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Cerebrovascular and antihyperlipidemic properties of sour cherry (*Prunus cerasus*) fruit

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ABSTRACT

Background: Ischemic diseases are major global health burden, contributing significantly to cardiovascular and cerebrovascular disorders, with ischemic stroke being a leading cause. The main risk factors for ischemia are vascular wall damage, atherosclerosis progression, lipid metabolism disturbances, and increased platelet aggregation. Current medications targeting these mechanisms may lead to side effects, especially when they are used for prevention, driving interest in plant-derived compounds with cardiovascular and cerebrovascular benefits. Sour cherry (*Prunus cerasus*) has attracted attention for its high levels of polyphenols, anthocyanins, flavonoids, and organic acids, known for their blood pressure-regulating, anti-inflammatory, and sleep-modulating properties. These compounds protect the vascular system by reducing oxidative stress, inflammation, and endothelial dysfunction. Investigation of sour cherry cerebrovascular and anti-hyperlipidemic properties may contribute to integrating plant-based therapies into existing cardiovascular treatment strategies, offering new opportunities for disease prevention and management.

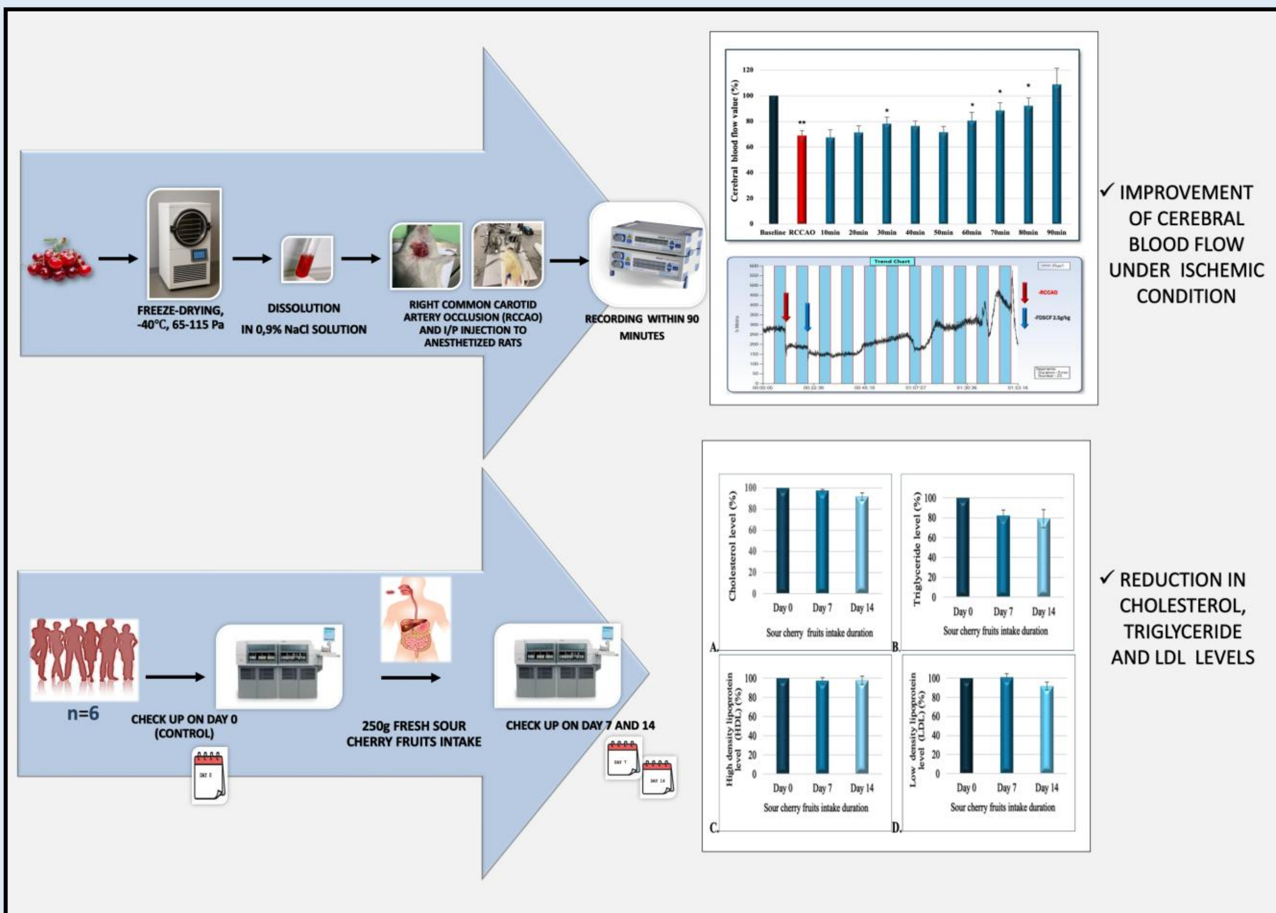
Objectives: The purpose of this observation was to investigate the antihyperlipidemic activity and discover the new cerebrovascular effects of sour cherry fruits.

