [t]p<0.001 statistical comparison between SG with SCG and SNG. [u]p<0.01 statistical comparison between SG with SMG. [v]p<0.001 statistical comparison between SOG with SG and SNG. [w]p<0.01 statistical comparison between SOG with SMG. [x]p<0.001 statistical comparison between SXG with SCG and SNG. [y]p<0.01 statistical comparison between SXG with SMG. [z]p<0.001 statistical comparison between SMG with SCG and SG. [*]p<0.01 statistical comparison between SMG with SG, SOG, and SXG. [$]$p$<0.001 statistical comparison between SNG with SG, SOG, SXG, and SMG.

Level of S100β and NSE in plasma

S100β and NSE are neuronal biochemical markers used to measure ischemic brain injuries (Makovec *et al*, 2025). In clinical settings, the levels of serum S100β and NSE were used to predict the outcomes of patients after stroke (Khandare *et al*., 2022). These neuronal markers showed correlation with the infarct size and post-stroke disabilities (Khandare *et al*., 2022). There was no significant difference in the level of S100β between SCG and SNG. The level of S100β in group SCG was significantly reduced compared to SG (p<0.001), SOG (p<0.001), SXG (p<0.001), and SMG (p<0.01). Meanwhile, the level of S100β in SG, SOG, and SXG was significantly higher compared to SCG (p<0.001), SMG (p<0.001), and SNG (p<0.001). The level of S100β was significantly reduced in SMG compared to SG (p<0.001), SOG (p<0.001), and SXG (p<0.001) but significantly higher compared to SCG (p<0.001) and SNG (p<0.001). However, the level of S100β was lowest in SNG and significantly different compared to other stroke groups, SG(p<0.001), SOG (p<0.001), SXG (p<0.001), and SMG (p<0.01), as shown in Figure 3. SCG showed significantly reduced levels of NSE compared to SG (p<0.001), SOG (p<0.001), SXG (p<0.001), SMG (p<0.001), and SNG (p<0.01). Furthermore, SG and SOG showed significantly higher levels of NSE compared to SCG (p<0.001), SCG (p<0.001), SMG (p<0.001) and SNG (p<0.001). Other than that, SXG and SMG also showed significantly higher levels of NSE compared to SCG (p<0.001) and SNG (p<0.001) but significantly lower levels of NSE compared to SG (p<0.001) and SOG (p<0.001). But the lowest level of NSE was shown in SNG, that significantly different compared to all other stroke groups, SG (p<0.001), SOG (p<0.001), SXG (p<0.01), and SMG (p<0.01) as shown in Figure 4. Both results of S100β and NSE showed no significant difference between the sham group, SCG with astaxanthin nanoemulsion group, SNG. This proved that supplementation of astaxanthin nanoemulsion can minimize the brain injuries to reduce the adverse effects of ischemic stroke insults.

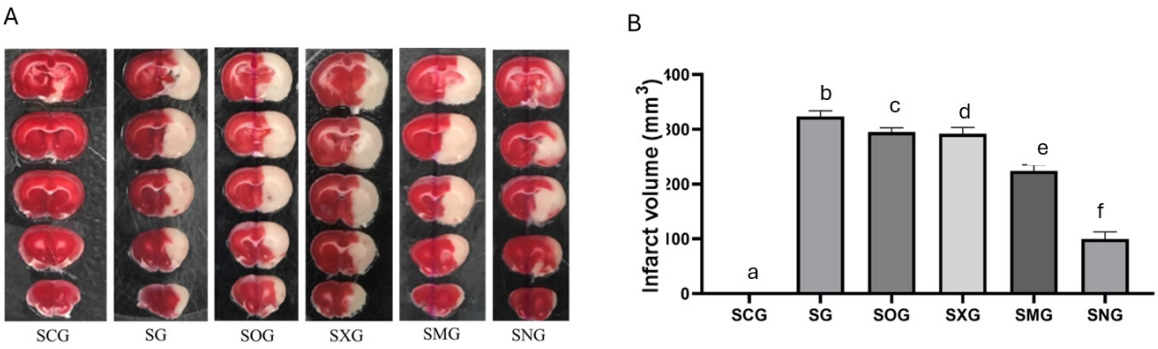

Fig. 2. (A) The infarcted area in the five brain slices of each group. The white part is the dead cells (ischemic core), and the red part is the viable cells (penumbra). **(B)** The infarct volume of each group (*n*=7). The data showed was mean ± S.E.M. [a]*p*<0.001 statistical comparison between SCG with all groups. [B]*p*<0.001 statistical comparison between SG with SCG, SMG, and SNG. [C]*p*<0.001 statistical comparison between SOG with SCG, SMG, and SNG. [D]*p*<0.001 statistical comparison between SXG with SCG, SMG, and SNG. [E]*p*<0.001 statistical comparison between SMG with all groups. [F]*p*<0.001 statistical comparison between SNG with all groups.

