



Artículo Original | Original Article

Antihypoxic and anti-ischemic properties of the North Caucasus flora plant extracts

[Propiedades antihipóxicas y antiisquémicas de los extractos de plantas de la flora del norte del Cáucaso]

Dmitry I Pozdnyakov, Anastasiya E Pozdnyakova, Similya L Adzhiahmetova, Nadezhda M Chervonnaya, Denis S Zolotych, Nataliya S Lyakhova & Kirill A Miroshnichenko

Pyatigorsk Medical and Pharmaceutical Institute, Pyatigorsk, Russia

Contactos / Contacts: Dmitry I POZDNYAKOV - E-mail address: pozdniackow.dmitry@yandex.ru

Abstract: Nowadays it is established that ischemic brain damage like ischemic stroke is one of the leading cause of death and disability in the population that assumes relevance development of anti-ischemic drugs. The work studied the anti-hypoxic and anti-ischemic effect of 7 plant extracts. Antihypoxic activity was assessed on models of hypobaric, hypercapnic, histotoxic, hematotoxic hypoxia. Anti-ischemic activity of test-extracts was studied on the focal cerebral ischemia model. Administration of *Tagetes patula*, *Gaillardia pulchella*, *Sorbaria sorbifolia*, *Grossularia reclinata*, *Ribes nigrum*, *Rubus caesius* and *Lysimachia punctata* extracts contributed to the necrosis zone reduction by 56.6% ($p<0.05$); 37.3% ($p<0.05$); 73.2% ($p<0.05$); 49.4% ($p<0.05$); 42.5% ($p<0.05$); 85.5% ($p<0.05$); 44.2% ($p<0.05$) and also restored aerobic metabolism in brain tissue. Test - objects increased of the animal lifespan under hypoxia conditions. Based on the data obtained, it is assumed that further studies of North Caucasus flora plant extracts as cerebro-protective agents are promising.

Keywords: Hypoxia; Brain aschemia; Plant extracts; North Caucasus

Resumen: Hoy en día, se ha establecido que el daño cerebral isquémico, como el accidente cerebrovascular isquémico, es una de las principales causas de muerte y discapacidad en la población lo cual hace relevante el desarrollo fármacos antiisquémicos. En este trabajo se estudió el efecto antihipóxico y antiisquémico de siete extractos de plantas. La actividad antihipóxica se evaluó en modelos de hipoxia hipocrática, hipercápnic, histotóxica y hematotóxica. La actividad antiisquémica de los extractos de prueba se estudió en el modelo de isquemia cerebral focal. La administración de los extractos de *Tagetes patula*, *Gaillardia pulchella*, *Sorbaria sorbifolia*, *Grossularia reclinata*, *Ribes nigrum*, *Rubus caesius* y *Lysimachia punctata* contribuyeron a la reducción de la zona de necrosis en un 56,6% ($p<0,05$); 37,3% ($p<0,05$); 73,2% ($p<0,05$); 49,4% ($p<0,05$); 42,5% ($p<0,05$); 85,5% ($p<0,05$); 44,2% ($p<0,05$), respectivamente, además, de restaurar el metabolismo aeróbico en el tejido cerebral. Comparado con el control, se observó un aumento en el tiempo de supervivencia del animal en condiciones de hipoxia. Sobre la base de los interesantes datos obtenidos, se sugiere estudios adicionales de extractos de plantas de la flora del Cáucaso Norte como agentes protectores del cerebro.

Palabras clave: Hipoxia; Isquemia cerebral; Extracto de plantas; Cáucaso norte

Recibido | Received: March 1, 2019

Aceptado | Accepted: May 25, 2019

Aceptado en versión corregida | Accepted in revised form: June 30, 2019

Publicado en línea | Published online: September 30, 2019

Este artículo puede ser citado como / This article must be cited as: DI Pozdnyakov, AE Pozdnyakova, SL Adzhiahmetova, NM Chervonnaya, DS Zolotych, NS Lyakhova, KA Miroshnichenko. 2019 Antihypoxic and anti-ischemic properties of the North Caucasus flora plant extracts. *Bol Latinoam Caribe Plant Med Aromat* 18 (5): 504 – 517.

<https://doi.org/10.35588/blacpma.19.18.5.33>

INTRODUCTION

Ischemic stroke is a pathological condition that develops as a result of obstruction of cerebral vessels and restriction of the level of cerebral blood flow. In the general structure of cerebral circulatory disorders, the proportion of ischemic stroke accounts for about 87% of cases of cerebrovascular pathology (Roger *et al.*, 2012). Under conditions of limited blood flow in the brain tissue, irreversible processes are observed, leading to a cerebral infarction and the formation of a ischemic necrosis zone, represented by a dead functionally inactive tissue (Jin *et al.*, 2010). The pathogenesis of ischemic stroke is complex and is provided by interrelated pathophysiological processes that form an «ischemic cascade» of brain tissue damage, the main elements of which include: hypoxia, glutamate excitotoxicity, oxidative stress, inflammation, BBB damage and edema, endothelial dysfunction (Luan *et al.*, 2013; Scherbakov *et al.*, 2017). Recently, in the pathogenesis of cerebral ischemia, a relatively new pathogenetic mechanism - mitochondrial dysfunction with limited macroergs synthesis - has been released (Nicholls, 2004). However, in addition to restricting the production of high-energy compounds, mitochondrial dysfunction plays a significant role in the initiation of apoptosis and the generation of reactive oxygen species, which implies a direct relationship between mitochondrial dysfunction and the other elements of the ischemic cascade (Yang *et al.*, 2018).

Nowadays, medicinal plants are widely used in the treatment of a number of diseases, including diabetes mellitus (Kooti *et al.*, 2016), infectious pathology Alamgeer *et al.*, 2018), oncological diseases (Chen *et al.*, 2016), HIV infection Mazzari & Prieto, 2014), nephropathy (Zhang *et al.*, 2013), and dyslipidemia (Fan *et al.*, 2018). An increasing number of people use herbal medicines. This is primarily due to the optimal ratio of efficacy/safety of use, as well as a wide spectrum of pharmacological activity and the presence of pleiotropic effects (Phondani *et al.*, 2010). According to various data, phytotherapy in Asia and Africa for the purpose of primary health care, phytotherapy is preferred by about 80% of the population. In China, phytopreparations account for about 30-50% of cases of using medicines, which in economic terms amounts to about 1.8 billion dollars, and globally the turnover of the market for medicines of plant origin is more than 80 billion dollars (Rastogi *et al.*, 2015). In

addition, the World Health Organization (WHO) encourages the combined use of synthetic drugs with herbal medicines (mainly local flora) for the treatment of various diseases (Njume y Goduka, 2012). However, despite the advantages of using herbal remedies, the scientific community and practitioners are skeptical about the use of natural medicines in the treatment of a number of pathologies, including ischemic stroke. First of all, this may be due to the lack of a specific pharmacological target for phytopreparations, which is why it is not always possible to determine the «point of application» for the action of phytopreparations (Solehi *et al.*, 2018). Thus, it seems relevant to search for new pharmacological targets for the action of phytopreparations, with the aim of expanding the range of their possible applications, which include mitochondrial dysfunction. In previous studies, a high level of pharmacological activity of the North Caucasus flora plant extracts was established, which served as a criterion for the selection of test-objects (Voronkov *et al.*, 2017; Pozdnyakov *et al.*, 2018).