Results: Obtained data evidenced that cholesterol levels in blood samples after 7 days of fruit intake were decreased by 2.74±1.52% compared with baseline parameters (day 0). Continued sour cherry fruit intake for 14 days led to further reduction in cholesterol levels by 8.1±3.30%, nearly three times exceeding the reduction level registered on day 7. Assessment of sour cherry fruits on triglyceride levels showed a significant reduction on days 7 and 14 by 17.86 ± 5.57% and 21.01 ± 9.25%, respectively, compared to baseline. No notable changes were observed in high-density lipoprotein (HDL) levels following sour cherry fruit intake. In contrast, low-density lipoprotein (LDL) levels decreased by 8.1 ± 4.24% after 14 days of consumption compared with control values.

Registration of brain blood flow enabled the discovery of cerebrovascular activity in sour cherry fruits. It is noteworthy that its ability of sour cherry fruits to increase the cerebral blood flow (CBF) appears especially in case of cerebral blood flow impairment. Thus, under the normal condition, cerebral blood flow after intraperitoneal injection of 2.5 g/kg freeze-dried sour cherry fruit solution was not changed significantly. After the ligation of right common carotid artery, when CBF was decreased by 31.07±3.96% ($p<0.05$) intraperitoneal administration of the freeze-dried sour cherry fruits solution (2.5 g/ kg) was accompanied by the elevation of CBF, being above from occlusion levels by 13.6±5.19% ($p<0.05$) at 30 minutes, and by 33.8±6.06% -at 80 minutes, compared to occlusion values. Notably, by the end of the experiment, CBF levels exceeded not only the occlusion level by 40.04±12.51%, as well as the baseline level.

Conclusion: The findings of this study suggest that sour cherry fruits possess cerebrovascular and antihyperlipidemic activity, as based on their ability to improve impaired cerebral blood flow and reduce cholesterol and triglyceride levels. These results highlight the potential of sour cherry as a natural and effective plant-based agent for reducing cardiovascular disease risk factors, including hypercholesterolemia.

Keywords: common carotid artery occlusion, new cerebrovascular activity, lipid spectrum changes, freeze-dried sour cherry.



Graphical Abstract: Cerebrovascular and antihyperlipidemic properties of sour cherry fruits

INTRODUCTION

Hyperlipidemia is one of the major modifiable risk factors in the development of ischemic diseases [1-2], especially ischemic heart disease, the second leading cause of mortality and third leading cause of disability worldwide [3-4]. Notably, in up to 45% of ischemic stroke cases, the primary cause of vascular occlusion is atherothrombotic events [5]. Endothelial dysfunction, resulting from hyperlipidemia, creates favorable conditions for subsequent thrombotic events or mechanical occlusion of the vascular wall [6-7]. Accordingly, effective management of hyperlipidemia is considered a cornerstone in treating and reducing the risk of cardiovascular and cerebrovascular pathologies, such as myocardial infarction and stroke [8]. Thus, currently used antihyperlipidemic drugs, particularly statins, are widely applied for the treatment of hyperlipidemia and have been shown to reduce the incidence of cardiovascular and cerebrovascular diseases. However, they are not free of adverse effects, including rhabdomyolysis, myalgia, and various hepatic and gastrointestinal disorders [9]. Moreover, standard statin therapy is sometimes ineffective, depending on individual response to treatment [10], which highlights the need for alternative therapeutic approaches.

