

Analysis of the neuroprotective activity of myrtle secondary metabolites in an ischemia-reperfusion model in rats: Overall electrical activity and neurological deficit

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Abstract

Introduction: The aim of the study was to investigate the effects of myrtle secondary metabolites (hydrolate, syrup I, and syrup II) on overall brain electrical activity and the severity of neurological deficits in rats in simulated ischemia-reperfusion.

Materials and Methods: Ischemia-reperfusion was modeled in 60 male Wistar rats using 30-minute occlusion of the left common carotid artery followed by restoration of blood flow. The animals were divided into six groups (n=10): sham-operated (SO); ischemia control (I/C); a comparison group Thiocetam; and experimental groups that received intragastrically administered myrtle hydrolate (Hydrolate), syrup I (Syrup I), and syrup II (Syrup II) at a dose of 2.5 mL/kg daily for 21 days.

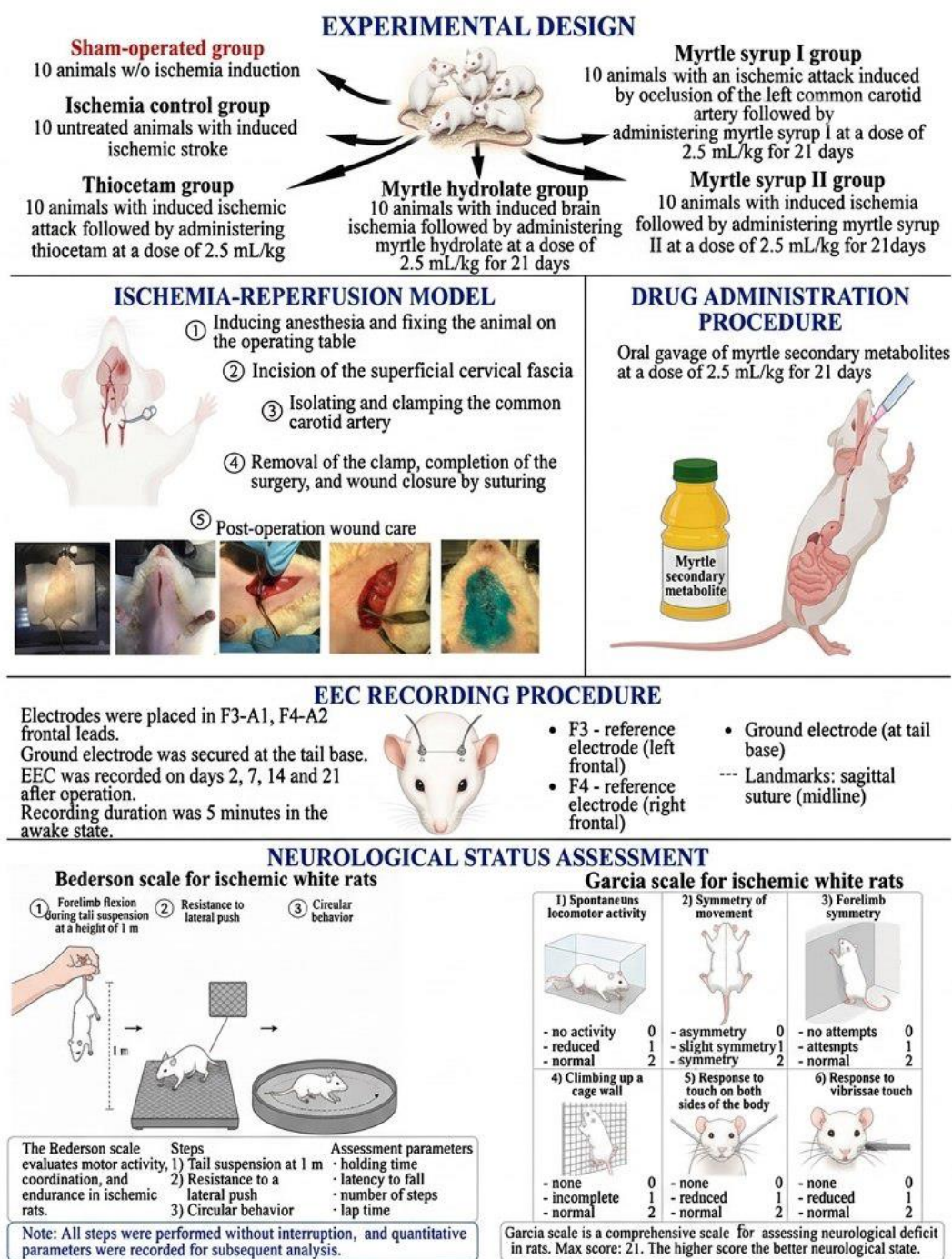
Results: Ischemia caused persistent suppression of EEG amplitudes (by 40–61%), spectrum restructuring with a predominance of delta (+47–63%) and theta rhythms (+41–119%) and suppression of alpha rhythm (–54–75%), as well as severe neurological deficit. Hydrolate had a moderate effect in the early period, but by day 21, amplitudes had fallen by 12–14% below the ischemia control (I/C) levels. Syrup I restored amplitudes (+29–82%), spectrum (alpha rhythm +151–345%, delta rhythm –36%), and neurological status by days 7–14. Syrup II restored neurological deficit by day 2 (0.08 points on the Bederson scale versus 1.67 in I/C); by day 7, the neurological deficit was fully restored, with EEG amplitudes increasing by 17–63% and alpha rhythm – by 104–272%.

Discussion: The differences revealed in the neuroprotective activity of the three myrtle secondary metabolite compositions correlate with their chemical profiles. In terms of the intensity of their neuroprotective effect, the myrtle secondary metabolites were ranked in the following order: Hydrolate < Syrup I ≈ Thiocetam < Syrup II. **Conclusion:** Myrtle secondary metabolites, particularly Syrup II with its high flavonoid content, exert a complex neuroprotective effect, which manifests in the coordinated restoration of electrophysiological and behavioral parameters.



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Graphical Abstract



Keywords

ischemia-reperfusion; *Myrtus communis*; secondary metabolites; electroencephalography; neurological deficit; myricetin; phenolic acids; neuroprotection; rats

Introduction

Ischemic stroke is the second leading cause of death worldwide, with an annual mortality rate of over 5.5 million. Up to 50% of surviving patients become disabled due to persistent neurological

deficits, including motor, sensory, and cognitive impairments (Aggul et al. 2022). In the Russian Federation, stroke is the leading cause of disability: approximately 30% of stroke survivors require assistance, and 20% are unable to move independently (Skvortsova et al. 2018). The pathogenesis of ischemic brain injury is based on a decrease in cerebral blood flow to below 20 mL/100 g/min, which leads to hypoxia, neuronal energy deficit, oxidative stress, and neuroinflammation (Al-Snafi et al. 2024). Microglial activation, the release of proinflammatory cytokines (IL-1 β , IL-6, TNF- α), and the accumulation of reactive oxygen species play a key role in the secondary injury cascade, which leads to apoptotic and necrotic neuronal death in the ischemic penumbra (Alsbrook et al. 2023; Ren et al. 2025).