Investigate antihypoxic and anti-ischemic properties of the North Caucasus flora plant extracts. **The originality** of the research lies in the fact that for the first time will be studied antihypoxic properties of plant extracts of the North Caucasus flora, and the impact of these extracts on necrotic processes in the brain tissue and functional conditions of mitochondria under the focal cerebral ischemia.

MATERIALS AND METHODS

Test-objects

The choice of the test-objects and their dose was based on the previous study, in which the high pharmacological potential of the studied extracts was established (Voronkov *et al.*, 2017; Pozdnyakov *et al.*, 2018).

The study was carried out as extracts: inflorescence *Tagetes patula* L. (Asteraceae), *Gaillardia pulchella* Foug. (Asteraceae), and *Sorbaria sorbifolia* L. Mill. (Rosaceae); leaves of *Grossularia reclinata* L. Mill. (Grossulariaceae), *Ribes nigrum* L. (Grossulariaceae) and *Rubus caesius* L. (Rosaceae); herb of *Lysimachia punctata* L. (Primulaceae). The studied samples were collected on the Western slope of *Beshtau* mountain (44°4'37"N, 43°0'54"E) and were confirmed by specialists of the Department of Pharmacognosy and

Botany with the Course of Phytopreparation Technology of Pyatigorsk Medical and Pharmaceutical Institute (Head of Department Doctor of Science (Pharm.) Konovalov DA).

Extraction

1 g (precise weight) of the crushed raw material is placed in a 100 ml round-bottom flask with a thin grind, 30 ml of 40% ethyl alcohol is added, connect with the reflux condenser and heated in a boiling water bath for 1 hour. After cooling, the resulting extract is filtered through a filter paper into a 100 ml volumetric flask. Extraction is repeated twice under the conditions described above. The extract is filtered through the same filter into the same volumetric flask. After cooling, the volume is adjusted with ethyl alcohol 40% to the mark and mix.

Method of total flavonoids determination (State Pharmacopoeia of the Russian Federation)

The analytical sample of the raw material is ground to a particle size passing through a sieve with a hole diameter of 1 mm. About 1 g (precise weight) of the crushed raw material is placed in a 100 ml round-bottom flask, added 30 ml of 95% ethyl alcohol containing 2.0 ml of 10% hydrochloric acid. The flask with the contents is attached to a reflux condenser and heated in a boiling water bath for 60 minutes, periodically shaking to wash the particles of the raw material from the walls. After cooling, the resulting extract is filtered through a filter paper into a 100 ml volumetric flask, so that no particles of the raw material get into the filter. Extraction is repeated twice under the conditions described above. The extract is filtered through the same filter into the same volumetric flask. After cooling, the volume is adjusted with 95% ethyl alcohol to the mark and mixed (solution A).

2.0 ml of solution A is placed in a volumetric flask with a capacity of 25 ml, added 3 ml of 2% aluminum chloride alcohol solution, with 3 drops of 10% hydrochloric acid solution and adjusted to 25 ml volume by 95% ethyl alcohol (solution B).

After 30 min, the absorbance of the solution B is measured on an «Aquilon» spectrophotometer at a wavelength of 430±2 nm in a cell with a layer thickness of 10 mm. As a reference solution, a solution consisting of 2.0 ml of solution A, 3 drops of 10% hydrochloric acid solution and adjusted to 25 ml volume by 95% ethyl alcohol is used. The content of

the sum of flavonoids in absolutely dry raw materials is calculated by the formula:

$$X_1 = \frac{A \cdot 25 \cdot 100 \cdot 100}{A_{1\%}^{1\text{cm}} \cdot a \cdot 2 \cdot (100 - W)}$$

where:

A = is the absorbance of the test solution

ΔA = specific absorption of the complex of quercetin (rutin, luteolin, patuletin) with aluminum chloride at a wavelength of 430 nm, equal to 764,6 (248; 549,41; 679,9)

a = weight of raw material, g

W = is the humidity of the raw material, %

Biological model

The study was performed on 120 Balb/c male mice weighing 20-22 grams and 90 Wistar male rats weighing 220-240 grams. Placement, maintenance and all manipulations with animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for experimental and other national purposes (Strasbourg, 1986). The animals were kept in standard vivarium conditions with a natural change of light and dark regime, an ambient temperature of 22±2°C and a relative humidity of 65±5%. Animals received food and water *ad libitum*.

Study design

The study design is presented in Figure No. 1. The antihypoxic activity of the studied extracts was evaluated after a course of 14 days oral administration of the test objects at a dose of 100 mg/kg, while outbred mice served as a biological model at this stage of the study (n=10 for each experimental groups). At this stage of the study, the following experimental groups of animals were formed: negative control (NC) - without pharmacological support and a group of mice treated with the studied extracts. Anti-ischemic activity of the studied extracts was evaluated on the rat model of focal cerebral ischemia (n=10 for each experimental groups). The extracts were administered *per os* after the reproduction of cerebral ischemia for 4 days at a

dose of 100 mg/kg. At this stage of the study, the following experimental groups of animals were formed: pseuro-operated rats (PO), negative control (NC) - without pharmacological support and a group

of animals treated by the test-extracts. The study was approved by the Local Ethics Committee of Pyatigorsk Medical and Pharmaceutical Institute (Protocol N° 29 from 15.10.2018).