In this regard, plant-derived agents have attracted considerable interest. Several medicinal plants—including garlic [11], artichoke [12], cinnamon [13], green tea [14], moringa [15-16], strawberry [17], black rice [18-20], and others—have been shown to exert lipid-regulating effects. The use of plant-based agents is associated with several advantages, such as a rich profile of biologically active agents, fewer adverse effects, and suitability for long-term or chronic administration [21].

The mechanisms underlying the lipid-lowering properties of plants are diverse, including inhibition of cholesterol absorption, enhancement of cholesterol excretion, suppression of lipid synthesis, and acceleration of lipid metabolism [22-23]. These effects are largely attributed to the presence of bioactive compounds such as resveratrol, quercetin, cyanidin, rutin [24], and other polyphenolic constituents.

Considering these findings, this observational study was conducted to evaluate cerebrovascular effects and antihyperlipidemic properties of sour cherry fruits (*Prunus cerasus*), being a functional food with the presence of cyanidin, quercetin, and other bioactive compounds possessing above-mentioned effects.

MATERIALS AND METHODS:

Participants: The investigation of lipid spectrum changes involved 6 men and women aged 38 to 68 years.

Fasting blood samples were collected from all participants at the start of the study (baseline, 0 day for antihyperlipidemic effect evaluation). Blood samples were collected after 7 and 14 days of 250g (about 8.82 oz) fresh sour cherry fruits' continuous intake up to 14 days to assess changes in lipid profile. According to our previous investigation, sour cherry fruits contain approximately 224 mg anthocyanins of cyanidin-3-glucoside equivalent [25].

Animals: The cerebrovascular activity of sour cherry fruits was observed in the method of Laser Doppler flowmetry, using the model of cerebral blood flow impairment caused by right common carotid artery occlusion, on 13 inbred, white, male rats, weighing 180-220 g, using MoorVMS-LDFTM (Moor Inc., Axminster, Devon, UK) Laser-Doppler flowmeter.

The experiments were performed between 12 and 15 h at a laboratory room temperature of 22±1°C. Food and water were available *ad libitum*. Animals were kept in standard laboratory vivarium conditions in accordance with the PHS Guide for the Care and Use of Laboratory Animals.

Experiments were carried out under the chloral hydrate anesthesia (350 mg/kg dose, injected intraperitoneally).

Antihyperlipidemic activity evaluation: To evaluate changes in the blood lipid profile, the following enzymatic and detergent-based methods were used: total cholesterol was measured using an enzymatic colorimetric method; triglycerides were determined using the glycerol phosphate oxidase method; HDL and LDL cholesterols were measured using the accelerator selective detergent and liquid selective detergent methods, respectively.

Blood sampling for lipid profile analysis was performed using Li-heparin-containing tubes according to the demands of Biosafety in Microbiological and Biomedical Laboratories (U.S. Department of Health and Human Services, Public Health Service, 6th edition 2020). Immediately after collection, samples were centrifuged for 10–15 minutes at 3000 rpm. The obtained plasma was placed into special racks and then placed into the ABBOTT Architect ci4100 machine, which is fully automated. The system began the process by scanning each sample's barcode, identifying the required type of analysis, and automatically selecting and dispensing the necessary reagents from the refrigerated compartment. All procedures were conducted under controlled conditions at 37°C. The results were transmitted to a computer connected to the analyzer, verified through initial and repeat quality control steps, and finally entered a central database. Lipid profile components were quantitatively expressed in mmol/L.

Method of Laser-Doppler flowmetry: The effect of cherry fruit on local cerebral blood flow was observed using a model of cerebral circulation disorder induced by right common carotid artery occlusion, applying the laser Doppler flowmetry method [4]. The operating principle of laser Doppler flowmetry is to direct light from the sensor onto moving blood components, particularly red blood cells. The reflected light, scattered by the red blood cells, is captured by the recording part of the probe. The frequency shift between the emitted and reflected light is used to calculate the velocity of blood cell movement, which represents the Doppler effect.