Despite some advances in the development of therapeutic approaches, the efficacy of the existing neuroprotective methods remains insufficient. The only approved pharmacological agent to treat acute ischemic stroke is tissue plasminogen activator, the use of which is limited by a narrow therapeutic window (up to 4.5 hours) and a high risk of hemorrhagic complications (Dabbaghi et al. 2023). Numerous attempts to introduce neuroprotective drugs that have demonstrated efficacy in preclinical studies have failed in clinical trials (Alsbrook et al. 2023); hence the relevance of searching for new natural compounds with multitarget cerebroprotective effects and the ability to modulate the key links in the pathogenesis of ischemic injury (Paul and Candelario-Jalil 2021; Ren et al. 2025).

A promising source of biologically active substances is myrtle (*Myrtus communis* L.), an evergreen plant of the Myrtaceae family, endemic to the Mediterranean and widely cultivated on the southern coast of Crimea (Hui et al. 2021). Myrtle secondary metabolites contain a complex of phenolic compounds, flavonoids (myricetin, quercetin, myrtillin, catechin), tannins, essential oils (1,8-cineole, α -pinene, linalool), and organic acids with antioxidant, anti-inflammatory, antibacterial, and neuroprotective properties (Uysal et al. 2023; Al-Snafi et al. 2024). Myrtle extracts have previously been shown to improve cognitive functions in experimental hypertension, to exhibit anxiolytic and antidepressant activity, and to protect neurons from oxidative stress *in vitro* (Ma et al. 2020). The flavonoid myricetin, found in myrtle fruits and leaves, inhibits activation of the NF- κ B signaling pathway, reduces the production of proinflammatory cytokines, activates the Nrf2/ARE antioxidant defense pathway, and prevents neuronal apoptosis (Imran et al. 2021; Narayan et al. 2021).

We studied the vasoactive effect of secondary metabolites of common myrtle (*Myrtus communis* L.), the predominant components of which are phenolic acids (Syrup I), flavonoids (Syrup II), and myrtle essential oil (hydrolate) (Chuyan et al. 2024). The vasotropic activity of Syrups I and II was shown to depend on the duration of administration to animals: on the first day, the response of skin microvessels involved a decrease in precapillary tone, vasodilation, and a decrease in blood pressure (BP); after a 7-fold administration of Syrup I and 14-fold administration of Syrup II, microvascular vasoconstriction and an increase in blood pressure developed; by the 21st day of observation, vasoconstrictor responses had weakened, and the values of the studied parameters had approached the control values, i.e., physiological norms. Throughout the entire study, the hydrolate only caused a tendency toward vasodilation of microvessels and a decrease in blood pressure in rats. These results demonstrated the potential for further research on myrtle secondary metabolites in models of pathological processes associated with circulatory disorders, such as ischemic stroke.

This study proposes a comprehensive assessment of neurological status and brain bioelectrical activity, which makes it possible to objectively characterize the severity of the central nervous system (CNS) damage during ischemic injury and the effectiveness of therapeutic interventions, such as the administration of myrtle secondary metabolites. Electroencephalography (EEG) is a sensitive, noninvasive method that reflects the functional state of neurons, and quantitative analysis of the spectral power of EEG rhythms allows for the assessment of the severity of cerebral dysfunction during ischemia (Pu et al. 2020; Salatin et al. 2023). Behavioral tests (the Garcia scale, the Bederson scale) allow for the quantitative assessment of the severity of sensorimotor deficits during the post-ischemic period and are widely used in experimental stroke studies in animal (Imran et al. 2021).

The aim of the present study was to study the effect of myrtle secondary metabolites (hydrolate, syrup I and syrup II) on the overall electrical activity of the brain and the severity of neurological deficit in rats in an induced ischemia-reperfusion model.

Materials and Methods

The experimental part of the study was carried out at the Experimental Physiology and Biophysics Core Facilities Center in the Department of Human and Animal Physiology and Biophysics at V. I. Vernadsky Crimean Federal University (Russia).

Animals

The experiments were performed on 60 mature white male Wistar rats weighing 220–280 g. The animals were raised and provided by the Rappolovo Laboratory Animal Nursery (Leningrad region, Russia).

Animal housing and maintenance

The animals were maintained under standard vivarium conditions in accordance with the Russian Federation State Standard (GOST 33216-2014 “Guidelines for Accommodation and Care of Animals. Species-specific Provisions for Laboratory Rodents and Rabbits”). Temperature conditions (18–22°C) and relative humidity (no more than 55% of the established norm according to GOST 33216-2014) in the vivarium provided an adequate living environment for the animals, which were kept under combined lighting with a controlled 12-hour light-dark cycle, with free access to water (GOST 33215-2014 “Guidelines for Accommodation and Care of Animals. Environment, Housing and Management”) and complete granulated feed. All manipulations with animals were carried out in accordance with GOST R 53434-2009 dated December 2, 2009, the Rules of Laboratory Practice for Preclinical Studies, ethical standards approved by legal acts of the Russian Federation, the principles of the Basel Declaration and the recommendations of the Ethics Committee on Bioethics of V.I. Vernadsky Crimean Federal University (Minutes No. 10 dated December 6, 2022).

Compounds and substances used

The study object was the following pharmaceutical substances: two types of extracts and myrtle hydrolate prepared at the Nikitsky Botanical Garden – National Scientific Center of the Russian Academy of Sciences (Russia) from dried myrtle leaves (*Myrtus communis* L.), collected during the fruiting period. Raw material extraction and extract preparation were performed in accordance with the method described in detail by Takasugi et al. (2024).

The test samples and drinking water (for the sham-operated and control groups) were administered orally to the animals by intragastric gavage at a dose of 2.5 mL/kg in the morning (9:00–11:00 Moscow time) once for 21 days postoperatively. The choice of a 2.5 mL/kg dose was based on the results of preliminary pharmacological studies (Chuyan et al. 2024).

The reference drug Thiocetam® (ART-PHARM LLC, Russia) was administered intragastrically at a dose of 250 mg/kg (2.5 mL/kg) daily for 21 days postoperatively. The choice of the dose was based on the literature data on the pronounced cerebroprotective and antioxidant effects of Thiocetam at a dose of 200–250 mg/kg (2.5 mL/kg) in experimental ischemia (Kalchuk 2013; Sikiryńska et al. 2021).

Study design

The animals were divided into 6 groups: 1) Sham-operated (SO, n=10) – the animals underwent surgery according to the ischemia-reperfusion modeling protocol, but without clamping the common carotid artery, followed by 21 intragastric administrations of drinking water; 2) Ischemia control (I/C, n=10) – the animals underwent surgical modeling of ischemia-reperfusion followed by 21 intragastric administrations of drinking water; 3) Myrtle hydrolate (n=10) – animals underwent surgical modeling of ischemia-reperfusion followed by 21 intragastric administrations of myrtle hydrolate; 4) Myrtle syrup I group – the animals (n=10) underwent surgical modeling of ischemia-reperfusion followed by administration of myrtle syrup I (containing 39.6 mg/L of phenolic (gallic and ellagic) acids); 5) Myrtle syrup II group – the animals (n=10) underwent surgical modeling of ischemia-reperfusion followed by administration of myrtle syrup II (containing flavonoids (187 mg/L of myricetin)); 6) Reference therapy group, which received the comparison drug Thiocetam.