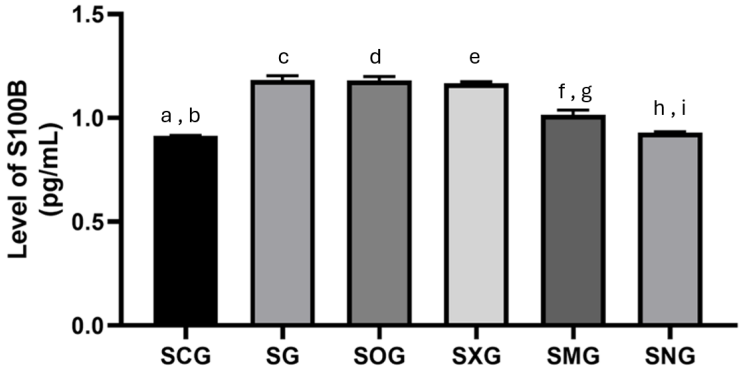

Fig. 3. The level of S100β in each group (*n*=7). The data showed was mean ± S.E.M. [a]*p*<0.001 statistical comparison between SCG with SG, SOG, and SXG. [b]*p*<0.01 statistical comparison between SCG with SMG. [c]*p*<0.001 statistical comparison between SG with SCG, SMG, and SNG. [d]*p*<0.001 statistical comparison between SOG with SCG, SMG, and SNG. [e]*p*<0.001 statistical comparison between SXG with SCG, SMG, and SNG. [f]*p*<0.01 statistical comparison between SMG with SCG and SXG. [g]*p*<0.001 statistical comparison between SMG with SNG. [h]*p*<0.001 statistical comparison between SNG with SG, SOG, and SXG. [i]*p*<0.01 statistical comparison between SNG with SMG.

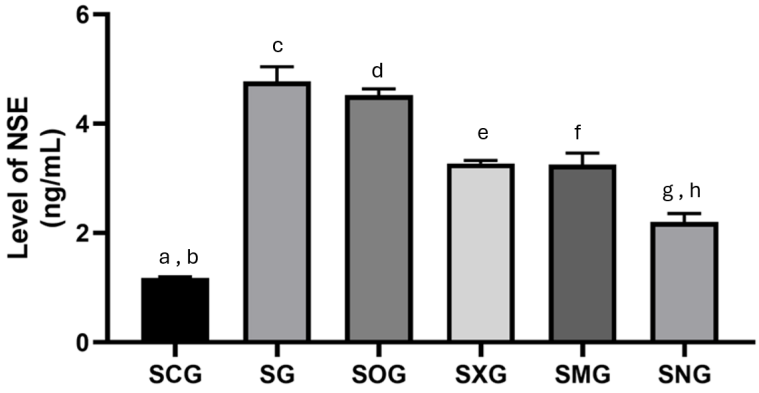

Fig. 4. The level of NSE in each group (*n*=7). The data showed was mean ± S.E.M. [a]*p*<0.001 statistical comparison between SCG with SG, SOG, SXG, and SMG. [b]*p*<0.01 statistical comparison between SCG with SNG. [c]*p*<0.001 statistical comparison between SG with SCG, SOG, SXG, SMG, and SNG. [d]*p*<0.001 statistical comparison between SOG with SCG, SG, SXG, SMG, and SNG. [e]*p*<0.001 statistical comparison between SXG with SCG, SOG, SXG, and SNG. [f]*p*<0.001 statistical comparison between SMG with SCG, SG, SOG, and SNG. [g]*p*<0.01 statistical comparison between SNG with SCG. [h]*p*<0.001 statistical comparison between SNG with SG, SOG, SXG, and SMG.

DISCUSSION

This study demonstrates that formulating astaxanthin into a nanoemulsion improved neurological function, reduced apoptosis, and minimized neuronal injuries following ischemic stroke in a rat model of pMCAO. Stroke is a neurological disease that causes injuries in the brain cells, such as apoptosis and inflammation, after the insults (Maida *et al*., 2024). Nowadays, neuroprotection is one of the strategies in reducing the adverse effects of ischemic stroke, including rapid loss of muscle functions and death of brain cells (Paul & Candelario-Jalil, 2020). Neuroprotection involves the process of retaining, preserving, recovering, and regenerating the nervous system, including the cells, structure, and functions (Haupt *et al*., 2023). Thus, more therapeutic agents and drugs were formulated to create neuroprotection in the brain to minimize the damage after an ischemic stroke attack (Frank *et al*., 2022). However, the therapeutic agents or drugs must be able to cross the blood-brain barrier (BBB) to be effective in building neuroprotection. BBB is a protective layer that encapsulates the brain to protect its homeostasis and neuronal function (J. Li *et al*., 2021). However, the tight protection of the BBB has caused limitations on the delivery and penetration of drugs and therapeutic agents, especially for neurological diseases and conditions (Achar *et al*., 2021). The limited penetration of drugs and therapeutic agents through the BBB caused insufficient concentration of drugs and therapeutic agents to exert their neuroprotective abilities (Achar *et al*., 2021). The size of therapeutic agents plays an important role in determining the ability to penetrate the blood-brain barrier (BBB) (J. Li *et al*., 2021). Moreover, BBB is also highly insoluble to large lipophilic and hydrophobic compounds (J. Li *et al*., 2021).