The animals were divided into two groups: Group 1 (n=8): under conditions of right common carotid artery occlusion, rats received an intraperitoneal injection of a solution obtained from freeze-dried cherry fruits (0.5 g in 0.5 ml 0.9% NaCl), at a dose of 2.5 g/kg and Group 2 (n=5): rats received the same solution of freeze-dried cherry fruits intraperitoneally (0.5 g in 0.5 ml. 0.9% NaCl, 2.5 g/kg) without induction of ischemia.

A midline incision was made on the rats' necks. After separation of the fascia and muscles, the right common carotid artery was exposed at the wound surface. A Vaseline-coated silk thread was passed under the artery, and a ligature was placed without knot tension. The animal was then positioned in a stereotaxic frame. The head was fixed using appropriate ear, tooth, and nose bars. The scalp over the parietal region was shaved and disinfected, followed by a midline incision of approximately 15 mm. The incised tissues were separated, and the skull was cleared of soft tissues and membranes. Using a dental drill, a round craniotomy (~2 mm in diameter) was performed at the parietal region ipsilateral to the ligated artery, without damaging the dura mater. To prevent sensor displacement during the experiment, an intraperitoneal catheter was inserted, through which the solution of freeze-dried cherry fruits was administered. Following craniotomy and catheter placement, a needle-shaped probe from the moorVMS-LDF™ laser Doppler flowmeter (Moor Instruments, Axminster, Devon, UK) was fixed using a special holder and placed within the created cranial window, ensuring it did not record from large-diameter vessels. After obtaining stable baseline values in the initial recording phase, the ligature on the right common carotid artery was tightened to induce ischemia. Once ischemia and baseline post-occlusion values were stabilized, the freeze-dried cherry fruit solution was administered intraperitoneally. Changes in local cerebral blood flow were monitored over a period of 90 minutes following administration.

Ethical considerations: Considering that sour cherry fruits, which are a part of the daily diet, and venipuncture for the blood collection from healthy adults, this study falls under the category of "minimal risk". Participants were given both written and verbal information about the study. They were also informed that participation was voluntary and that they could withdraw at any time without consequences.

These study protocols were approved by the Ethics Committee of the Science Department of Yerevan State Medical University after Mkhitar Heratsi.

Statistical analysis: Statistical analysis of the received data was performed by SPSS 20.0 using the student t-test to determine the significance of the observed differences between groups. A p-value of less than 0.05 was considered statistically significant. 0-day results of lipid profile and occlusion, and baseline level results of cerebral blood flow served as negative controls.

RESULTS

According to the obtained results, a reduction in cholesterol concentration by 2.74±1.52% compared to baseline was observed in blood samples collected 7 days after daily fruit intake. Further observations revealed that continued fruit intake for up to 14 days led to a more pronounced decrease in cholesterol levels, exceeding the 7-day reduction by more than 3-fold, with a total decrease of 8.1±3.30% compared to baseline.

The analysis of cherry fruit's influence on triglyceride levels revealed expressed changes. Thus, the obtained data were evident that triglyceride concentration consistently decreased on both day 7 and day 14, with reductions by 17.86±5.57 ($p<0.05$) and 21.01±9.25%, respectively, compared to initial values.

For HDL levels, no statistically significant changes were recorded under the influence of cherry fruit. In contrast, LDL levels showed a different trend: a 8.1±4.24% decrease was observed after 14 days of daily fruit intake compared to control values.