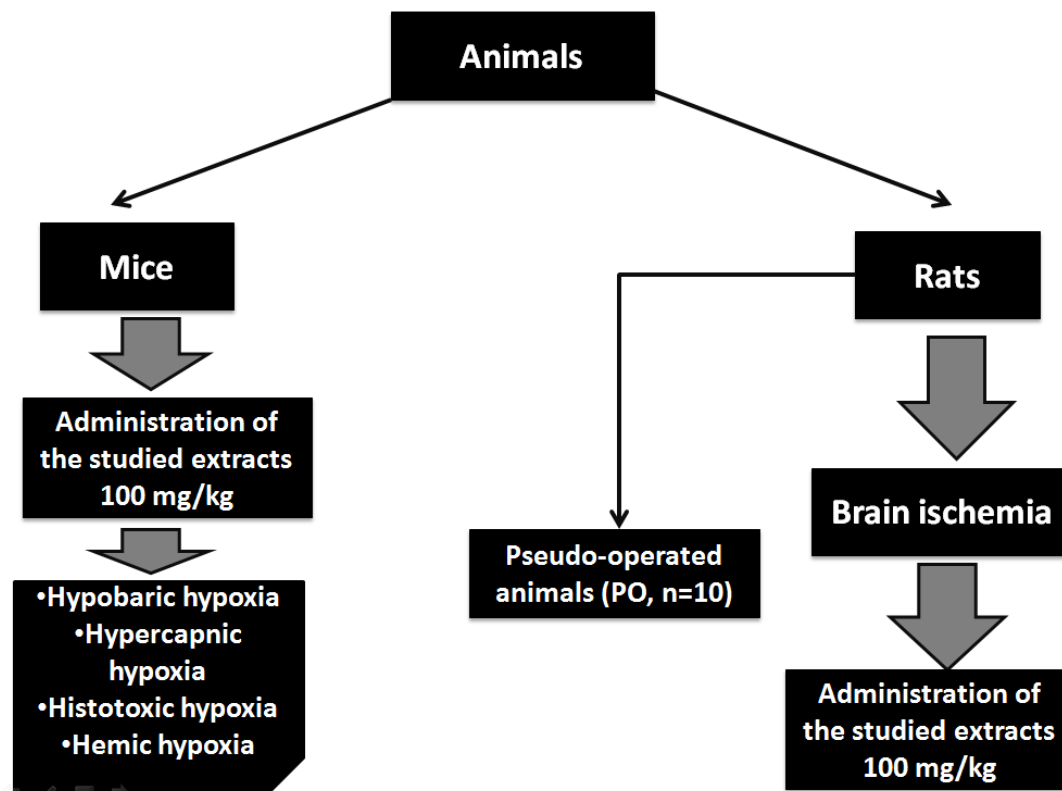


Figure No. 1
Study design

Hypoxia models

The antihypoxic properties of the test-extracts were evaluated using Balb/c mice as biomodel. Hypobaric hypoxia was reproduced by «raising» experimental animals «to a height of 11000 m» in a pressure chamber at a speed of 25-50 m/s. Hypercapnic hypoxia was modeled by placing the mice in 200 cm³ sealed containers. Hemic and histotoxic hypoxia was reproduced by single intraperitoneal administration to mice of sodium nitrite at a dose of 250 mg/kg and sodium nitroprusside at a dose of 400 mg/kg, respectively. At the same time, in all models of hypoxia, the estimated parameter was the lifespan of experimental animals (in sec).

Model of focal cerebral ischemia

Focal cerebral ischemia was modeled by irreversible right-sided thermocoagulation of the middle cerebral artery under chloral hydrate anesthesia (350 mg/kg, intraperitoneal). The area below and to the right of the eye was depilated, an incision was made and the soft tissues were moved apart, exposing the scion of the zygomatic bone, which was removed. After that trepanation gap was drilled and a thermocoagulator burned through the middle cerebral artery under its intersection with the olfactory tract. Later, as far as possible, the topography of soft tissues was restored. The seam is processed by 5% iodine solution. The biomaterial was taken on the 4th day after the reproduction of focal ischemia (Bederson *et al.*, 1986).

Biomaterial sample preparation

Rat brain was used as a biomaterial for respirometric analysis. The animals were decapitated under chloral hydrate anesthesia (350 mg/kg), organs were collected, after which the biomaterial was homogenized in a mechanical Potter homogenizer in a selection medium in the ratio 1:5 (1 mmol EDTA, 215 mmol mannitol, 75 mmol sucrose, 0.1% BSA solution, 20 mmol HEPES, with a pH of 7.2). The cell population was obtained by differential centrifugation, for which the obtained biogenic homogenate was centrifuged in the mode of 1.400 g → 3 min. at 4°C, after which the supernatant was transferred to 2 ml tubes. Next, the resulting supernatant was centrifuged at 13.000g → 10 min and the supernatant (culture contains native mitochondria) was removed for analysis (Sullivan *et al.*, 2007). The ELISA study was performed in the supernatant of rat brain tissue obtained by centrifuging the HEPES brain homogenate in the 10,000 g mode for 5 minutes according to the recommendations of the manufacturer of the reagent kit for ELISA analysis. Blood samples were obtained by centrifugation of citrate blood at 3.500 RPM → 10 min.

ELISA study

In this study, the concentration ATP was determined by ELISA in a rat brain supernatant. We used standard kits for ELISA analysis produced by Cloud Clone corp (USA). The course of the analysis corresponded to the instructions attached to each kit.

Determining the size of the necrosis zone

The size of the necrosis zone was determined by the triphenyltetrazolium method. The brain was removed, cut off the cerebellum, divided the hemispheres. Both hemispheres were weighed, then separately homogenized and placed in cups with 10 ml of a 1% solution of triphenyltetrazolium chloride in phosphate buffer (pH 7.4). Sample bottles were placed in a water bath for 20 minutes at 37°C. Next, the brain tissue was precipitated by centrifugation at 5000 RPM/10 min. The supernatant was removed and 3 ml of cooled chloroform were added to the precipitate. Shake for 2 minutes. Chloroform formazan extract was obtained for 15 min at 4°C, shaking the mixture every 5 min for 30 s. Centrifuged and measured optical density (492 nm) against pure chloroform. The calculation of the necrosis zone was expressed as

a percentage of the total mass of the hemispheres:

$$x = 100 - \frac{\varepsilon_1 M_1 + \varepsilon_2 M_2}{\varepsilon_1 (M_1 + M_2)} 100$$

Where:

x = the size of the zone of necrosis as a percentage of the total mass of the brain;

ε_1 = is the optical density of the sample with an intact hemisphere;

ε_2 = is the optical density of the sample with a damaged hemisphere;

M_1 = is the mass of the intact hemisphere;

M_2 = is the mass of the damaged hemisphere.

Respirometric analysis

Analysis of the state of the respiratory function of mitochondria was carried out by the method of respirometry using the AKPM1-01L laboratory respirometer system (Alfa Bassens, Russia). The mitochondrial respiratory function was assessed by the change in oxygen consumption in the medium against the introduction of mitochondrial respiratory uncouplers. The last in the work were: oligomycin 1 µg/ml; 4 - (trifluoromethoxy) phenyl) hydrazono) malononitrile (FCCP-1 µM); rotenone - 1 µM; sodium azide - 20 mmol. The oxidation substrates were: glucose - 15 mmol. The overall assessment of mitochondrial function was determined by the level of oxygen consumption in the medium after sequential addition of oligomycin, FCCP and rotenone to the medium, and the ATP-generating ability was determined (by the difference in oxygen consumption after the addition of FCCP and oligomycin); the maximum level of respiration (according to the difference in oxygen consumption after the addition of FCCP and rotenone) and the respiratory capacity (according to the difference in oxygen consumption after the addition of FCCP and the basal level of oxygen consumption). The activity of glycolysis processes was evaluated when glucose was used as an oxidation substrate during the registration of oxygen consumption under the conditions of sequential addition of glucose, oligomycin and sodium azide to the medium. The intensity of glycolysis was determined (according to the difference in oxygen consumption after adding glucose and the basal level of oxygen consumption), glycolytic capacity (according to the difference in oxygen consumption after adding oligomycin and

glucose) and glycolytic reserve (according to the difference in oxygen consumption after adding glucose and sodium azide). During the analysis, the biosample volume was 275 µl, and 25 µl of injected analyzers. Oxygen consumption was determined in ppm (Sauerbeck *et al.*, 2011).