Astaxanthin is a lipid-soluble compound with low oral bioavailability (Abdol Wahab *et al*., 2022). Astaxanthin has been known to have anti-apoptotic and anti-inflammatory benefits toward human health against various diseases (Kohandel *et al*., 2022). Various studies have proven the protective value of astaxanthin, including in eye, skin, cardiovascular, and gastrointestinal diseases (Lin *et al*., 2020; Shatoor & Al Humayed, 2021; Lee *et al*., 2022; Gao & Zheng, 2024). However, neurological and neurodegenerative diseases such as ischemic stroke require specific modifications to allow the therapeutic agents and drugs to be facilitated and absorbed through the highly selective BBB. Thus, with the advancement of nanotechnology, the formulation of drugs and therapeutic agents for extreme conditions into nanoemulsions in the pharmaceutical field can improve the delivery and penetration for specific drug delivery (Wilson *et al*., 2022). An early study by Wan Chik *et al* (2022) has shown the presence of astaxanthin nanoemulsion in the rat's brain region, including cortex, hippocampus, and cerebellum, after supplementation of oral astaxanthin nanoemulsion for 30 days at 160 mg/kg bw. This showed that astaxanthin nanoemulsion can be used for neurological and neurodegenerative diseases as a supplement to build neuroprotection or as a treatment. In this study, the sham group represents the normal and healthy rats that were given free flow of food pellets and water for 7 days before being subjected to sham surgery by only creating an incision on the neck area under anaesthesia without inducing permanent middle cerebral artery occlusion (pMCAO). Furthermore, this study also incorporates the group SOG that received supplementation of palm oil as the vehicle group, as palm oil is one of the substances used for the formulation in astaxanthin extract, macroemulsion, and nanoemulsion. The vehicle group was used to eliminate the possible neuroprotective effect of palm oil that might mask the actual neuroprotective effect of astaxanthin. The supplementation of palm oil, astaxanthin extract, astaxanthin macroemulsion, and nanoemulsion was given 7 days before and 3 hr after the induction of pMCAO. The 3 hr after pMCAO is the duration of the neuroprotective window of ischemic stroke before the brain cells become completely untreatable after the insult (Chia *et al*., 2023). The main purpose of supplementing astaxanthin 7 days before and 3 hr after pMCAO is to build neuroprotection to the brain cells to reduce the insult by aiming to improve the reversible damage of the penumbra area and reduce the irreversible damage of the ischemic core in the brain cells

This study showed that supplementation of astaxanthin macroemulsion and nanoemulsion can demonstrate neuroprotective effects in reducing neurological impairment, infarcted volume in the brain, and neuronal markers. However, the supplementation of astaxanthin nanoemulsion provided significantly better protection compared to all other stroke groups, including those with macro-emulsion. These can be reflected from the neurobehavior test, such as the stroke score of mNSS, percentage of grid walking, and latency of rotarod. Although the stroke score of mNSS in all stroke groups should be mild injury scores, which were between 1 to 6 but SNG showed the lowest stroke scores mean at 3.167 compared to SMG at 5.333, SXG and SOG at 6.000, and SG at 6.167. The mNSS test includes various aspects of motor and coordination points, including the elevation fixation test, open space test, abnormal movement test, sensory test, and reflex test (Ruan & Yao, 2020). This proved that SNGs have lower overall neurological deficits, that able to retain better motor function and coordination following the ischemic stroke, indicating a potential protective or therapeutic effect of the nanoemulsion. Similarly, SNG showed the lowest percentage of grid slipping in the grid walking test compared to all other stroke groups, including SG, SOG, SXG, and SMG. Although SMG also showed a significantly lower percentage of slipping compared to SG, SOG, and SMG but it has a higher percentage of slipping compared to SNG. This proved that SNG has better motor coordination to move around the grids without falling between the grids. Furthermore, the latency of the rotarod also showed that SNG has the significantly longest latency at almost 200 sec, which was the maximum time set for the rotarod test, compared to all other stroke groups. Although SMG also showed significantly longer latency compared to other stroke groups, such as SG, SOG, and SXG but it still has significantly shorter latency on the rotarod compared to SNG. SMG might record significantly longer latency compared to SG, SOG, and SXG, as the astaxanthin macroemulsion might be able to exert its neuroprotective effect after ischemic insult, but SMG only recorded a mean latency of 39.05 sec compared to SNG at 198.30 sec. Thus, the supplementation of astaxanthin nanoemulsion in SNG exerts better neuroprotection capabilities after ischemic stroke insults in maintaining the muscle movement and coordination. These results of neurological tests proved that SNG showed better neuroprotection ability compared to SMG, as SNG exhibited the lowest stroke scores, the lowest percentages of grid falling, and the longest latency on the rotarod. This also might show that the SNG group might maintain better motor coordination and function after the ischemic insult compared to SMG. Thus, these findings suggest that nanoemulsion supplementation may contribute to enhanced neuroprotection, potentially reducing the severity of motor impairment and lowering the risk of paralysis following ischemic stroke.Similar trends were observed in the result of infarcted volume and level of S100β and NSE, reflecting the degree of neuronal injury. Although SMG differed significantly from other stroke groups except SNG, SNG demonstrated even better protection by producing the smallest infarcted area and the lowest level of S100β and NSE in serum. SNG recorded the smallest infarcted volume after 24 hr of pMCAO compared to all