Ischemia-reperfusion modeling

Ischemia-reperfusion was induced by 30-minute occlusion of the left common carotid artery followed by reperfusion (Ma et al. 2020). Surgery was performed under general anesthesia using zoletil (0.03 mg/kg, intramuscularly, Zoetis, USA) and meditin (0.06 mg/kg, intramuscularly, API-SAN LLC, Russia). After anesthesia onset, a skin incision was made in the neck extending from the level of the thyroid cartilage to the upper third of the sternum; the left common carotid artery was isolated and a vascular clamp was applied for 30 minutes, upon which the clamp was removed, restoring blood flow. The wound was sutured layer by layer and treated with an antiseptic. Postoperatively, the animals were administered 0.2 mL of diclofenac (Diclofenac 25 mg/mL, Solofarm, Grotex LLC, Russia) for analgesia. Animals in the sham-operated group (SO) underwent similar surgical procedures, but without clamping the artery.

EEG recording

EEG was recorded on days 2, 7, 14, and 21 postoperatively using a 32-channel Neuron-Spectrum-5 electroencephalograph (Neurosoft LLC, Russia), equipped with an ADC with a sampling frequency of 5000 Hz and a 16-bit resolution; the bandwidth was 0.5-60 Hz. Cup electrodes with a diameter of 7-10 mm, filled with electrode paste to ensure their firm contact with the skin, were used. The electrodes were placed in the F3-A1 and F4-A2 (frontal) leads. The ground electrode was fixed at the base of the tail. The recording duration was 5 minutes. The amplitude parameters (minimum, maximum, mean, and total amplitude in μV^2) and spectral power of EEG rhythms (delta: 0.5-4 Hz, theta: 4-8 Hz, alpha: 8-13 Hz, low-frequency beta: 13-20 Hz, high-frequency beta: 20-35 Hz) were analyzed using built-in software.

Neurological deficit assessment

Neurological deficit was assessed on days 2, 7, 14, and 21 postoperatively using the Garcia scale and the Bederson scale (Imran et al. 2021). The Garcia scale includes assessment of six parameters (on a scale from 0 to 3): spontaneous activity in the cage for 5 minutes, symmetry of four-limb movements, symmetry of forelimb extension when suspended by the tail, ability to climb a wire cage wall, response to touch on each side of the body, and response to vibrissae touch. The maximum score is 18 (no abnormalities) and the minimum is 3 (maximum abnormalities). The Bederson scale assesses three parameters (on a scale from 0 to 3): forelimb flexion during tail suspension at a height of 1 m, resistance to lateral push (prevention of slipping), and circular behavior. A score of 0 corresponds to normal state, while a score of 3 points indicates the most severe impairment.

Statistical data processing

Statistical data were processed using Statistica 10.0 software package (StatSoft, USA). Normality of distribution was tested using the Shapiro-Wilk test. Due to the lack of a normal distribution, the nonparametric Mann-Whitney U-test was used to assess the significance of differences between the groups. Data are presented as the arithmetic mean $\pm$ standard error of the mean ($M \pm m$). Differences were considered significant at $p < 0.05$.

Results

Ischemia-reperfusion modeling resulted in a significant and persistent suppression of overall brain electrical activity in rats of the I/C group throughout the entire observation period (Table 1). On day 2 after occlusion, the minimum amplitude in the F3-A1 lead decreased by 53.3% ($p < 0.05$), the maximum – by 41.7% ($p < 0.05$), the mean – by 42.4% ($p < 0.05$), and the total – by 47.0% ($p < 0.05$) relative to such in the SO group. On day 7, a significant decrease of 34-44% ($p < 0.05$) persisted for all amplitude parameters. On day 14, the maximum suppression was observed: the minimum amplitude decreased by 56.4% ($p < 0.05$), maximum – by 60.8% ($p < 0.05$), mean – by 60.5% ($p < 0.05$), and total – by 58.3% ($p < 0.05$) relative to such in the SO group. On day 21, the parameters remained significantly reduced relative to the SO group: minimum amplitude – by 61.2%, maximum – by 41.6%, mean – by 42.7%, and total – by 44.6%. These patterns indicate a progressive impairment of neuronal functional activity and the development of secondary ischemic injury, which is consistent with literature data on the pathophysiology of cerebral ischemia (Tao et al. 2020).

Spectral analysis of EEG rhythms revealed a shift in the frequency spectrum characteristic of ischemic injury (Table 2). On day 2, delta rhythm power increased by 46.9% ($p < 0.05$), and theta rhythm power – by 41.2% ($p < 0.05$) relative to such in the SO group. At the same time, a significant inhibition of fast-wave activity was observed: the alpha rhythm decreased by 54.1% ($p < 0.05$), low-frequency beta – by 48.6% ($p < 0.05$), and high-frequency beta – by 72.1% ($p < 0.05$) relative to such in the SO group. On day 14, the rhythm imbalance reached its maximum: the delta rhythm exceeded the control values by 43.0% ($p < 0.05$), the theta rhythm – by 99.3% ($p < 0.05$), while the alpha rhythm reduced by 73.2% ($p < 0.05$), low-frequency (LF) beta – by 58.8% ($p < 0.05$), and high-frequency (HF) beta – by 69.9% ($p < 0.05$). On day 21, there was a significant predominance of slow-wave activity (delta rhythm +62.8%, theta rhythm +118.7%, $p < 0.05$) with profoundly suppressed fast rhythms (alpha -74.5%, low-frequency beta -72.5%, high-frequency beta -77.9%, $p < 0.05$). The increase in the power of slow rhythms reflects disruption of thalamocortical interactions and the development of structural changes in brain tissue, while the suppression of alpha and beta activity indicates a decrease in the functional activity of neurons (Salatin et al. 2023; Thapa et al. 2023).

Table 1. Overall brain electrical activity in rats in the ischemia-reperfusion model with the administration of myrtle secondary metabolites (F3-A1 lead)

Day	Group	Minimal amplitude, μV^2	Maximum amplitude, μV^2	Mean amplitude, μV^2	Total amplitude, μV^2
2	SO	10.31±1.02	154.95±3.70	82.63±1.35	218.66±7.46
	I/C	4.82±0.39*	90.36±7.12*	47.59±3.57*	115.89±4.37*
	Thiocetam	6.93±0.39*#	115.42±3.07*#	61.18±1.97*#	159.43±5.17*#
	Hydrolate	6.63±0.22*#	108.20±2.26*#	57.41±1.14*#	152.27±2.17*#
	Syrup I	7.75±0.38*#	124.16±5.30*#	65.96±2.65*#	176.21±2.52*#‡
	Syrup II	7.37±0.62*#	144.02±13.00#	75.70±6.57#	165.68±11.80*#
7	SO	11.21±0.26	164.23±7.13	87.72±3.48	222.66±8.46
	I/C	6.33±0.16*	108.22±3.70*	57.28±1.91*	132.43±3.58*
	Thiocetam	7.54±0.26*#	116.84±1.04*#	62.19±0.52*#	165.22±1.37*#
	Hydrolate	7.52±0.11*#	116.10±0.39*#	61.68±0.23*#	157.60±3.89*#
	Syrup I	7.40±0.41*#	121.89±2.67*#	64.65±1.39*#	170.23±3.84*#
	Syrup II	6.65±0.27*#	123.53±0.55*#	65.09±0.37*#	163.48±3.85*#
14	SO	11.40±1.89	188.74±7.83	100.07±4.63	252.03±12.22
	I/C	4.97±0.42*	74.07±5.94*	39.52±3.07*	105.11±10.33*
	Thiocetam	7.08±0.18*#	118.53±1.55*#	62.80±0.78*#	167.88±1.17*#
	Hydrolate	6.77±0.21*#	106.98±4.43*#	56.87±2.28*#	147.92±6.95*#
	Syrup I	7.94±0.34*#	135.09±6.75*#	71.51±3.4*#	178.37±10.95*#
	Syrup II	6.64±0.34*#	121.34±8.80*#	63.99±4.52*#	157.05±6.79*#
21	SO	12.46±0.61	202.29±9.99	107.37±4.70	269.06±22.85
	I/C	4.83±0.21*	118.17±4.15*	61.50±2.14*	149.09±2.94*
	Thiocetam	7.34±0.13*#	130.88±0.99*#	69.11±0.53*#	176.18±0.94*#
	Hydrolate	6.11±0.42*#	102.02±0.84*#‡	54.06±0.56*#‡	131.20±7.16*#‡
	Syrup I	8.16±0.77*#	159.51±7.02*#	83.84±3.72*#	192.54±4.36*#
	Syrup II	7.01±0.52*#	133.92±2.93*#	70.47±1.60*#	174.11±8.85*#