Determination lactic acid concentration

The concentration of lactate in blood serum was determined in the enzymatic reaction with the formation of quinomine, the concentration of which is proportional to the content of lactic acid in the sample. Incubation medium: phosphate buffer (pH 6.8), Pipes 50 mmol/L, 4-chlorophenol 6 mmol/L, 4-AAP 0.4 mmol/L, 2000 U/L lactoxydase, U/L peroxidase. The volume of the test sample is 10 µl. Sampling was carried out at 500 nm. Calculation of lactic acid was carried out according to the formula:

$$C = Ex / E0 * 3.34 \mu\text{mol} / L$$

where

Ex - absorbance of the test sample;

E0 - absorbance calibration sample.

Determination pyruvic acid concentration

The content of pyruvic acid in blood serum was determined by the decrease in NADH in the lactate dehydrogenase reaction. Incubation medium: Good's buffer 1000 µL, NADH 200 µL, LDH (2000 U/L) 20 µl. The volume of the sample was 600 µl. Samples were extruded at 340 nm. Calculation of the content of pyruvic acid was carried out according to the formula:

$$C = Ex / E0 * 1.25 \mu\text{mol} / L.$$

Ex - absorbance of the test sample;

E0 - absorbance calibration sample.

Statistical analysis methods

Statistical processing of the obtained results was performed using the stat-analysis package STATISTICA 6.0. Data are presented as $M \pm \text{SEM}$. Comparison of medium groups was performed using the ANOVA method with the post-test of Newman-Keuls at $p < 0.05$.

RESULTS

The data obtained in assessing the total content of flavonoids in the test-extracts are presented in Table No. 1.

Table No. 1
Results of total flavonoids determination in test-objects

No	Raw material	Standart sample	Extragent	Total flavonoids, %
11	inflorescence <i>Tagetes patula</i> L	Patuletin	Ethanol 40%	2,98±0,06%
22	inflorescence <i>Gaillardia pulchella</i> Foug	Luteolin	Ethanol 40%	0,32±0,01%
33	Inflorescence <i>Sorbaria sorbifolia</i> (L.)	Quercetin	Ethanol 40%	0,822±0,02%
44	leaves of <i>Grossularia reclinata</i> L. Mill	Quercetin	Ethanol 40%	0,51±0,01%
55	leaves of <i>Ribes nigrum</i> L.	Rutin	Ethanol 40%	0,68±0,01%
66	leaves of <i>Rubus caesius</i> L	Rutin	Ethanol 40%	0,94±0,02%
77	herb of <i>Lysimachia punctata</i> L.	Quercetin	Ethanol 40%	0,59±0,01%,

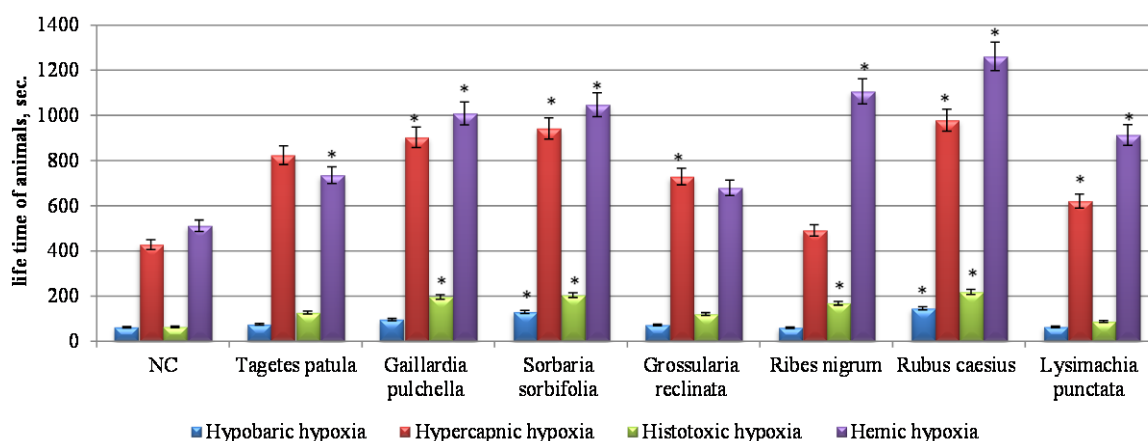
As you can see, the largest number of flavonoids contained in inflorescence *Tagetes patula* L - 2,98±0,06%

Evaluating the antihypoxic activity of the studied extracts (Figure No. 2), it was found that under conditions of hypobaric hypoxia, the use of extracts of *Sorbaria sorbifolia* and *Rubus caesius* contributes to an increase in the lifespan of mice compared to the NC group of animals by 110.4%

($p < 0.05$) and 135.9% ($p < 0.05$) respectively. The introduction of the rest of the studied objects had no significant effect on the lifespan of animals under hypobaric hypoxia (Figure No. 2). On the background of hypercapnic hypoxia, the introduction of extracts of *Tagetes patula*; *Gaillardia pulchella*;

Sorbaria sorbifolia; *Grossularia reclinata*; *Rubus caesius* and *Lysimachia punctata* contributed to an increase in the lifespan of animals relative to the NC group of mice by 92.6% ($p<0.05$); 111% ($p<0.05$); 120.2% ($p<0.05$); 70.3% ($p<0.05$); 128.8% ($p<0.05$) and 45.1% ($p<0.05$), respectively. In conditions of histotoxic hypoxia, use of extracts of *Gaillardia pulchella*; *Sorbaria sorbifolia*; *Ribes nigrum* and *Rubus caesius* contributed to an increase in the lifespan of mice in comparison with the NC group of animals by 210.4% ($p<0.05$); 223.4% ($p<0.05$); 166.5% ($p<0.05$) and 245.6% ($p<0.05$), while

administering *Tagetes patula* extracts to mice; *Grossularia reclinata* and *Lysimachia punctata* had no significant effect on the change in the parameter being assessed. In modeling hemic hypoxia, the use of the extract of *Tagetes patula* contributed to an increase in the life expectancy of animals in relation to the NC group of mice by 43.7% ($p<0.05$); *Gaillardia pulchella* - 97.2% ($p<0.05$); *Sorbaria sorbifolia* - 104.7% ($p<0.05$); *Rubus caesius* - 146.4% ($p<0.05$); *Ribes nigrum* - 116.3% ($p<0.05$) and *Lysimachia punctata* - 78.5% ($p<0.05$).