other stroke groups, including SMG. Although SMG also showed a significant reduction of infarcted volume compared to SG, SOG, and SXG but SMG still showed significantly higher infarcted volume compared to SNG. Thus, the supplementation of SNG showed better results in reducing the infarcted volume in the brains of the rats. Infarcted volume represents the ischemic core, which is a dead brain cell. As shown in Figure 2 (A), the ischemic core is the white part of the brain that has already undergone irreversible death and has lost all its functions (Salaudeen *et al*., 2024). The death of brain cells after an ischemic attack led to loss of muscle functions and coordination, which causes paralysis (Qi *et al*., 2023). Although apoptotic markers were not quantified in this study, TTC staining of the brain slices provides a visual representation of the apoptotic damage, as indicated by the formation of white areas in the brain following an ischemic stroke (Wang *et al*., 2023). In this study, TTC staining was used as a rapid and practical histological technique to estimate and differentiate the viable and non-viable brain tissue based on the mitochondrial enzyme activity (Li *et al*, 2018). The red-stained areas represent the viable and healthy brain tissue; meanwhile, the unstained or white areas represent the non-viable and infarcted areas where cell death occurred (Li *et al*, 2018). However, TTC staining analysis also has limitations as it does not measure apoptosis in the brain tissue. Therefore, future studies will aim to quantify apoptotic biomarkers such as Bcl-2 and BAX using ELISA analysis to obtain a comprehensive understanding of the apoptotic response following ischemic stroke. Furthermore, this study also demonstrated that the level of S100β was significantly lower in SNG compared to all other stroke groups, including SMG. Although SMG also showed a significantly low level of S100β compared to SG, SOG, and SXG but it was significantly higher compared to SNG. Similarly, SNG showed a significantly low level of NSE compared to all stroke groups. However, SMG only showed a significantly low level of NSE compared to SG and SOG, but there was no significant difference between SMG with SXG, and a significantly higher level of NSE compared to S100β. The result of neuronal markers proved that supplementation of astaxanthin nanoemulsion in SNG showed better neuroprotection effect as it was able to significantly reduce both levels of S100β and NSE compared to all stroke groups, compared to astaxanthin macroemulsion that only showed significant differences in S100β for all stroke groups but not in NSE level. Thus, supplementation of astaxanthin nanoemulsion might serve better neuroprotection in reducing the infarct volume in the brain and reducing the neuronal markers of S100β and NSE after ischemic stroke insults compared to supplementation of astaxanthin macroemulsion. This is consistent with the studies by (M. Zhang *et al*., 2017; Y. Wu *et al*., 2024), which suggest that astaxanthin may help in reducing apoptosis and inflammation by inhibiting the activation of cytokines such as Bax, IL-6, and p38 in astrocytes and macrophages. Astaxanthin supplementation in other neurological diseases, such as traumatic brain injury, Alzheimer's disease, and seizures, has shown a reduction in inflammatory reactions with the decreased production of pro-inflammatory genes and cytokines (Y. Chang *et al*., 2018; Rahman *et al*., 2019; X. S. Zhang *et al*., 2021).

This study showed that SNG has better neuroprotection effects in reducing neurological impairments, infarcted volume, and neurological damage compared to other treatments in SXG and SMG. This is because formulation into nanoemulsion reduces particle size to less than 100 nm compared to 100 µm only for macro emulsion (Singh & Pulikkal, 2022). Thus, astaxanthin nanoemulsion formulation produces fine and uniform particle size that increases the chances of penetrating the BBB (Smejkal *et al*., 2021). Additionally, the fine particles of nanoemulsion increase the interfacial area of astaxanthin, improving its bioavailability throughout the body system (Domínguez-Hernández *et al*., 2016). Nanoemulsions also help in stabilizing and maintaining the physicochemical properties of drugs and therapeutic agents that are crucial for drug delivery (Mushtaq *et al*., 2023). Formulation of nanoemulsion produces fine particles with ultra-low interfacial tension, larger oil and water interfacial areas, and can reduce the enzymatic hydrolysis in the gastrointestinal tract for drugs and therapeutic agents' absorption (Sabjan *et al*., 2019). Furthermore, the formulation of astaxanthin nanoemulsion also improved its bioavailability, permeability, and absorption (Chen *et al*., 2023). Additionally, the fast and effective neuroprotective effects shown in SNG correlate with the pharmacokinetic study by (Meor Mohd Affandi *et al*., 2012), demonstrated that astaxanthin nanoemulsion has higher bioavailability compared to macro-emulsion, as indicated by higher values of maximum observed plasma concentration (C^{max}) and area under the curve ($AUC^{0-\infty}$), reflecting the total amount of drug reaching systemic circulation. Moreover, astaxanthin nanoemulsion reaches maximum concentration (T^{max}) faster and has a longer half-life ($t^{1/2}$) compared to macro-emulsion (Meor Mohd Affandi *et al*., 2012). The results of a pharmacokinetics study have proved that astaxanthin in nanoemulsion is a better solution compared to macro-emulsion for therapeutic potential, especially in neurological diseases as ischemic stroke (Meor Mohd Affandi *et al*., 2012). The past study by Meor Mohd Affandi *et al*. (2012) proved that the formulation of astaxanthin into nanoemulsion improved its bioavailability, which is a vital aspect, especially in designing and formulating drugs for clinical use, compared to astaxanthin in the form of extract and macroemulsion. Other than that, the formulation of astaxanthin nanoemulsion can produce higher surface zone particles and more free energy compared to macro-emulsion for better absorption in the stomach during digestion (Sabjan *et al*., 2019). Other than that, the formulation of astaxanthin nanoemulsion also helps in better absorption as it can avoid hepatic first-pass metabolism, as it follows lipid-based drug delivery (Preeti *et al*., 2023).

The positive results of supplementation of astaxanthin nanoemulsion for 7 days before and 3 hr after pMCAO might be due to the building of neuroprotection in the brain cells before the ischemic insults. The astaxanthin nanoemulsion accumulation in the brain might help in reducing the activation of signalling pathways such as TLR4, NF-kB, and MAPK that are involved in the activation of apoptotic and inflammatory cytokines that elicit apoptotic and inflammatory responses (Wang & Qi, 2022; Briones-Valdivieso *et al*., 2024). Thus, accumulation of astaxanthin nanoemulsion in the brain cells before the ischemic attack and before the end of the neuroprotective window might be a solution for neuroprotection to reduce the adverse effects, especially reducing the percentage of paralysis and improving the quality of life of the affected patient. Furthermore, astaxanthin nanoemulsion has better commercial values as therapeutic agents compared to macroemulsion, as it can mask the metallic and bitter taste of medicine and drugs that could prevent side effects such as vomiting and diarrhea (Preeti *et al*., 2023). Moreover, astaxanthin can also be formulated into various forms, including gels, creams, aerosols, and sprays, which can be administered by multiple routes such as oral, topical, intravenous, and intramuscular (Preeti *et al*., 2023). Lastly, macro-emulsion also usually has the limitation of sedimentation, flocculation, and creaming during long storage after its formulation, that unlikely to happen in nanoemulsion formulation (Meor Mohd Affandi *et al*., 2012; Sabjan *et al*., 2019), making astaxanthin nanoemulsion a better choice to be formulated into therapeutic agents and drugs.