Note: * – significant differences at $p<0.05$ compared to such in SO; # – significant differences at $p<0.05$ compared to such in I/C; ‡ – $p<0.05$ compared to such in Thiocetam, SO – sham-operated, I/C – ischemia control.

Table 2. Spectral power indices of EEG rhythms in rats in an ischemia-reperfusion model with the administration of myrtle secondary metabolites (F3-A1 lead)

Day	Group	Delta-rhythm	Theta-rhythm	Alpha-rhythm	Low-frequency beta	High-frequency beta
2	SO	122.03±0.41	37.52±1.78	32.74±0.59	9.68±0.66	6.16±0.60
	I/C	179.24±3.55*	52.96±1.23*	15.03±0.98*	4.98±0.34*	1.72±0.09*
	Thiocetam	140.78±2.96#	40.55±0.73#	25.33±1.12#	3.36±0.11#	2.22±0.15#
	Hydrolate	157.62±1.79#	38.70±0.66#	17.78±0.94#	2.00±0.12#	2.09±0.08#
	Syrup I	158.52±2.92#	47.60±0.67#	20.74±1.17#	3.99±0.27#	2.42±0.22#
	Syrup II	148.82±3.47#	46.66±2.05#	17.07±0.48#	4.54±0.03#	2.37±0.14#
7	SO	120.33±0.01	34.16±0.29	30.51±0.54	9.37±0.57	9.65±0.49
	I/C	166.91±2.74*	59.76±0.70*	11.20±0.56*	4.24±0.18*	1.79±0.22*
	Thiocetam	135.67±4.10*#	44.59±2.01#	28.15±2.08#	5.27±0.18*#	3.44±0.33*#
	Hydrolate	146.50±2.65*#	51.84±0.71*#	21.27±0.56*#	4.96±0.21*#	3.68±0.22*#
	Syrup I	147.56±4.38*#	42.24±2.50#	28.16±2.08#	5.14±0.18*#	3.24±0.49*#
	Syrup II	134.52±3.19#	32.09±1.96#	22.84±1.33*#	6.30±0.44*#	4.94±0.31*#
14	SO	110.93±1.90	32.82±0.48	34.33±1.62	11.19±0.65	10.28±0.43
	I/C	158.66±2.41*	65.42±1.43*	9.21±0.75*	4.61±0.34*	3.09±0.08*
	Thiocetam	117.98±0.27#	34.33±0.78#	33.16±3.10#	7.07±0.60#	4.59±0.34#
	Hydrolate	133.88±3.23*#	52.87±1.50*#	27.52±0.47*#	5.66±0.25*#	3.99±0.31*#
	Syrup I	117.03±4.95#	32.66±2.00#	34.95±3.58#	6.61±0.39*#	4.14±0.20*#
	Syrup II	105.35±3.20#	18.84±0.37#	34.28±1.07#	7.83±0.39*#	5.13±0.25*#
21	SO	97.75±0.19	26.16±0.72	39.70±1.19	12.66±1.07	10.98±0.93
	I/C	159.09±2.65*	57.20±2.25*	10.12±0.73*	3.48±0.29*	2.43±0.13*
	Thiocetam	99.92±1.84#	24.71±0.55#	41.63±1.70#	8.98±0.57#	6.00±0.49*#
	Hydrolate	110.39±1.97#	39.70±3.02#	32.54±2.11#	7.47±0.64*#	4.38±0.36*#
	Syrup I	101.18±3.01#	23.56±1.28#	45.03±2.63#	8.93±0.57*#	6.26±0.58*#
	Syrup II	104.74±3.96#	23.16±1.72#	33.48±1.44#	7.06±0.32*#	5.49±0.49*#

Note: * – significant differences at $p<0.05$ compared to such in SO; # – significant differences at $p<0.05$ compared to such in I/C; ‡ – $p<0.05$ compared to such in Thiocetam; SO – sham-operated; I/C – ischemia control.

Neurological deficit in the I/C group was at its maximum on day 2 after surgery (Table 3). According to the Garcia scale, spontaneous activity decreased by 55.6% ($p<0.05$), symmetry of movements – by 50.0% ($p<0.05$), forelimb symmetry – by 44.4% ($p<0.05$), climbing up a cage wall – by 55.6% ($p<0.05$), response to body touch – by 51.5% ($p<0.05$), and response to vibrissae touch – by 50.0% ($p<0.05$). According to the Bederson scale, on day 2 the indicators were the following: tail suspension – 1.83 ± 0.11 points, prevention of slipping – 1.50 ± 0.15 points, and circular behavior – 1.66 ± 0.22 points ($p<0.001$ compared to such in SO). By day 7, partial functional recovery was observed: according to the Garcia scale, indicators reached 61-94% of the SO level. By day 14, most indicators approached the control values (83-100%). By day 21, the following residual deficits persisted: symmetry of movements and forelimb symmetry – 94.4% of such in SO ($p<0.05$), tail suspension and prevention of slipping according to the Bederson scale – 0.16 ± 0.11 points ($p<0.05$). These data are consistent with the results obtained by other scientists who demonstrated the persistence of neurological deficit for 21 days after 30-minute carotid artery occlusion (Imran et al. 2021; Ma et al. 2020).