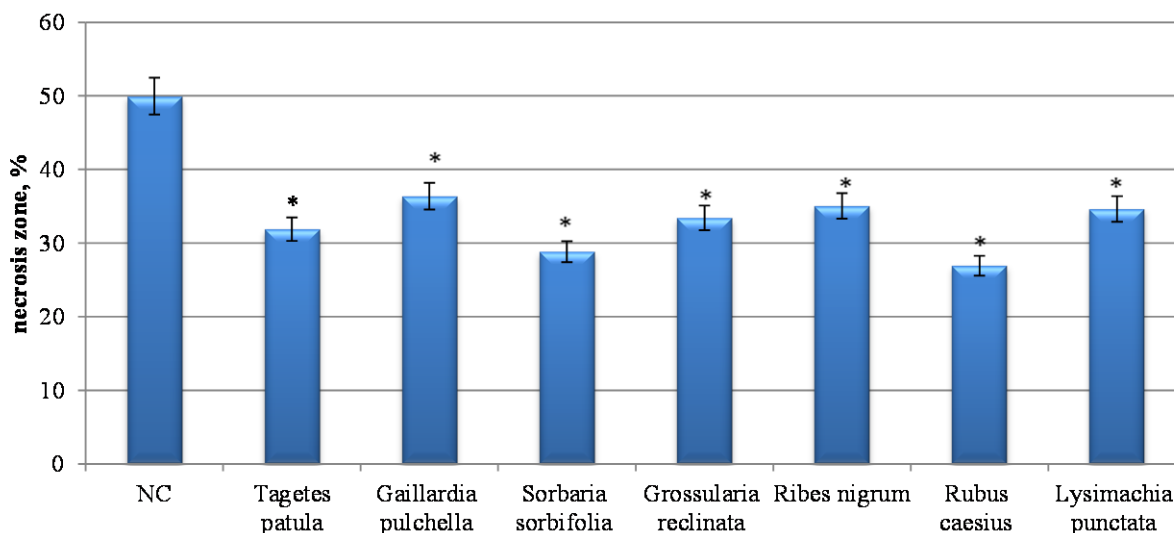


Note: * - statistically significant relative to the NC group of animals

Figure No. 2
Antihypoxic activity of the studied extracts

At the second stage of the study, the evaluation of the anti-ischemic activity of the studied extracts revealed that in the presence of cerebral ischemia, the use of extracts of *Tagetes patula*; *Gaillardia pulchella*; *Sorbaria sorbifolia*; *Grossularia reclinata*; *Ribes nigrum*; *Rubus caesius* and *Lysimachia punctata* contributed to a decrease in the necrosis zone (Figure No. 3) of the brain tissue in relation to the NC group of rats by 56.6% ($p<0.05$); 37.3% ($p<0.05$); 73.2% ($p<0.05$); 49.4% ($p<0.05$); 42.5% ($p<0.05$); 85.5% ($p<0.05$) and 44.2% ($p<0.05$). At the same time, the necrotic zone size of

the brain against the background of the administration of *Sorbaria sorbifolia* extract to animals was lower than that when using *Gaillardia pulchella*; *Ribes nigrum* and *Lysimachia punctata* extracts by 26.2% ($p<0.05$); 21.6% ($p<0.05$) and 20.2% ($p<0.05$), respectively. In addition, when the *Rubus caesius* extract was administered to rats, the degree of necrosis of the brain tissue was lower than that of *Gaillardia pulchella*; *Grossularia reclinata*; *Ribes nigrum* and *Lysimachia punctata* extracts by 35.1% ($p<0.05$); 24.1% ($p<0.05$); 30.2% ($p<0.05$) and 28.6% ($p<0.05$), respectively.



Note: * - statistically significant relative to the NC group of animals.

Figure No. 3

The change in the value of the zone of necrosis with the introduction of the studied extracts in conditions of cerebral ischemia

Under conditions of cerebral ischemia in NC group rats with respect to pseudo-operated animals, there was a decrease in ATP concentration (Table No. 1) in the brain and pyruvic acid (Table No. 2) in blood serum by 4.8 ($p<0.05$) and 4.68 ($p<0.05$) times, respectively, followed by an increase in the plasma content of lactic acid (Table No. 2) by 12 ($p<0.05$) times. The use of the *Tagetes patula* extract (Table No. 2) contributed to a decrease in the concentration of lactic acid relative to the NC group of rats 2.8 ($p<0.05$) times and an increase in the content of ATP and pyruvate by 1.67 ($p<0.05$) and 2.04 times respectively. The administration of *Gaillardia pulchella* extract to animals resulted in a decrease in the lactate content, as well as an increase in the concentration of ATP and pyruvate in 3.07 ($p<0.05$); 1.68 ($p<0.05$) and 2.09 ($p<0.05$) times, respectively. Similar changes were characteristic for groups of rats who were administered *Sorbaria sorbifolia* extract: in comparison with the NC group of rats, there was an increase in the content of ATP and pyruvate 2.5 ($p<0.05$) times and 2.76 times ($p<0.05$), respectively, and a decrease in the concentration of lactic acid in

3.53 ($p<0.05$) times. With the introduction of the extract of *Grossularia reclinata* to animals relative to the NC group of animals, the level of ATP and pyruvate increased by 1.34 ($p<0.05$) times and 1.73 ($p<0.05$) times, respectively, while the concentration of lactic acid decreased by 2.9 times ($p<0.05$). Against the background of the use of *Ribes nigrum* extract in rats compared to the NC group of animals, a decrease in the lactate content was observed by 2.9 times ($p<0.05$), followed by an increase in the level of pyruvate and ATP by 1.74 ($p<0.05$) times and 1.33 times ($p<0.05$) respectively. When *Rubus caesius* extract was administered to rats, the content of ATP and pyruvate relative to the NC of the group of animals increased 2.35 times ($p<0.05$) and 2.79 ($p<0.05$) times, respectively, while the concentration of lactic acid decreased 4.1 times ($p<0.05$). In animals treated with *Lysimachia punctata* extract in relation to the NC group of rats, there was a decrease in the level of lactate by 2.93 times ($p<0.05$), as well as an increase in the level of ATP and pyruvate by 1.34 times ($p<0.05$) and 1.82 times, respectively (Table No. 2).

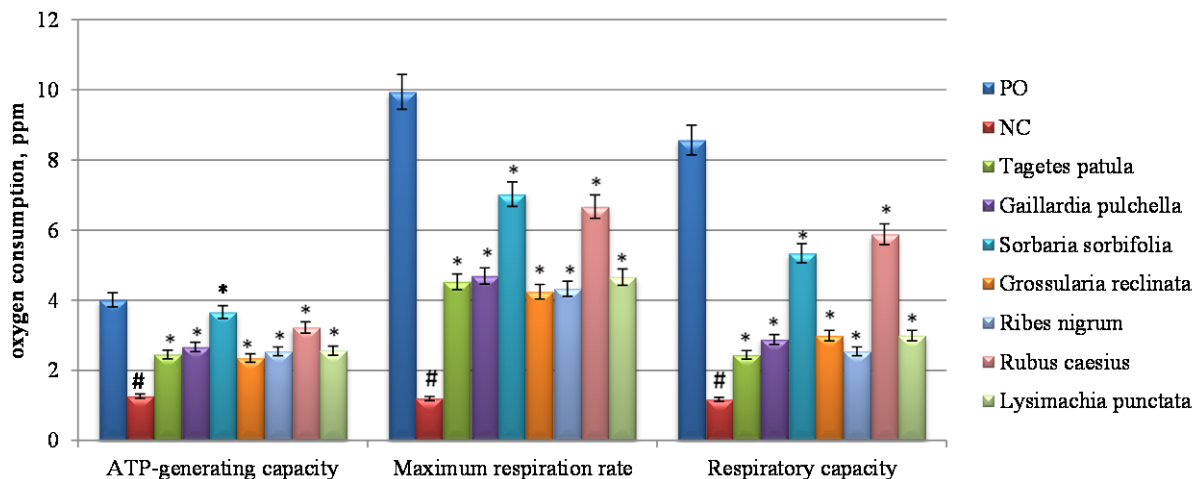
Table No. 2
Changes in the concentration of ATP, lactic and pyruvic acids in conditions of correction of cerebral ischemia by the introduction of the studied extracts