CONCLUSION

This study provides evidence that pre-supplementation with astaxanthin in nanoemulsion for 7 days before and 3 hr following ischemic stroke was significantly more effective in mitigating the negative effects of ischemic stroke in rats compared to using only astaxanthin extract or astaxanthin macro-emulsion. The formulation of astaxanthin into nanoemulsion appears to enhance bioavailability, permeability, and penetration through the blood-brain barrier (Abdelazim *et al*., 2023). These findings underscore the potential of astaxanthin nanoemulsion as a promising therapeutic approach for enhancing neuroprotection against ischemic stroke.

ACKNOWLEDGEMENTS

This study was supported by FRGS Grant (FRGS/1/2021/SKK0/UITM/03/11). Special acknowledgement to Centre of Neuroscience Research (NeuRON), Faculty of Medicine, UiTM Sungai Buloh, Institute of Medical Molecular Biotechnology, UiTM Sungai Buloh, Laboratory Animal Control Unit, UiTM Sungai Buloh, and Faculty of Pharmacy, UiTM Puncak Alam.

ETHICAL STATEMENT

This study was approved by the Animal Ethics Committee of Universiti Teknologi MARA (UiTM CARE: 398/2023).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdelazim, K., Ghit, A., Assal, D., Dorra, N., Noby, N., Khattab, S.N., El Feky, S.E. & Hussein, A. 2023. Production and therapeutic use of astaxanthin in the nanotechnology era. Pharmacological Reports, 75(4): 771-790. https://doi.org/10.1007/s43440-023-00488-y

Abdol Wahab, N.R., Meor Mohd Affandi, M.M.R., Fakurazi, S., Alias, E. & Hassan, H. 2022. Nanocarrier system: state-of-the-art in oral delivery of astaxanthin. Antioxidants, 11(9): 1676. https://doi.org/10.3390/antiox11091676

Abdu, H., Tadese, F. & Seyoum, G. 2021. Comparison of ischemic and hemorrhagic stroke in the medical ward of Dessie Referral Hospital, Northeast Ethiopia: a retrospective study. Neurology Research International, 2021: 9996958. https://doi.org/10.1155/2021/9996958

Achar, A., Myers, R. & Ghosh, C. 2021. Drug delivery challenges in brain disorders across the blood–brain barrier: novel methods and future considerations for improved therapy. Biomedicines, 9(12): 1834. https://doi.org/10.3390/biomedicines9121834

Ambati, R.R., Moi, P.S., Ravi, S. & Aswathanarayana, R.G. 2014. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications - a review. Marine Drugs, 12(1): 128-152. https://doi.org/10.3390/md12010128

Briones-Valdivieso, C., Briones, F., Orellana-Urzúa, S., Chichiarelli, S., Saso, L. & Rodrigo, R. 2024. Novel multi-antioxidant approach for ischemic stroke therapy targeting the role of oxidative stress. Biomedicines, 12(3): 501. https://doi.org/10.3390/biomedicines12030501

Cai, W., Wu, Q., Yan, Z.Z., He, W.Z., Zhou, X.M., Zhou, L.J., Zhang, J.Y. & Zhang, X. 2021. Neuroprotective effect of ultrasound triggered astaxanthin release nanoparticles on early brain injury after subarachnoid hemorrhage. Frontiers in Chemistry, 9: 775274. https://doi.org/10.3389/fchem.2021.775274

Chang, J.C. 2020. Stroke classification: critical role of unusually large von Willebrand factor multimers and tissue factor on clinical phenotypes based on novel "two-path unifying theory" of hemostasis. Clinical and Applied Thrombosis/Hemostasis, 26: 1076029620913634. https://doi.org/10.1177/1076029620913634

Chang, Y., Lu, C.W., Chen, Y.J., Lin, T.Y., Huang, S.K. & Wang, S.J. 2018. Astaxanthin protects against kainic acid-induced seizures and pathological consequences. Neurochemistry International, 116: 85-94. https://doi.org/10.1016/j.neuint.2018.02.008

Chen, S., Wang, J., Feng, J. & Xuan, R. 2023. Research progress of Astaxanthin nano-based drug delivery system: Applications, prospects and challenges?. Frontiers in Pharmacology, 14: 1102888. https://doi.org/10.3389/fphar.2023.1102888

Chia, G.Y.Y., Yeo, S., Ho, J.S.Y., Jou, E., Yeo, L.L.L. & Tan, B.Y.Q. 2023. Neuroprotective agents in acute ischemic stroke. Exploration of Neuroprotective Therapy, 3(1): 47-70. https://doi.org/10.37349/ent.2023.00037

Domínguez-Hernández, C.R., García-Alvarado, M.A., García-Galindo, H.S., Salgado-Cervantes, M.A., Beristáin, C.I., Stability, C.I., Whitaker, S., Domínguez-Hernández, C.R., García-Alvarado, M.A., García-Galindo, H.S., Salgado-Cervantes, M.A. & Beristáin, C.I. 2016. Stability, antioxidant activity and bioavailability of nano-emulsified astaxanthin. Revista Mexicana de Ingeniería Química, 15(2): 457-468.