Table 3. Neurological deficit indicators in rats according to the Garcia scale in the ischemia-reperfusion model with the administration of myrtle secondary metabolites (scores)

Day	Group	Spontaneous activity	Symmetry of movements	Forelimb symmetry	Climbing up a cage wall	Response to body touch	Response to vibrissae touch
2	SO	3.00±0	3.00±0	3.00±0	3.00±0	2.75±0.13	3.00±0
	I/C	1.33±0.22*	1.50±0.15*	1.67±0.22*	1.33±0.22*	1.33±0.14*	1.50±0.15*
	Thiocetam	2.50±0.25#	2.20±0.20#	2.00±0.00	2.75±0.25#	2.00±0.20*#	2.25±0.25#
	Hydrolate	2.67±0.14#	2.16±0.21#	2.00±0.25	2.50±0.23#	2.50±0.15*#	2.67±0.14#
	Syrup I	1.50±0.30	1.75±0.13	2.25±0.25	2.75±0.13#	2.00±0.21#	2.25±0.25#
	Syrup II	3.00±0‡	2.00±0.00#	2.00±0.00	2.75±0.11#	2.33±0.13#	3.00±0.00‡
7	SO	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0
	I/C	2.50±0.23*	2.33±0.33*	2.50±0.34*	2.83±0.11	1.83±0.32*	2.00±0.25*
	Thiocetam	3.00±0	2.50±0.00	2.50±0.00	3.00±0	2.75±0.00	3.00±0
	Hydrolate	2.83±0.11	2.50±0.15	2.17±0.21	2.83±0.11	2.50±0.15#	2.67±0.14#
	Syrup I	3.00±0	2.50±0.15	2.50±0.15	3.00±0	2.75±0.13#	3.00±0#
	Syrup II	3.00±0	2.25±0.13	2.25±0.13	2.75±0.13	2.75±0.13#	3.00±0#
14	SO	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0
	I/C	2.83±0.11	2.83±0.11	2.83±0.11	3.00±0	2.50±0.23*	2.67±0.14*
	Thiocetam	3.00±0	2.75±0.25	2.75±0.25	3.00±0	3.00±0	3.00±0
	Hydrolate	2.83±0.11	2.83±0.11	2.33±0.14	2.83±0.11	2.83±0.11	2.83±0.11
	Syrup I	3.00±0	2.75±0.13	2.50±0.15	3.00±0	3.00±0	3.00±0
	Syrup II	3.00±0	3.00±0.00	2.50±0.15	2.75±0.13	3.00±0	3.00±0
21	SO	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0
	I/C	3.00±0	2.83±0.11*	2.83±0.11*	3.00±0	2.83±0.11	3.00±0
	Thiocetam	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0
	Hydrolate	3.00±0	2.83±0.11	2.83±0.11	3.00±0	3.00±0	3.00±0
	Syrup I	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0	3.00±0
	Syrup II	3.00±0	3.00±0.00	3.00±0.00	3.00±0	3.00±0	3.00±0

Note: * – significant differences at $p<0.05$ compared to such in SO; # – significant differences at $p<0.05$ compared to such in I/C; ‡ – $p<0.05$ compared to such in Thiocetam; SO – sham-operated, I/C – ischemia control.

Effect of thiocetam on EEG and neurological deficit parameters

The use of the reference drug Thiocetam resulted in pronounced cerebroprotective effects at all stages of the post-ischemia period (Tables 1-4). On day 2 after ischemia-reperfusion, in animals that had received Thiocetam, the total EEG amplitude in the F3-A1 lead increased by 37.6% ($p<0.05$) relative to such in the I/C group, and the maximum amplitude increased by 27.7% ($p<0.05$) (Table 1). Spectral analysis on day 2 demonstrated a significant decrease in delta rhythm power by 21.4% ($p<0.05$) and theta rhythm power – by 23.4% ($p<0.05$) compared to such in the control. Moreover, alpha rhythm power was 68.6% higher than such in I/C ($p<0.05$) (Table 2).

By day 7, further recovery of bioelectrical activity was observed in the Thiocetam group: the total amplitude exceeded the control values by 24.8% ($p<0.05$), and alpha rhythm power increased by 151.3% ($p<0.05$), reaching $28.15\pm2.08 \mu V^2$, which was virtually identical to the values in sham-operated animals. By days 14-21, all amplitude and spectral parameters in the Thiocetam group remained at the level which was significantly higher than such in the I/C, approaching the physiological norms.

Table 4 – Neurological deficit indicators in rats according to the Bederson scale in the ischemia-reperfusion model with the administration of myrtle secondary metabolites (scores)

Day	Group	Suspension by tail at a height of 1 m	Prevention of slipping	Circular behavior	Average score
2	SO	0	0	0	0
	I/C	1.83±0.11*	1.50±0.15*	1.66±0.22*	1.67
	Thiocetam	0.50±0.00	0.50±0.00	0.33±0.33	0.33
	Hydrolate	0.66±0.22	0.50±0.23	0.33±0.14	0.50
	Syrup I	0.25±0.13	0.50±0.15	0.75±0.25	0.50
	Syrup II	0.75±0.13	0.50±0.15	0	0.08‡
7	SO	0	0	0	0
	I/C	0.50±0.15*	0.33±0.14*	0.66±0.28*	0.50
	Thiocetam	0	0	0	0
	Hydrolate	0.66±0.14	0.16±0.11	0.16±0.11	0.33
	Syrup I	0	0	0	0
	Syrup II	0.25±0.13	0.25±0.13	0	0.17
14	SO	0	0	0	0
	I/C	0.33±0.14*	0.33±0.22	0.33±0.14*	0.33
	Thiocetam	0	0	0	0
	Hydrolate	0.50±0.15	0.16±0.11	0	0.22
	Syrup I	0	0	0	0
	Syrup II	0	0	0	0
21	SO	0	0	0	0
	I/C	0.16±0.11	0.16±0.11	0	0.11
	Thiocetam	0	0	0	0
	Hydrolate	0.16±0.11	0	0	0.05
	Syrup I	0	0	0	0
	Syrup II	0	0	0	0

Note: * – $p < 0.05$ compared to such in SO; # – $p < 0.05$ compared to such in I/C; ‡ – $p < 0.05$ compared to such in Thiocetam; SO – sham-operated, I/C – ischemia control.

The obtained electrophysiological data are consistent with the dynamics of neurological status (Tables 3-4). By day 2 after induced ischemia, the average Bederson scale score in the Thiocetam group was 0.33 (versus 1.67 in the I/C group, $p < 0.05$), and the Garcia score was 14.5 out of 18 possible. By day 7, in the Thiocetam group there was no neurological deficit (the average Bederson score of 0, Garcia score of 18). Statistical analysis showed that, for most neurological status indicators (Garcia scale, Bederson scale), the animals in the Thiocetam group had no significant differences from sham-operated animals, which indicates restoration of central nervous system functions to the level of physiological norm by day 7.

Effect of myrtle hydrolate on EEG and neurological deficit parameters

Administration of myrtle hydrosol resulted in a moderate improvement in EEG parameters in the early post-ischemic period (Tables 1-4). On day 2, the total amplitude increased by 31.4% ($p < 0.05$), the minimum – by 37.6% ($p < 0.05$), the maximum – by 19.7% ($p < 0.05$), and the mean – by 20.6% ($p < 0.05$) relative to such in the I/C group. On day 7, the amplitude increase was 7-19% ($p < 0.05$). On day 14, a further increase in parameters was observed: the total amplitude – by 40.7% ($p < 0.05$), the maximum – by 44.4% ($p < 0.05$), and the mean – by 43.9% ($p < 0.05$) relative to such the I/C group. However, on day 21, a drop in amplitudes below the I/C level was recorded: the maximum amplitude decreased by 13.7% ($p < 0.05$), the mean amplitude – by 12.1% ($p < 0.05$), and the total amplitude – by 12.0% ($p < 0.05$), indicating the development of delayed secondary brain injury. Spectral analysis showed that the hydrolate helped to increase alpha rhythm power by 18-222% ($p < 0.05$) and decrease delta rhythm by 12-31% ($p < 0.05$) relative to such in I/C; however, by day 21, there was no normalization of the rhythms.