Group	PO	NC	<i>Tagetes patula</i>	<i>Gaillardia pulchella</i>	<i>Sorbaria sorbifolia</i>	<i>Grossularia reclinata</i>	<i>Ribes nigrum</i>	<i>Rubus caesius</i>	<i>Lysimachia punctata</i>
ATP, ng/ml	1237± 10,225	275,87± 7,596#	460,85± 9,119*	462,74± 12,741*	680,5± 6,117*	369,75± 14,008*	367± 13,705*	649,5± 4,805*	370± 11,262*
Lactic acid, mmol/l	1,12± 0,114	13,46± 2,282#	4,839± 0,208*	4,327± 0,361*	3,805± 0,465*	4,628± 0,203*	4,588± 0,118*	3,28± 0,331*	4,587± 1,201*
Pyruvic acid, µl	113,2± 1,971	24,21± 4,071#	49,375± 3,383*	50,82± 0,432*	66,76± 2,804*	41,79± 6,907*	42,08± 4,493*	67,54± 2,526*	44,08± 2,983*

Note: * - statistically significant relative to the NC group of animals
- statistically significant relative to PO group of animals

When assessing mitochondrial function, it was found that in rats of the NC group under conditions of cerebral ischemia compared with pseudo-operated animals, there was a decrease in ATP-generating ability by 3.2 times ($p<0.05$), the maximum level of mitochondrial respiration by 8.3 times ($p<0.05$), respiratory capacity (Figure No. 4) by 7.3 times ($p<0.05$), accompanied by an increase in the intensification of glycolysis (Figure No. 5) by 5.5 times ($p<0.05$), as well as a decrease in glycolytic capacity and glycolytic reserve in 5.68 times ($p<0.05$) and 8.36 times ($p<0.05$), respectively. When using the extract of *Tagetes patula* (Figure No. 4 and Figure No. 5) in comparison with the NC group of rats, there was an increase in ATP-generating ability, maximum respiration rate, respiratory capacity by 1.94 times ($p<0.05$); 3.78 times ($p<0.05$) and 2.08 times ($p<0.05$), respectively, while the glycolysis activity decreased by 77% ($p<0.05$) and the glycolytic reserve and glycolytic capacity increased by 1.91 times ($p<0.05$) and 2.29 times respectively.

The administration of the *Gaillardia pulchella* extract to animals showed a decrease in the intensity of the glycolysis process (Figure No. 5) compared to the NC group of rats by 1.87 times ($p<0.05$) with an increase in glycolytic capacity 2.17 times ($p<0.05$) and glycolytic reserve 2.32 times ($p<0.05$), respectively. Also, when using *Gaillardia pulchella* extract, there was an increase in ATP-generating ability, respiratory capacity and maximum respiration level relative to the NC animals group by 2.1 times ($p<0.05$); 2.46 times ($p<0.05$) and 3.92 times ($p<0.05$), respectively. In terms of administering *Sorbaria sorbifolia* extract to rats relative to rats lacking pharmacological support, an increase in ATP-generating ability, maximum respiration rate and respiratory capacity by 2.89 times ($p<0.05$); 5.86 times ($p<0.05$) and 4.54 times ($p<0.05$), respectively, the glycolysis intensity decreased by 3.18 times ($p<0.05$), with an increase in glycolytic capacity and glycolytic reserve by 3.18 times ($p<0.05$); 4.06 times ($p<0.05$) and 6.04 times ($p<0.05$), respectively.



Note: * - statistically significant relative to the NC group of animals

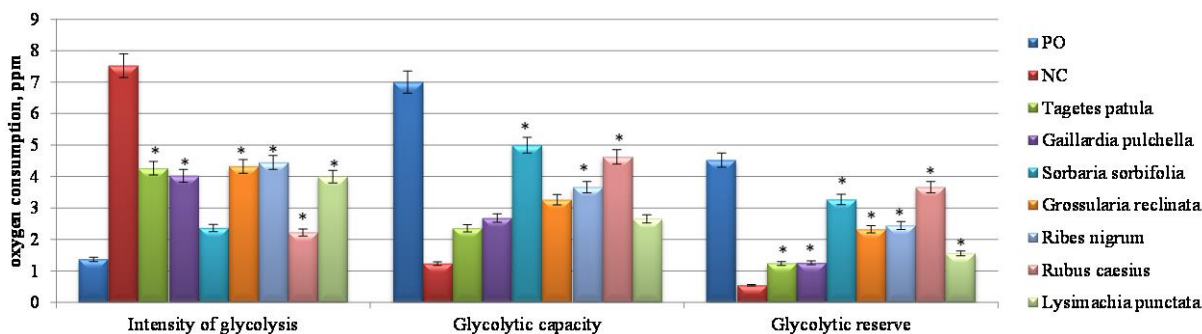
- statistically significant relative to PO group of animals

Figure No. 4

Changes in the total respiratory function of mitochondria against the background of the introduction of the studied extracts in conditions of cerebral ischemia

With the administration of the *Grossularia reclinata* extract to animals, an increase in the ATP-generating activity, the maximum level of respiration and respiratory capacity was observed in comparison with the NC group of rats in 1.86 ($p<0.05$); 3.54 times ($p<0.05$) and 2.55 times ($p<0.05$), respectively. Also, in animals with the use of the extract of *Grossularia reclinata*, an increase in glycolytic capacity and glycolytic reserve by 2.65 times ($p<0.05$) and 4.29 times ($p<0.05$), respectively, the intensity of

glycolysis decreased by 1.74 times ($p<0.05$). The introduction of *Ribes nigrum* extract into animals relative to the NC group of rats showed an increase in the ATP-generating activity, the maximum level of respiration, respiratory capacity, glycolytic capacity and glycolytic reserve by 2 times ($p<0.05$); 3.62 times ($p<0.05$); 2.17 times ($p<0.05$); 2.97 ($p<0.05$) and 4.51 times, respectively, while the intensity of glycolysis decreased by 69% ($p<0.05$).



Note: * - statistically significant relative to the NC group of animals

- statistically significant relative to PO group of animals

Figure No. 5

Changes in the process of glycolysis with the introduction of the studied extracts under conditions of cerebral ischemia

When comparing the group of animals NC and rats treated with the extract of *Rubus caesius*, it was established that the introduction of the *Rubus caesius* extract to animals showed an increase in the ATP-generating ability of 2.55 times ($p<0.05$), the maximum level of respiration 5.57 times ($p<0.05$) and respiratory capacity by 5.01 times ($p<0.05$), as well as a decrease in the intensity of glycolysis by 3.39 times ($p<0.05$), followed by an increase in glycolytic capacity and glycolytic reserve by 3.75 times ($p<0.05$) and 6.77 times ($p<0.05$), respectively. In rats treated with *Lysimachia punctata* extract relative to the NC group of animals, there was an increase in ATP-generating activity by 3.02 times ($p<0.05$), a maximum respiratory rate by 3.89 times ($p<0.05$) and respiratory capacity by 2.55 times ($p<0.05$), while reducing the intensity of glycolysis by 1.88 times ($p<0.05$) and increasing the glycolytic capacity and glycolytic reserve by 2.15 times ($p<0.05$) and 2.87 times ($p<0.05$), respectively.