Donoso, A., González-Durán, J., Muñoz, A.A., González, P.A. & Agurto-Muñoz, C. 2021. Therapeutic uses of natural astaxanthin: an evidence-based review focused on human clinical trials. Pharmacological Research, 166: 105479. https://doi.org/10.1016/j.phrs.2021.105479

Eltokhi, A., Kurpiers, B. & Pitzer, C. 2021. Comprehensive characterization of motor and coordination functions in three adolescent wild-type mouse strains. Scientific Reports, 11(1): 6434. https://doi.org/10.1038/s41598-021-85858-3

Frank, D., Zlotnik, A., Boyko, M. & Gruenbaum, B.F. 2022. The development of novel drug treatments for stroke patients: a review. International Journal of Molecular Sciences, 23(10): 5796. https://doi.org/10.3390/ijms23105796

Gao, H. & Zheng, H. 2024. Astaxanthin expedites the healing of acute skin wounds in rats by facilitating M2 macrophage polarization and enhancing collagen secretion. Discovery Medicine, 36(185): 1180. https://doi.org/10.24976/discov.med.202436185.108

Giannaccare, G., Pellegrini, M., Senni, C., Bernabei, F., Scorcia, V. & Cicero, A.F.G. 2020. Clinical applications of astaxanthin in the treatment of ocular diseases: emerging insights. Marine Drugs, 18(5): 239. https://doi.org/10.3390/md18050239

Haupt, M., Gerner, S.T., Bähr, M. & Doeppner, T.R. 2023. Neuroprotective strategies for ischemic stroke—future perspectives.