Neurological deficit in the Hydrolate group significantly decreased as early as day 2: according to the Garcia scale, spontaneous activity increased by 100.8% ($p < 0.05$), climbing up the wall – by 87.5% ($p < 0.05$), response to body touch – by 87.5% ($p < 0.05$), response to vibrissae touch – by 77.8% ($p < 0.05$) relative to such in the I/C group. According to the Bederson scale, the average score on day 2 was 0.5 ($p < 0.05$) versus 1.67 in the I/C group. By day 7, the response to body touch increased by 36.4% ($p < 0.05$), and the response to vibrissae touch – by 33.3% ($p < 0.05$). However, by days 14-21, no significant differences were observed from the I/C group in most indicators.

A comparative analysis of efficacy revealed that in animals that received myrtle hydrolate on days 14-21, amplitude parameters were significantly lower than such in the Thiocetam group

($p < 0.05$), which confirms the development of secondary brain injury and the absence of a sustained neuroprotective effect.

When assessing neurological deficits in animals in the Hydrolate group, no significant differences were found compared to animals in the SO group, which indicates the restoration of behavioral responses to physiological norms despite persistent electrophysiological disturbances.

Thus, myrtle hydrolate exerts a moderate neuroprotective effect primarily in the first week of the post-ischemic period, but does not prevent the development of secondary injury.

Effect of myrtle syrup I on EEG and neurological deficit parameters

Use of myrtle syrup I led to a steady and progressive increase in EEG amplitudes throughout the whole observation period (Table 1). On day 2, the total amplitude increased by 52.1% ($p < 0.05$), the minimum – by 60.8% ($p < 0.05$), and the maximum – by 37.4% ($p < 0.05$) relative to such in I/C. On day 14, the increase was maximal: the total amplitude grew by 69.7% ($p < 0.05$), the maximum – by 82.4% ($p < 0.05$), and the mean – by 81.0% ($p < 0.05$). It is notable that on day 21, in contrast to the Hydrolate group, the animals which had received syrup I maintained a significant increase in all amplitude parameters: the total amplitude – by 29.1% ($p < 0.05$), the minimum – by 68.9% ($p < 0.05$), and the maximum – amplitude by 35.0% ($p < 0.05$).

Spectral analysis revealed significant frequency spectrum normalization under the influence of syrup I (Table 2). On day 7, the alpha rhythm power increased by 151.4% ($p < 0.05$), and high-frequency beta – by 81.0% ($p < 0.05$) relative to such in I/C. On day 14, the alpha rhythm exceeded such in I/C by 279.5% ($p < 0.05$), low-frequency beta – by 43.4% ($p < 0.05$), and high-frequency beta – by 34.0% ($p < 0.05$). On day 21, the alpha rhythm power was 345.0% higher ($p < 0.05$), low-frequency beta – by 156.6% ($p < 0.05$), and high-frequency beta – by 157.6% ($p < 0.05$), while the delta rhythm decreased by 36.4% ($p < 0.05$) and the theta rhythm – by 58.8% ($p < 0.05$) compared to control. Such dynamics suggest the restoration of the normal pattern of bioelectrical activity of the brain.

Neurological deficit (Tables 3–4) in the Syrup I group significantly decreased on day 2: according to the Garcia scale, climbing up a cage wall increased by 106.2% ($p < 0.05$), the response to vibrissae touch – by 50.0% ($p < 0.05$), and the response to body touch – by 50.0% ($p < 0.05$) compared to such in I/C. According to the Bederson scale, the average score was 0.5 ($p < 0.05$). By day 7, neurological status indicators reached the SO level, and no neurological deficit was detected afterwards until day 21.

A comparative analysis of efficacy revealed that myrtle syrup I is not inferior to Thiocetam in most bioelectrical activity and neurological status indicators. On day 2, the total EEG amplitude in the Syrup I group significantly exceeded that in the Thiocetam group ($176.21 \pm 2.52 \mu V^2$ versus $159.43 \pm 5.17 \mu V^2$, $p < 0.05$), which indicates a more rapid recovery of the overall brain electrical activity. The absence of significant differences from the SO group in the Garcia scale and Bederson scale indicators at any observation period indicates a complete neurological recovery to physiological norms, which suggests an improvement in functional indicators of neurogenesis.

Effect of myrtle II syrup on EEG and neurological deficits parameters

Myrtle II syrup demonstrated the most pronounced therapeutic effect (Tables 1–4). On day 2, the total amplitude increased by 43.0% ($p < 0.05$), the minimum – by 52.9% ($p < 0.05$), and the maximum – by 59.4% ($p < 0.05$) relative to such in I/C. On day 7, the amplitudes increased by 5–23% ($p < 0.05$). On day 14, the total amplitude exceeded such in I/C by 49.4% ($p < 0.05$), the maximum – by 63.8% ($p < 0.05$), and the mean – by 61.9% ($p < 0.05$). On day 21, significant increases in all parameters persisted: the total amplitude – by 16.8% ($p < 0.05$), the minimum amplitude – by 45.1% ($p < 0.05$), and the maximum – by 13.3% ($p < 0.05$).

Spectral analysis revealed a rapid restoration of the normal frequency spectrum (Table 2). On day 7, alpha rhythm power increased by 103.9% ($p < 0.05$), high-frequency beta – by 176.0% ($p < 0.05$), and low-frequency beta – by 48.6% ($p < 0.05$), while the theta rhythm decreased by 46.3% ($p < 0.05$). On day 14, the alpha rhythm exceeded such in I/C by 272.2% ($p < 0.05$), LF beta – by 69.9% ($p < 0.05$), and HF beta – by 66.0% ($p < 0.05$). The delta rhythm decreased by 33.6% ($p < 0.05$) and the theta rhythm – by 71.2% ($p < 0.05$). On day 20, the alpha rhythm power was higher by 230.8% ($p < 0.05$), LF beta – by 103.0% ($p < 0.05$), and HF beta – by 125.9% ($p < 0.05$) compared to control.

Neurological deficit in the Syrup II group was the lowest among all the experimental groups. On day 2, according to the Garcia scale, spontaneous activity increased by 125.6% ($p < 0.05$) compared to such in I/C, climbing up a cage was – by 106.2% ($p < 0.05$), and the response to vibrissae touch – by 100.0% ($p < 0.05$). According to the Bederson scale, the average score on day 2 was 0.08 ($p < 0.05$). By day 7, neurological deficit was completely absent in both scales.

The pronounced effect of syrup II is probably due to the high content of the flavonoid myricetin (187 mg/L), which, according to the literature, inhibits the activation of NF- κ B, reduces the production of proinflammatory cytokines, activates the Nrf2/ARE signaling pathway of antioxidant protection and protects neurons from oxidative stress (Ren et al. 2025; Medda et al. 2021).

Comparative analysis of the efficacy showed that the animals in the Myrtle syrup II group demonstrated the most rapid neurological recovery: as early as on day 2, the average score according to the Bederson scale was 0.08, which was significantly ($p < 0.05$) lower than such in the Thiocetam group (0.33). According to the Garcia scale, spontaneous activity and response to vibrissae touch in the Syrup II group reached their maximum values on day 2 (3.00 ± 0 points), while in the Thiocetam group these indicators were 2.50 ± 0.25 and 2.25 ± 0.25 points, respectively ($p < 0.05$). By day 7, the differences between the Syrup II and Thiocetam groups in neurological tests were leveled out (both groups achieved full recovery). No statistically significant differences were found between Syrup II and Thiocetam groups in most electrophysiological parameters (EEG amplitude, power of delta and theta rhythms) ($p > 0.05$), which suggests comparable efficacy of these two agents in restoring brain bioelectrical activity. No significant differences were recorded in sham-operated animals in behavioral tests, which confirms full restoration of neurological function to physiological norms within two days of the post-ischemic period.