DISCUSSION

Ischemic stroke is a pathological condition that develops during occlusion of a cerebral vessel, resulting in a cascade of pathophysiological reactions, leading to irreversible damage to neuronal tissue and the formation of a necrotic zone. A functionally active tissue is located around the zone of cerebral necrosis, in which, however, there is a significant limitation of the cerebral blood flow level - a zone of ischemic penumbra (Jiang *et al.*, 2016). This area is the main site of action of cerebroprotective agents with anti-ischemic and antihypoxic activity (Huang *et al.*, 2015). In the area of the ischemic penumbra, due to a decrease in blood supply and insufficient intake of oxidation substrates, in particular, glucose and oxygen, there is an increase in anaerobic oxidation processes with an increase in lactic acid concentration, as well as a decrease in pyruvate content, which causes a significant decrease in ATP synthesis and, as a result, the development of hypoxia and cell death, which increases the area of necrosis of the brain. Thus, it can be assumed that the use of agents capable of restoring mitochondrial function and the synthesis of ATP can reduce the degree of cerebronecrosis (Deng, 2016).

Currently, more and more attention is paid to herbal remedies, both by scientists and specialists of practical medicine, combining high therapeutic efficiency and low systemic toxicity of use. At the

same time, a significant role is played by means of natural origin, derived from plants, of the region in which the use of the drug is planned, i.e. locally growing plants (Grochowski *et al.*, 2016). In this regard, a study was conducted to evaluate the antihypoxic and anti-ischemic properties of extracts obtained from plants of the North Caucasus flora. In a series of experiments, it was found that the studied extracts of the inflorescences of *Tagetes patula* L., *Gaillardia pulchella* Foug. and *Sorbaria sorbifolia* L. Mill.; leaves of *Grossularia reclinata* L. Mill., *Ribes nigrum* L. and *Rubus caesius* L.; *Lysimachia punctata* L. herb have antihypoxic activity. At the same time, the most pronounced antihypoxic properties were observed in extracts of *Sorbaria sorbifolia* and *Rubus caesius*, the prophylactic administration of which increased the lifespan of mice under conditions of hypobaric, hypercapnic, histotoxic and hematotoxic hypoxia. Further, at the second stage of the study, it was established that the use of the studied extracts contributed to a decrease in the area of necrosis of the brain tissue, accompanied by the restoration of the aerobic metabolism of macroergs, which was confirmed by respiratory analysis, as well as a decrease in the concentration of lactic acid and an increase in the ATP content in the brain supernatant and pyruvate in the blood serum. The high anti-ischemic and antihypoxic activity of the studied extracts may be related to their rich chemical composition. So in extracts from *Tagetes patula* L., patuletin, patuletrin, luteolin, sesquiterpene lactones with an extensive spectrum of pharmacological activity are found includes antioxidant, anti-inflammatory, anti-cancer and cytoprotective properties (Chkhikvishvili *et al.*, 2016). In the extract obtained from *Gaillardia pulchella* Foug, the main biologic active substances are represented by vicenin-2, vitexin, luteolin, apigeninone and 6-methoxylyl-tetolin, exhibiting antioxidant, anti-inflammatory and hepatoprotective activity (Moharram *et al.*, 2017). Extracts based on *Sorbaria sorbifolia* L. Mill. characterized by the presence of anthocyanins, which have a cytoprotective effect (Sun *et al.*, 2018). Extracts obtained from *Grossularia reclinata* L. Mill., *Ribes nigrum* L. and *Rubus caesius* L.; *Lysimachia punctata* L. herb are characterized by the presence of flavonoids and terpenoids, as well as cinnamic acid derivatives, exhibiting anticancer, antioxidant, anti-inflammatory properties (Monteiro *et al.*, 2014;

Antolak et al., 2016; Grochowski et al., 2016). In addition, it is described that test- objects can be used for the treatment of ischemic stroke, while the therapeutic effect of the extracts is realized through the normalization of endothelial function and antioxidant properties (Voronkov et al., 2017). Thus, the high therapeutic potential of the studied extracts, aimed at restoring mitochondrial function in the conditions of ischemic brain damage, as well as increasing tissue resistance to hypoxia, makes these plant extracts promising means of correcting ischemic-hypoxic conditions.

CONCLUSION

The study showed the presence of extracts of inflorescences *Tagetes patula* L., *Gaillardia pulchella* Foug. and *Sorbaria sorbifolia* L. Mill.; leaves of *Grossularia reclinata* L. Mill., *Ribes nigrum* L. and *Rubus caesius* L.; Herb of *Lysimachia punctata* L. Antihypoxic and anti-ischemic properties evaluated under conditions of focal cerebral ischemia. The introduction of the studied extracts contributed to a decrease in the size of the necrosis zone of the brain in rats, and also restored neuronal metabolism, which was confirmed by the data of respiratory and ELISA studies. Based on the obtained results, it is assumed that further study of the studied objects is promising in order to expand the spectrum of cerebrotropic drugs used for the therapy of ischemic stroke.