International Journal of Molecular Sciences, 24(5): 4334. https://doi.org/10.3390/ijms24054334
Kadry, H., Noorani, B. & Cucullo, L. 2020. A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity. Fluids and Barriers of the CNS, 17(1): 69. https://doi.org/10.1186/s12987-020-00230-3
Kamarudin, S.N., Iezhitsa, I., Tripathy, M., Alyautdin, R. & Ismail, N.M. 2020. Neuroprotective effect of poly(lactic-co-glycolic acid) nanoparticle-bound brain-derived neurotrophic factor in a permanent middle cerebral artery occlusion model of ischemia in rats. Acta Neurobiologiae Experimentalis, 80(1): 1-18. https://doi.org/10.21307/ane-2020-001
Kato, T., Kasai, T., Sato, A., Ishiwata, S., Yatsu, S., Matsumoto, H., Shitara, J., Murata, A., Shimizu, M., Suda, S., Hiki, M., Naito, R. & Daida, H. 2020. Effects of 3-month astaxanthin supplementation on cardiac function in heart failure patients with left ventricular systolic dysfunction-a pilot study. Nutrients, 12(6): 1896. https://doi.org/10.3390/nu12061896
Khandare, P., Saluja, A., Solanki, R.S., Singh, R., Vani, K., Garg, D. & Dhamija, R.K. 2022. Serum S100B and NSE levels correlate with infarct size and bladder-bowel involvement among acute ischemic stroke patients. Journal of Neurosciences in Rural Practice, 13(2): 218-225. https://doi.org/10.1055/s-0042-1743214
Kohandel, Z., Farkhondeh, T., Aschner, M., Pourbagher-Shahri, A.M. & Samarghandian, S. 2022. Anti-inflammatory action of astaxanthin and its use in the treatment of various diseases. Biomedicine and Pharmacotherapy, 145: 112179. https://doi.org/10.1016/j.biopha.2021.112179
Kriegel, C., Festag, M., Kishore, R.S.K., Roethlisberger, D. & Schmitt, G. 2020. Pediatric safety of polysorbates in drug formulations. Children, 7(1): 1. https://doi.org/10.3390/children7010001
Kuriakose, D. & Xiao, Z. 2020. Pathophysiology and treatment of stroke: present status and future perspectives. International Journal of Molecular Sciences, 21(20): 7609. https://doi.org/10.3390/ijms21207609
Lafarga, T., Sánchez-Zurano, A., Morillas-España, A. & Acién-Fernández, F.G. 2021. Extremophile microalgae as feedstock for high-value carotenoids: a review. International Journal of Food Science and Technology, 56(10): 4934-4941. https://doi.org/10.1111/ijfs.15069
Landon, R., Gueguen, V., Petite, H., Letourneur, D., Pavon-Djavid, G. & Anangnostou, F. 2020. Impact of astaxanthin on diabetes pathogenesis and chronic complications. Marine Drugs, 18(357): 357. https://doi.org/10.3390/md18070357
Lee, J., Kim, M.H. & Kim, H. 2022. Anti-oxidant and anti-inflammatory effects of astaxanthin on gastrointestinal diseases. International Journal of Molecular Sciences, 23(24): 15471. https://doi.org/10.3390/ijms232415471
Li, J., Zheng, M., Shimoni, O., Banks, W.A., Bush, A.I., Gamble, J.R. & Shi, B. 2021. Development of novel therapeutics targeting the blood–brain barrier: from barrier to carrier. Advanced Science, 8(16): 2101090. https://doi.org/10.1002/advs.202101090
Li, L., Yu, Q. & Liang, W. 2018. Use of 2,3,5-triphenyltetrazolium chloride-stained brain tissues for immunofluorescence analyses after focal cerebral ischemia in rats. Pathology Research and Practice, 214(1): 174-179. https://doi.org/10.1016/j.prp.2017.11.016
Lin, W.N., Kapupara, K., Wen, Y.T., Chen, Y.H., Pan, I.H. & Tsai, R.K. 2020. Haematococcus pluvialis-derived astaxanthin is a potential neuroprotective agent against optic nerve ischemia. Marine Drugs, 18(2): 85. https://doi.org/10.3390/md18020085
Maida, C.D., Norrito, R.L., Rizzica, S., Mazzola, M., Scarantino, E.R. & Tuttolomondo, A. 2024. Molecular pathogenesis of ischemic and hemorrhagic strokes: background and therapeutic approaches. International Journal of Molecular Sciences, 25(12): 6297. https://doi.org/10.3390/ijms25126297
Makovec, M., Skitek, M., Šimnovec, L. & Jerin, A. 2025. Neuron-specific enolase and S100B as biomarkers of ischemic brain injury during surgery. Clinics and Practice, 15(4): 74. https://doi.org/10.3390/clinpract15040074
Meléndez-Martínez, A.J., Mandić, A.I., Bantis, F., Böhm, V., Borge, G.I.A., Brnčić, M., Bysted, A., Cano, M.P., Dias, M.G., Elgersma, A., Fikselová, M., García-Alonso, J., Giuffrida, D., Gonçalves, V.S.S., Hornero-Méndez, D., Kljak, K., Lavelli, V., Manganaris, G.A., Mapelli-Brahm, P. & O'Brien, N. 2022. A comprehensive review on carotenoids in foods and feeds: status quo, applications, patents, and research needs. Critical Reviews in Food Science and Nutrition, 62(8): 1999-2049. https://doi.org/10.1080/10408398.2020.1867959
Meor Mohd Affandi, M.M.R., Julianto, T. & Majeed, A.B.A. 2012. Enhanced oral bioavailability of astaxanthin with droplet size reduction. Food Science and Technology Research, 18(4): 549-554. https://doi.org/10.3136/fstr.18.549
Mushtaq, A., Mohd Wani, S., Malik, A.R., Gull, A., Ramniwas, S., Ahmad Nayik, G., Ercisli, S., Marc, R.A., Ullah, R. & Bari, A. 2023. Recent insights into nanoemulsions: their preparation, properties and applications. Food Chemistry: X, 18: 100684. https://doi.org/10.1016/j.fochx.2023.100684
Nabi, F., Arain, M.A., Rajput, N., Alagawany, M., Soomro, J., Umer, M., Soomro, F., Wang, Z., Ye, R. & Liu, J. 2020. Health benefits of carotenoids and potential application in poultry industry: a review. Journal of Animal Physiology and Animal Nutrition, 104(6): 1809-1818. https://doi.org/10.1111/jpn.13375
Nishida, Y., Berg, P.C., Shakersain, B., Hecht, K., Takikawa, A., Tao, R., Kakuta, Y., Uragami, C., Hashimoto, H., Misawa, N. & Maoka, T. 2023. Astaxanthin: past, present, and future. Marine Drugs, 21(10): 514. https://doi.org/10.3390/md21100514
Paul, S. & Candelario-Jalil, E. 2021. Emerging neuroprotective strategies for the treatment of ischemic stroke: an overview of clinical and preclinical studies. Experimental Neurology, 335: 113518. https://doi.org/10.1016/j.expneurol.2020.113518
Preeti, Sambhakar, S., Malik, R., Bhatia, S., Al Harrasi, A., Rani, C., Saharan, R., Kumar, S., Geeta & Sehrawat, R. 2023. Nanoemulsion: an emerging novel technology for improving the bioavailability of drugs. Scientifica, 2023: 6640103. https://doi.org/10.1155/2023/6640103
Qi, H., Tian, D., Luan, F., Yang, R. & Zeng, N. 2024. Pathophysiological changes of muscle after ischemic stroke: a secondary consequence of stroke injury. Neural Regeneration Research, 19(4): 737-746. https://doi.org/10.4103/1673-5374.382221
Rahman, S.O., Panda, B.P., Parvez, S., Kaundal, M., Hussain, S., Akhtar, M. & Najmi, A.K. 2019. Neuroprotective role of astaxanthin in hippocampal insulin resistance induced by Aβ peptides in animal model of Alzheimer's disease. Biomedicine and Pharmacotherapy, 110: 47-58. https://doi.org/10.1016/j.biopha.2018.11.043
Ruan, J. & Yao, Y. 2020. Behavioral tests in rodent models of stroke. Brain Hemorrhages, 1(4): 171-184. https://doi.org/10.1016/j.hest.2020.09.001

Sabjan, K.B., Munawar, S.M., Rajendiran, D., Vinoji, S.K. & Kasinathan, K. 2019. Nanoemulsion as oral drug delivery - a review. Current Drug Research Reviews, 12(1): 4-15. https://doi.org/10.2174/2589977511666191024173508

Saini, V., Guada, L. & Yavagal, D.R. 2021. Global epidemiology of stroke and access to acute ischemic stroke interventions. Neurology, 97(20): S6-S16. https://doi.org/10.1212/WNL.0000000000012781

Salaudeen, M.A., Bello, N., Danraka, R.N. & Ammani, M.L. 2024. Understanding the pathophysiology of ischemic stroke: the basis of current therapies and opportunity for new ones. Biomolecules, 14(3): 305. https://doi.org/10.3390/biom14030305

Shatoor, A.S. & Al Humayed, S. 2021. Astaxanthin ameliorates high-fat diet-induced cardiac damage and fibrosis by upregulating and activating SIRT1. Saudi Journal of Biological Sciences, 28(12): 7012-7021. https://doi.org/10.1016/j.sjbs.2021.07.079