Discussion

The data obtained in this study demonstrate that modeling ischemia by 30-minute occlusion of the left common carotid artery in rats leads to the development of a characteristic complex of electrophysiological and behavioral disturbances that persist for 21 days of the post-ischemic period. A 40-61% reduction in EEG amplitudes and a restructuring of the frequency spectrum with a predominance of slow-wave activity (delta and theta rhythms) and simultaneous suppression of fast rhythms (alpha and beta) are classic signs of ischemic brain injury (Pu et al. 2020; Salatin et al. 2023; Thapa et al. 2023). A decrease in alpha rhythm power reflects a disruption in the functional integrity of thalamocortical connections and a decrease in integrative brain function, which is clinically manifested by inhibition of spontaneous motor activity, movement asymmetry, and impaired sensory responses (Uysal et al. 2023). The increase in delta rhythm power recorded in the I/C group throughout the observation period indicates the development of structural changes in brain tissue and functional thalamocortical decoupling (Yildirim et al. 2015).

So, analysis of the connection between EEG parameters and the severity of neurological deficits revealed a clear correlation between electrophysiological and behavioral disturbances in the ischemia-reperfusion model and their coordinated dynamics under the influence of myrtle secondary metabolites (Table 5). This confirms that restoration of normal brain bioelectrical activity is a prerequisite for functional recovery after ischemic injury.

Table 5. Correlation of changes in electrophysiological and behavioral parameters in experimental groups

Group	EEG amplitude dynamics	Alpha-rhythm dynamics	Neurological deficit dynamics	Secondary injury (by day 21)
I/C	Persistent suppression ($\downarrow 40-61\%$)	Persistent suppression ($\downarrow 54-75\%$)	Evident deficit for 14-21 days	Present
Thiocetam	Sustained increase ($\uparrow 25-38\%$)	Marked recovery ($\uparrow 68-151\%$ as compared to I/C)	Recovery by day 7	Absent
Hydrolate	Improvement by days 7-14 ($\uparrow 20-44\%$), decline on day 21 ($\downarrow 12-14\%$)	Partial recovery ($\uparrow 18-222\%$ as compared to I/C)	Reduction by days 2-7; residual impairments	Marked (amplitude decline)
Syrup I	Sustained increase ($\uparrow 29-82\%$)	Pronounced recovery ($\uparrow 151-345\%$ as compared to I/C)	Recovery by days 7-14	Absent
Syrup II	Rapid and sustained increase ($\uparrow 17-63\%$)	Rapid recovery ($\uparrow 104-272\%$ as compared to I/C)	Recovery by days 2-7	Absent

Note: $\uparrow$ – increase in the indicator, $\downarrow$ – decrease in the indicator

The increase in electrophysiological disturbances identified in the I/C group by day 14, followed by partial recovery by day 21, aligns with the dynamics of the post-ischemic period

described in the literature. As is known, after the acute phase of ischemic injury (days 1-3), a subacute phase develops (days 7-14), characterized by the activation of microglia, the development of neuroinflammation and apoptotic death of neurons in the penumbra region (Alsbrook et al. 2023; Ren et al. 2025). Thus, the maximum suppression of EEG amplitudes (a decrease in the total amplitude by 47-58%, $p < 0.05$) matched in time with the most pronounced neurological deficit (a decrease in indicators according to the Garcia scale by 44-56% on day 2, $p < 0.05$). An EEG frequency spectrum shift towards a predominance of slow-wave activity (an increase in the delta rhythm by 47-63%, theta rhythm – by 41-119%, $p < 0.05$) with simultaneous suppression of the alpha rhythm (by 54-75%, $p < 0.05$) and beta rhythms (by 49-82%, $p < 0.05$) matched the severity of motor and sensory disorders.

An increase in the delta rhythm power reflects the functional thalamocortical decoupling, while the suppression of alpha activity suggests a decrease in the integrative activity of the brain, which clinically manifested in a violation of spontaneous motor activity, movement asymmetry, and suppression of sensory responses (Ajčević et al. 2021). It is the very period when the maximum suppression of functional brain activity was observed, which was recorded in our study. By day 21, despite partial spontaneous recovery of behavioral parameters (up to 94-100% of the SO level), residual EEG changes persisted (alpha rhythm decreased by 74.5%, delta rhythm increased by 62.8%, $p < 0.05$), which indicated a higher sensitivity of electrophysiological methods to residual impairments of brain function. Partial spontaneous recovery by day 21 is due to neuroplasticity, reparative regeneration, and compensatory activation of intact neural networks (Hayani et al. 2024).

A comparative analysis of the efficacy of three myrtle secondary metabolite compositions revealed significant differences in their cerebroprotective effects. Myrtle hydrolate, containing predominantly water-soluble components and essential oils (Odeh et al. 2022), exerted a moderate neuroprotective effect in the early post-ischemic period (days 2-14), but did not prevent the development of secondary brain injury, as evidenced by a decrease in EEG amplitudes on day 21 and the persistence of residual neurological deficits. This dynamic indicates that myrtle hydrolate exerts only a short-term neuroprotective effect, but does not prevent the development of delayed secondary damage, likely caused by microglial activation and the development of neuroinflammation (Al-Snafi et al. 2024). In this case, electrophysiological changes preceded the deterioration in behavioral indicators, which emphasizes the predictive value of EEG monitoring. The obtained data are consistent with the results of other scientists who have shown that myrtle essential oils exert predominantly antibacterial and anti-inflammatory effect, but their neuroprotective potential is limited due to low bioavailability and rapid elimination (Zappasodi et al. 2014).

Myrtle syrup I containing 39.6 mg/L of phenolic acids (gallic and ellagic) demonstrated a sustained cerebroprotective effect, ensuring progressive restoration of EEG amplitudes and normalization of the frequency spectrum by day 21, which was accompanied by complete neurological recovery by day 7. A significant increase in alpha rhythm power, a marker of the functional integrity of thalamocortical connections, correlated with normalization of spontaneous motor activity and movement symmetry (Odeh et al. 2022). The absence of a secondary decrease in EEG amplitudes indicates the prevention of delayed brain injury, which may relate to the antioxidant and anti-inflammatory properties of phenolic acids (gallic and ellagic) contained in syrup I. Phenolic acids are known for their antioxidant activity: they are effective free radical scavengers, chelators of transition metal ions, and inhibitors of lipid peroxidation (Bakova et al. 2023). Gallic acid and its derivatives are able to penetrate the blood-brain barrier, suppress microglia activation, and reduce the production of proinflammatory cytokines (Dayneko et al. 2014). It is likely that the combined antioxidant and anti-inflammatory effects of phenolic acids are responsible for the neuroprotective effect of syrup I.