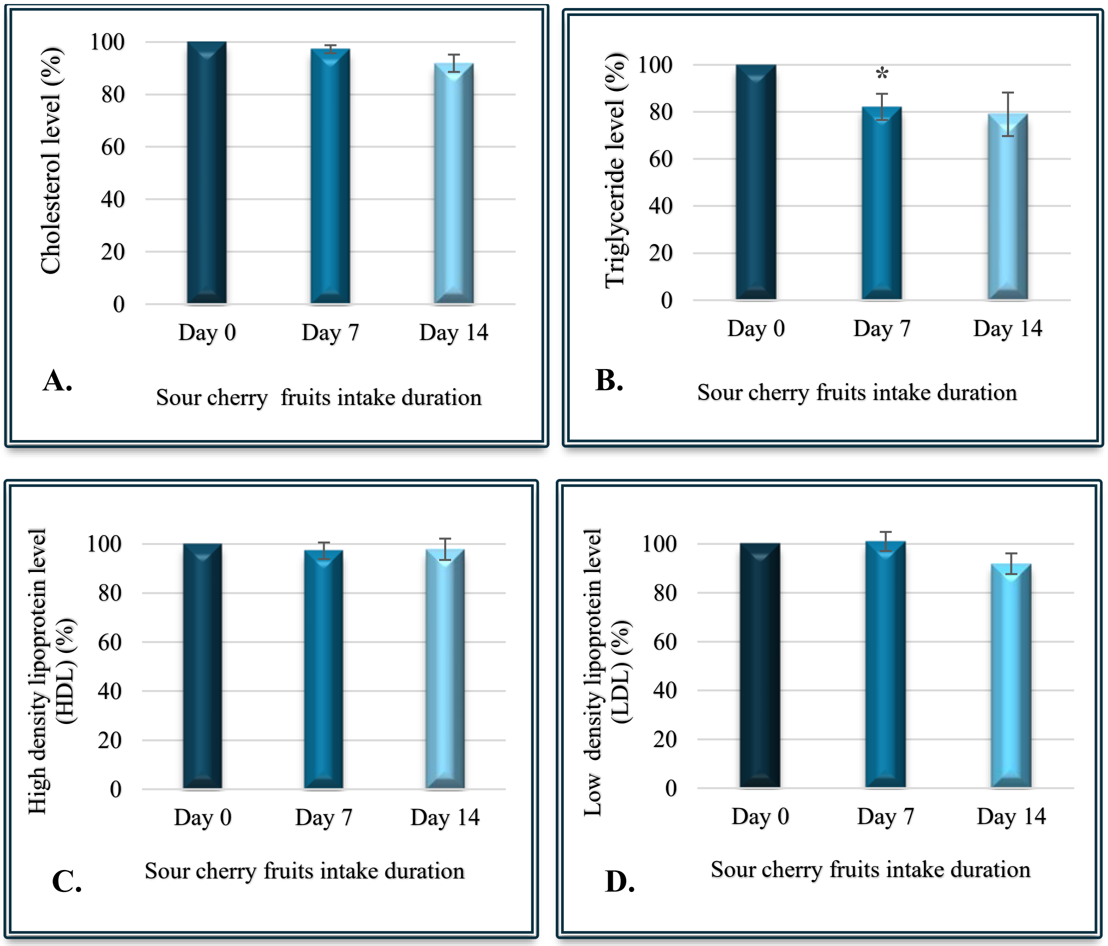


Figure 1. Effects of Sour cherry fruits on lipid profile parameters: cholesterol (A.), triglycerides (B.), high (HDL) (C.) and low (LDL) (D.) density lipoprotein changes (%) 7 and 14 days after 250g fresh Sour cherry fruits intake by the volunteers compared with 0-day results (n=6, M±SEM) *p<0.05 (paired t-test)

As demonstrated by the experimental results, intraperitoneal administration of freeze-dried cherry fruit at a dose of 2.5 g/kg to rats under physiological conditions did not produce a consistent change in local cerebral blood flow, as measured by laser Doppler flowmetry. Thus, a decrease in cerebral blood flow (CBF) by 16.17±7.39% was observed even 10 minutes after the injection. This reduction continued up to 30 minutes, reaching 25.42±13.90%. At 40 and 50 minutes, a slight increase in CBF was observed, though it remained below baseline levels by 22.88±12.46% and 22.20±9.76%, respectively. However, by the 60th minute, CBF again declined, resulting in a 30.82±7.81% reduction from baseline. Statistical analysis of the Doppler recordings indicated that at 70 and 80 minutes, the CBF fluctuated, and by 90 minutes post-administration, it was still 28.72±16.74% lower than the initial values (Table 1).