REFERENCES

- Alamgeer, Younis W, Asif H, Sharif A, Riaz H, Bukhari IA, Assiri AM. 2018. Traditional medicinal plants used for respiratory disorders in Pakistan: a review of the ethno-medicinal and pharmacological evidence. **Chin Med** 13: 48.
<https://doi.org/10.1186/s13020-018-0204-y>
- Antolak H, Czyzowska A, Kregiel D. 2016. Black Currant (*Ribes nigrum* L.) and Bilberry (*Vaccinium myrtillus* L.) Fruit Juices Inhibit Adhesion of *Asaia* spp. **Biomed Res Int** 2016: 3671306.
<https://doi.org/10.1155/2016/3671306>
- Chen SL, Yu H, Luo HM, Wu Q, Li CF, Steinmetz A. 2016. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. **Chin Med** 11: 37.
<https://doi.org/10.1186/s13020-016-0108-7>
- Chkhikvishvili I, Sanikidze T, Gogia N, Enukidze M, Machavariani M, Kipiani N, Vinokur Y, Rodov V. 2016. Constituents of French Marigold (*Tagetes patula* L.) flowers protect jurkat T-cells against oxidative stress. **Oxid Med Cell Longev** 2016: 4216285.
<https://doi.org/10.1155/2016/4216285>
- Deng YH, He HY, Yang LQ, Zhang PY. 2016. Dynamic changes in neuronal autophagy and apoptosis in the ischemic penumbra following permanent ischemic stroke. **Neural Regen Res** 11: 1108 - 1114.
<https://doi.org/10.4103/1673-5374.187045>
- Fan Y, Jin X, Man C, Gong D. 2018. Does adjuvant treatment with *Ginkgo biloba* to statins have additional benefits in patients with dyslipidemia?. **Front Pharmacol** 9: article 659.
<https://doi.org/10.3389/fphar.2018.00659>
- Grochowski DM, Paduch R, Wiater A, Dudek A, Pleszczyńska M, Tomczykowa M, Granica S, Polak P, Tomczyk M. 2016. *In vitro* antiproliferative and antioxidant effects of extracts from *Rubus caesius* leaves and their quality evaluation. **Evid Based Complement Alternat Med** 2016: 5698685.
<https://doi.org/10.1155/2016/5698685>
- Huang H, Chen YM, Zhu F, Tang ST, Xiao JD, Li LL, Lin XJ. 2015. Down-regulated Na⁺/K⁺-ATPase activity in ischemic penumbra after focal cerebral ischemia/reperfusion in rats. **Int J Clin Exp Pathol** 8: 12708 - 12717.
- Jiang MQ, Zhao YY, Cao W, Wei ZZ, Gu X, Wei L, Yu SP. 2016. Long-term survival and regeneration of neuronal and vasculature cells inside the core region after ischemic stroke in adult mice. **Brain Pathol** 27: 480 - 498.
<https://doi.org/10.1111/bpa.12425>
- Jin R, Yang G, Li G. 2010. Inflammatory mechanisms in ischemic stroke: Role of inflammatory cells. **J Leukoc Biol** 87: 779 - 789.
<https://doi.org/10.1189/jlb.1109766>
- Kim SW. 2012. Phytotherapy: emerging therapeutic option in urologic disease. **Transl Androl Urol** 1: 181 - 191.
- Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D, Asadi-Samani M. 2016. The role of medicinal plants in the treatment of diabetes:

- A systematic review. **Electron Physician** 8: 1832 - 1842.
<https://doi.org/10.19082/1832>
- Luan H, Kan Z, Xu Y, Lv C, Jiang W. 2013. Rosmarinic acid protects against experimental diabetes with cerebral ischemia: relation to inflammation response. **J Neuroinflammation** 10: 28.
<https://doi.org/10.1186/1742-2094-10-28>
- Mazzari ALDA, Prieto JM. 2014. Herbal medicines in Brazil: pharmacokinetic profile and potential herb-drug interactions. **Front Pharmacol** 5: article 162.
<https://doi.org/10.3389/fphar.2014.00162>
- Moharram FA, El Dib RAEM, Marzouk MS, El-Shenawy SM, Ibrahim HA. 2017. New apigenin glycoside, polyphenolic constituents, anti-inflammatory and hepatoprotective activities of *Gaillardia grandiflora* and *Gaillardia pulchella* aerial parts. **Pharmacogn Mag** 13: S244 - S249.
https://doi.org/10.4103/pm.pm_344_16
- Monteiro Lde S, Bastos KX, Barbosa-Filho JM, de Athayde-Filho PF, Diniz Mde F, Sobral MV. 2014. Medicinal plants and other living organisms with antitumor potential against lung cancer. **Evid Based Complement Alternat Med** 2014: 604152.
https://doi.org/10.4103/pm.pm_344_16
- Nicholls DG. 2004. Mitochondrial dysfunction and glutamate excitotoxicity studied in primary neuronal cultures. **Curr Mol Med** 4: 149 - 177.
<https://doi.org/10.2174/1566524043479239>
- Njume C, Goduka NI. 2012. Treatment of diarrhoea in rural African communities: an overview of measures to maximise the medicinal potentials of indigenous plants. **Int J Environ Res Public Health** 9: 3911 - 3933.
<https://doi.org/10.3390/ijerph9113911>
- Phondani PC, Maikhuri RK, Kal CP. 2010. Ethnoveterinary uses of medicinal plants among traditional herbal healers in Alaknanda catchment of Uttarakhand, India. **Afr J Tradit Complement Altern Med** 7: 195 - 206.
<https://doi.org/10.4314/ajtcam.v7i3.54775>
- Rastogi S, Pandey MK, Prakash J, Sharma A, Singh GN. 2015. Veterinary herbal medicines in India. **Pharmacogn Rev** 9: 155 - 163.
<https://doi.org/10.4103/0973-7847.162140>
- Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Litchman JH, Lisabeth LD, Makuc DM, Markus GM, Marelli A, Matchar DV, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. 2012. Heart disease and stroke statistics-2012 update: a report from the American Heart Association. **Circulation** 125: 2 - 220.
<https://doi.org/10.1161/cir.0b013e3182456d46>
- Salehi B, Kumar NVA, Şener B, Sharifi-Rad M, Kılıç N, Mahady GB, Vlaisavljevic S, Iriti M, Kobarfard F, Setzer WN, Ayatollahi SA, Ata A, Sharifi-Rad J. 2018. Cerebral artery occlusion: evaluation of the model and development of a neurologic examination. **Stroke** 17: 472 - 476.
<https://doi.org/10.3390/ijms19051459>
- Sauerbeck A, Pandya J, Singh I, Bittman K, Readnower R, Bing G, Sullivan P. 2011. Analysis of regional brain mitochondrial bioenergetics and susceptibility to mitochondrial inhibition utilizing a microplate based system. **J Neurosc Method** 198: 36 - 43.
<https://doi.org/10.1016/j.jneumeth.2011.03.007>
- Scherbakov N, Sandek A, Ebner N, Valentova M, Nave AH, Jankowska EA, Schefold JC, von Haehling S, Anker SD, Fietze I, Fiebach JB, Haeusler KG, Doehner W. 2017. Sleep-disordered breathing in acute ischemic stroke: A mechanistic link to peripheral endothelial dysfunction. **J Am Heart Assoc** 6: e006010.
<https://doi.org/10.1161/jaha.117.006010>
- Sullivan PG, Krishnamurthy S, Patel SP, Pandya JD, Rabchevsky AG. 2007. Temporal characterization of mitochondrial bioenergetics after spinal cord injury. **J Neurotrauma** 24: 991 - 999.
<https://doi.org/10.1089/neu.2006.0242>
- Sun J, Shi S, Li J, Yu J, Wang L, Yang X, Guo L,

- Zhou S. 2018. Phylogeny of Maleae (Rosaceae) based on multiple chloroplast regions: implications to genera circumscription. **Biomed Res Int** 2018: 7627191.
<https://doi.org/10.1155/2018/7627191>
- Yang JL, Mukda S, Chen SD. 2018. Diverse roles of mitochondria in ischemic stroke. **Redox Biol** 16: 263 - 275.
- <https://doi.org/10.1016/j.redox.2018.03.002>
- Zhang L, Mao W, Guo X, Wu Y, Li C, Lu Z, Su G, Li X, Liu Z, Guo R, Jie X, Wen Z, Liu X. 2013. *Ginkgo biloba* extract for patients with early diabetic nephropathy: A systematic review. **Evid Based Complement Alternat Med** 2013: 689142.
<https://doi.org/10.1155/2013/689142>