The most pronounced and rapid recovery of both electrophysiological and behavioral parameters was observed in the Syrup II group. As early as by day 2, the neurological deficit was minimal (the average score according to the Bederson scale was 0.08 versus 1.67 in I/C, $p < 0.05$), which was accompanied by a significant increase in EEG amplitudes on day 2 (the total amplitude increased by 43%, $p < 0.05$). By day 7, the alpha rhythm power increased by 104% ($p < 0.05$), beta rhythms – by 49-176% ($p < 0.05$), and the delta rhythm decreased by 19% ($p < 0.05$) compared to control, which suggests the restoration of the normal pattern of bioelectrical activity. At the same time, by day 7, all manifestations of neurological deficit completely resolved. A high correlation between EEG normalization and behavioral recovery in the Syrup II group suggests a strong cerebroprotective effect of this composition. This effect is likely due to the high content of the flavonoid myricetin at a concentration of 187 mg/L. Myricetin belongs to the class of flavonols with a strong antioxidant activity, superior to that of quercetin and other flavonoids (Imran et al. 2021). Numerous *in vitro* and *in vivo* studies have demonstrated that myricetin penetrates the blood-brain barrier, inhibits microglial activation, activates the Nrf2/ARE signaling pathway, which leads to increased expression of antioxidant enzymes (heme oxygenase-1, NAD(P)H:quinone oxidoreductase-1, glutathione-S-

transferase) (Narayan et al. 2021). Furthermore, myricetin inhibits NF- κ B activation by suppressing I κ B α phosphorylation and p65 nuclear translocation, which leads to the reduced production of proinflammatory cytokines (TNF- α , IL-1 β , IL-6) and chemokines (Damulin and Ekusheva 2014). It is notable that myricetin also modulates the mitochondrial apoptotic pathway, reducing the Bax/Bcl-2 ratio and preventing cytochrome c release and caspase-3 activation (Zakharov 2015).

The rapid neurological recovery in the Syrup II group (by days 2-7) may relate to the additional vasodilatory effect of myricetin. Myricetin has been shown to activate endothelial NO synthase (eNOS) via the Akt-dependent pathway, which leads to the increased production of nitric oxide and improved cerebral microcirculation (Logvinenko 2017). Improved perfusion in the ischemic penumbra facilitated the maintenance of neuronal viability and accelerated functional recovery.

A comparison of the obtained data with the results of other studies shows that the efficacy of myrtle syrup is comparable to that of known plant-based neuroprotectors. For example, ginkgo biloba extract, contains flavonoid glycosides, reduces infarct volume by 30-40% and improves neurological outcome in experimental ischemia (Novikova and Sharapova 2018). Curcumin, a turmeric polyphenol, reduces oxidative stress and neuroinflammation in cerebral ischemia, but its bioavailability after oral administration is extremely low (Skvortsova et al. 2018). Unlike curcumin, myricetin has good bioavailability and the ability to penetrate the blood-brain barrier, which makes it a promising candidate for the development of neuroprotective agents (Chuyan et al. 2024).

The completed study showed that the cerebroprotective activity of myrtle syrup enriched with myricetin is comparable to that of the reference combination neuroprotector Thiocetam, and even surpasses it in terms of the neurological recovery rate in the early post-ischemic period (day 2). Thiocetam (a combination of piracetam and thiotriazoline) exerts its effect through improved neurometabolism (activation of glycolysis, glucose utilization) and antioxidant protection (reactivation of SOD and catalase) (Kalchuk and Kirichev 2013; Demchenko and Belenichev 2015). In our study, Thiocetam demonstrated a stable neuroprotective effect, ensuring complete neurological recovery by day 7.

A key distinguishing feature of Syrup II is that it facilitates accelerated recovery on day 2: neurological deficit was minimal (0.08 points in the Bederson scale versus 0.33 in the Thiocetam group, $p < 0.05$), while spontaneous activity and sensory responses according to the Garcia scale reached their maximum values within the first two days (3.00 ± 0 points versus 2.50 ± 0.25 in the Thiocetam group). This is apparently due to the direct inhibitory effect of myricetin on the NF- κ B pathway and its strong vasodilatory effect through eNOS activation, whereas the effect of Thiocetam is mediated primarily by metabolic mechanisms that require a longer timeframe to be implemented (Imran et al. 2021; Chuyan et al. 2024).

The absence of statistically significant differences between Syrup II and Thiocetam in most electrophysiological parameters (EEG amplitude, alpha rhythm power on days 7-21) confirms that the myricetin-containing composition is not inferior to a modern combination neuroprotective agent and is a promising alternative to the synthetic combination drug, especially in the context of the therapeutic window of the first 72 hours after ischemic injury.

So, the analysis demonstrates a close correlation between brain electrical activity and behavioral manifestations of neurological deficit during ischemia-reperfusion. Secondary metabolites of myrtle, especially Syrup II with its high flavonoid content, exert a complex cerebroprotective effect, which manifests in the coordinated restoration of electrophysiological and behavioral parameters. Rapid normalization of EEG spectral characteristics in the Syrup I and Syrup II groups preceded full recovery of behavioral parameters, which indicates high sensitivity of electrophysiological methods to early signs of functional brain recovery. EEG monitoring along with behavioral testing represents an informative approach to the assessment of the effectiveness of neuroprotective interventions in experimental models of ischemic stroke.

Conclusion

This is the first comprehensive comparative study of the effects of three different myrtle secondary metabolite compositions (hydrolate, syrup I with phenolic acids, and syrup II with flavonoids) on the overall brain electrical activity and the severity of neurological deficits over a 21-day post-ischemic period in rats. It was established that modeling 30-minute ischemia-reperfusion in rats leads to persistent suppression of the overall electrical activity of the brain (a decrease in EEG amplitudes by 40-61%, $p < 0.05$), a frequency spectrum shift with a predominance of slow-wave activity (an increase in the delta rhythm by 47-63%, theta rhythm – by 41-119%, $p < 0.05$) and suppression of fast rhythms (alpha rhythm – by 54-75%, $p < 0.05$), as well as to the development of severe neurological deficit which peaks by days 2–7 with partial spontaneous recovery by day 21.

Administration of myrtle hydrolate exerts a moderate neuroprotective effect in the early post-ischemic period, but does not prevent the development of secondary brain injury, which manifests in a decrease in EEG amplitudes on day 21 (by 12-14% below the I/C level, $p < 0.05$). Compared to Thiocetam, hydrolate is significantly less effective by days 14-21 ($p < 0.05$). It has been shown that myrtle syrup I (phenolic acids 39.6 mg/L) promotes sustainable restoration of brain bioelectric activity (an increase in total amplitude by 29-70%, alpha rhythm – by 151-345%, $p < 0.05$) and neurological status which reaches control values by days 7-14. Syrup I is not inferior to Thiocetam in terms of effectiveness, and is significantly superior to it in terms of the total EEG amplitude on day 2 ($p < 0.05$). Myrtle II syrup, with a high content of the flavonoid myricetin (187 mg/L), exhibits the most pronounced and sustained cerebroprotective effect, ensuring accelerated neuronal – neurological recovery (by days 2-7), complete normalization of EEG amplitude and spectral characteristics, and preventing secondary ischemic brain injury. The findings support the potential for developing new phytotherapeutic agents based on myrtle II syrup for correcting post-ischemic disorders.

Additional Information

Conflict of interest

The authors declare that they have no conflicts of interest.

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Ethics statement

The animal study protocols were approved by the Ethics Committee of V.I. Vernadsky Crimean Federal University (Minutes No. 10 dated December 6, 2022).

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Data availability

All relevant data generated and analyzed during this study are included in this article.

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