A completely different pattern was observed under conditions of impaired cerebral blood flow. According to experimental results, after occlusion of the right common carotid artery in rats, intraperitoneal administration of freeze-dried Sour cherry fruit at a dose of 2.5 g/kg resulted in the gradual restoration of compromised cerebral blood.

Table 1. Effect of freeze-dried Sour cherry fruits (2.5 g/kg, i/p) on local cerebral blood flow under the normal condition and after right common carotid artery occlusion (*M±SEM)*

		Cerebral blood flow value (%)	
		Normal condition (n=5)	**After RCCAO (n=8)**
	Baseline	**100**	
	Before FDSCF	100±0.00	68.93± 3.96**
Time after FDSCF injection	10 min	83.83±7.39	67.59±5.86
	20 min	79.04±4.57**	71.47±5.17
	30 min	74.58±13.90	78.29±5.19*
	40 min	77.12±12.46	76.59±3.82
	50 min	77.80±9.76	71.66±4.5
	60 min	69.18±7.81**	80.84±6.36*
	70 min	74.04±7.32**	88.60±6.10*
	80 min	69.33±14.72	92.22±6.06*
	90 min	71.28±16.74	108.97±12.51

Notes: FDSCF-Freeze dried Sour cherry fruit, RCCAO-Right common carotid artery occlusion, *p<0.05 vs. RCCAO; **p<0.05 vs. baseline values

Following the carotid artery occlusion, cerebral blood flow in the cortex of the ipsilateral region decreased by 31.07 ± 3.96% (p<0.05) compared to baseline. Under these conditions, administration of the freeze-dried Sour cherry fruit led to a gradual and statistically significant improvement in local cerebral perfusion, surpassing the ischemic value by 13.6 ± 5.19% (p<0.05) at 30 minutes post-injection. This upward trend continued, and by 80 minutes, CBF was 33.8 ± 6.06% (p<0.05) higher than the ischemic level. Notably, by 90 minutes, CBF was not only higher than occlusion-induced ischemic value by 40.04±12.51%, but even exceeded baseline values (Figure 2, Table 1).