Shi, X., Bai, H., Wang, J., Wang, J., Huang, L., He, M., Zheng, X., Duan, Z., Chen, D., Zhang, J., Chen, X. & Wang, J. 2021. Behavioral assessment of sensory, motor, emotion, and cognition in rodent models of intracerebral hemorrhage. Frontiers in Neurology, 12: 667511. https://doi.org/10.3389/fneur.2021.667511

Si, P. & Zhu, C. 2022. Biological and neurological activities of astaxanthin. Molecular Medicine Reports, 26(4): 12816. https://doi.org/10.3892/mmr.2022.12816

Singh, I.R. & Pulikkal, A.K. 2022. Preparation, stability and biological activity of essential oil-based nano emulsions: a comprehensive review. OpenNano, 8: 100066. https://doi.org/10.1016/j.onano.2022.100066

Smejkal, G.B., Ting, E.Y., Nambi, K.N.A., Schumacher, R.T. & Lazarev, A.V. 2021. Characterization of astaxanthin nanoemulsions produced by intense fluid shear through a self-throttling nanometer range annular orifice valve-based high-pressure homogenizer. Molecules, 26(10): 2856. https://doi.org/10.3390/molecules26102856

Sodaei, F. & Shahmaei, V. 2020. Identification of penumbra in acute ischemic stroke using multimodal MR imaging analysis: a case report study. Radiology Case Reports, 15(10): 2041-2046. https://doi.org/10.1016/j.radcr.2020.07.066

Sun, S.Q., Zhao, Y.X., Li, S.Y., Qiang, J.W. & Ji, Y.Z. 2020. Anti-tumor effects of astaxanthin by inhibition of the expression of STAT3 in prostate cancer. Marine Drugs, 18(8): 415. https://doi.org/10.3390/MD18080415

Terao, J. 2023. Revisiting carotenoids as dietary antioxidants for human health and disease prevention. Food and Function, 14(17): 7799-7824. https://doi.org/10.1039/d3fo02330c

Wan Chik, M., Meor Mohd Affandi, M.M.R. & Singh, G.K.S. 2022. Detection of astaxanthin at different regions of the brain in rats treated with astaxanthin nanoemulsion. Journal of Pharmacy and Bioallied Sciences, 14(1): 25-30. https://doi.org/10.4103/jpbs.jpbs_464_21

Wang, S. & Qi, X. 2022. The putative role of astaxanthin in neuroinflammation modulation: mechanisms and therapeutic potential. Frontiers in Pharmacology, 13: 916653. https://doi.org/10.3389/fphar.2022.916653

Wang, Y., Zhang, Q., Zhang, S., Qi, J. & Li, L. 2023. The superiority and feasibility of 2,3,5-triphenyltetrazolium chloride–stained brain tissues for molecular biology experiments based on microglial properties. Animal Models and Experimental Medicine, 6(2): 111-119. https://doi.org/10.1002/ame2.12312

Wilson, R.J., Li, Y., Yang, G. & Zhao, C.X. 2022. Nanoemulsions for drug delivery. Particuology, 64: 85-97. https://doi.org/10.1016/j.partic.2021.05.009

Wu, D., Chen, Q., Chen, X., Han, F., Chen, Z. & Wang, Y. 2023. The blood–brain barrier: structure, regulation, and drug delivery. Signal Transduction and Targeted Therapy, 8(1): 217. https://doi.org/10.1038/s41392-023-01481-w

Wu, Y., Bashir, M.A., Shao, C., Wang, H., Zhu, J. & Huang, Q. 2024. Astaxanthin targets IL-6 and alleviates the LPS-induced adverse inflammatory response of macrophages. Food and Function, 15(8): 4207-4222. https://doi.org/10.1039/d4fo00610k

Xie, Q., Li, H., Lu, D., Yuan, J., Ma, R., Li, J., Ren, M., Li, Y., Chen, H., Wang, J. & Gong, D. 2021. Neuroprotective effect for cerebral ischemia by natural products: a review. Frontiers in Pharmacology, 12: 607412. https://doi.org/10.3389/fphar.2021.607412

Yang, S.H. & Liu, R. 2021. Four decades of ischemic penumbra and its implication for ischemic stroke. Translational Stroke Research, 12(6): 937-945. https://doi.org/10.1007/s12975-021-00916-2

Zeng, L., Hu, S., Zeng, L., Chen, R., Li, H., Yu, J. & Yang, H. 2023. Animal models of ischemic stroke with different forms of middle cerebral artery occlusion. Brain Sciences, 13(7): 1007. https://doi.org/10.3390/brainsci13071007

Zhang, M., Cui, Z., Cui, H., Wang, Y. & Zhong, C. 2017. Astaxanthin protects astrocytes against trauma-induced apoptosis through inhibition of NKCC1 expression via the NF-KB signaling pathway. BMC Neuroscience, 18(1): 67. https://doi.org/10.1186/s12868-017-0358-z

Zhang, X.S., Lu, Y., Li, W., Tao, T., Peng, L., Wang, W.H., Gao, S., Liu, C., Zhuang, Z., Xia, D.Y., Hang, C.H. & Li, W. 2021. Astaxanthin ameliorates oxidative stress and neuronal apoptosis via SIRT1/NRF2/Prx2/ASK1/p38 after traumatic brain injury in mice. British Journal of Pharmacology, 178(5): 1114-1132. https://doi.org/10.1111/bph.15346