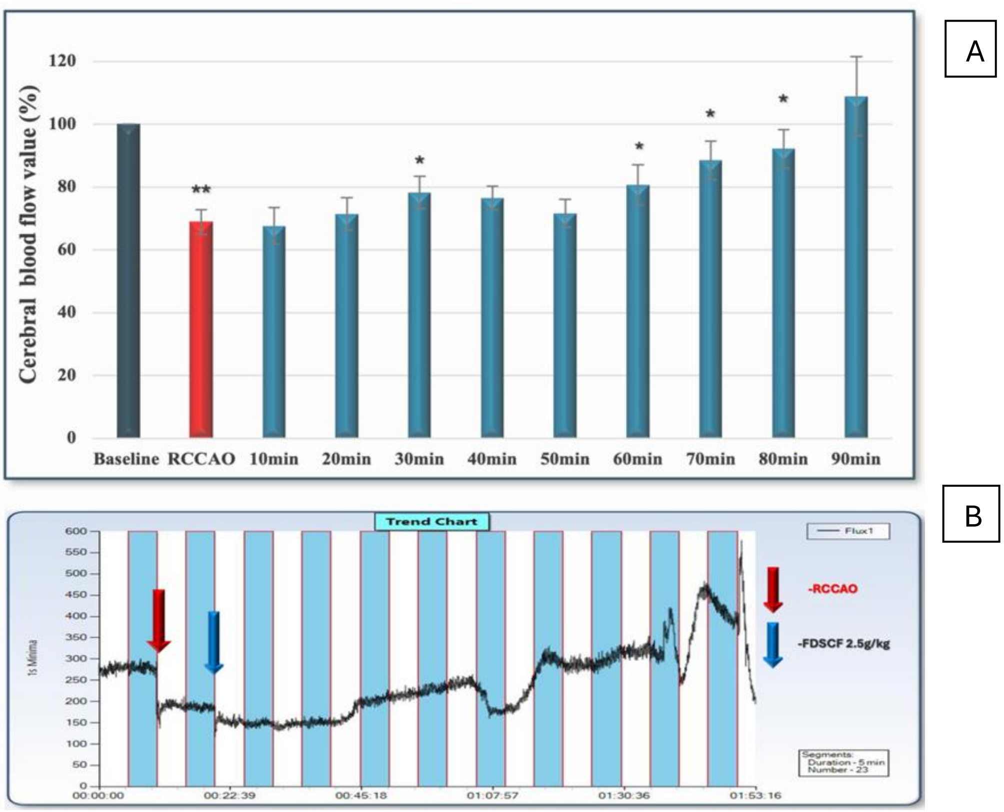


Figure 2. Effect of freeze-dried Sour cherry fruit solution (2.5 g/kg) on rats' local cerebral blood flow under the condition of right common carotid artery occlusion (RCCAO). **A.** dynamics of changes in % *(n = 8, M ± SEM), *p < 0.05 vs. carotid ligation values; ** p < 0.05 vs. baseline values B. rat's dopplergram*.

DISCUSSION

The development of effective agents for the prevention and treatment of cardiovascular diseases (CVDs), which remain among the leading causes of global mortality with a persistently increasing incidence, continues to be a significant challenge in biomedical research. It is well established that endothelial dysfunction, hyperlipidemia, and platelet overactivation contribute to vascular occlusion and constitute fundamental mechanisms underlying the development of atherothrombotic events, particularly ischemic stroke. Accordingly, the pharmacological management of hyperlipidemia [26], platelet overactivation [27], and hypertension [28] is considered a cornerstone of the treatment and prevention of ischemic diseases. In this context, sour cherry fruits (*Prunus cerasus*) (CF), known to be rich in biologically active compounds, have been investigated as a potential source for the development of novel agents aimed at modulating key risk factors involved in the pathogenesis of cardiovascular disorders.

In view of this, the antiplatelet and antihypertensive effects of fresh and freeze-dried sour cherry fruits were previously investigated [25], which formed the basis for the present study of fruits' cerebrovascular and antihyperlipidemic properties.

Evaluation of lipid profile parameters in the blood of volunteers showed that after 14 days of daily intake of 250 g of CF, there was a marked decrease in triglyceride levels, which by the end of the observation period were reduced by $21.01 \pm 9.25\%$ relative to baseline. Additionally, reductions in total cholesterol and low-density lipoprotein (LDL) levels were noted by $8.1 \pm 3.30\%$ and $8.1 \pm 4.24\%$, respectively.

These findings served as the basis for obtaining a freeze-dried cherry fruit powder, which was standardized for anthocyanin content (89.67 mg per 100 g of fresh cherry fruit) and used for further investigation of cerebrovascular activity [25].

In experiments evaluating local cerebral blood flow (LCBF) in rats using laser Doppler flowmetry, cherry fruits demonstrated the ability to improve cerebral perfusion under acute ischemic conditions. At the same time, intraperitoneal (i.p.) administration of freeze-dried cherry fruits at a dose of 2.5 g/kg did not show statistically significant changes in LCBF under normal conditions. In a model of impaired cerebral circulation induced by proper typical carotid artery occlusion (resulting in a $31.07 \pm 3.96\%$ ($p<0.05$) reduction in LCBF), administration of the same dose led to a $40.04 \pm 12.51\%$ increase in LCBF at 90 minutes post-injection relative to the ischemic value.

Thus, the results confirm the initial hypothesis that cherry fruits are an effective and safe agent for the prevention and treatment of cardiovascular diseases, owing to their hypolipidemic and cerebrovascular activities.

List of Abbreviations: Cardio-vascular diseases (CVD), Cerebral blood flow (CBF), Freeze-dried Sour cherry fruits (FDSCF), High density lipoprotein (HDL), Local cerebral blood flow (LCBF), Low density lipoprotein (LDL), Right common carotid artery occlusion (RCCAO).

Author's Contributions: All authors contributed to this study.

Competing Interests: The authors declare that they have no competing interests.

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