Choline accumulation in mistletoe leaves (*Viscum album* L.) and the effect of extracts based on them on the course of the sporadic form of Alzheimer's disease in experimental animals

Pozdnyakov Dmitry[1*], Adzhiakhmetova Simillа[2], Popova Olga[3], Oganesyan Eduard[2].

1- Department of pharmacology with course of clinical pharmacology, Pyatigorsk Medical and Pharmaceutical Institute

2- - Department of organic chemistry, Pyatigorsk Medical and Pharmaceutical Institute

3- Department of pharmacognosy and botany with course of phytomedicines technology, Pyatigorsk Medical and Pharmaceutical Institute

Abstract

Choline is an important vitamin-like compound, the deficiency of which is one of the bases of the pathogenesis of neurodegenerative diseases, in particular Alzheimer's disease. Aim of the study: to evaluate the content of choline in white mistletoe leaves and to study the effect of white mistletoe leaf extract on the course of the sporadic form of Alzheimer's disease in rats. White mistletoe leaves are collected in autumn and winter from the host tree - the apple tree. The concentration of choline is measured spectrophotometrically by the changes of choline rheinecate absorbance at 400 nm. The anti- amyloid properties of the received extracts are estimated *in vitro* to prevent the formation of beta-amyloid aggregates. Alzheimer's disease was modeled in Wistar rats. Changes in the cognitive functions, cerebral hemodynamics, and the level of beta-amyloid and tau protein in the hippocampus of rats were observed. In mistletoe leaves collected in winter, the concentration of choline at the tendency level was higher than in leaves harvested in autumn (3.07±1.84% versus 2.88±1.03%). *In vitro*, extracts from white mistletoe leaves decrease aggregation of beta-amyloid plaques. In rats with experimental Alzheimer's disease, the course administration of mistletoe extracts, improve cognitive functions, increase in cerebral blood flow and a decrease in the content of beta-amyloid and tau protein in hippocampal tissues.

Keywords: *mistletoe, choline, Alzheimer's disease, amyloid-beta, neuroprotection*

**Corresponding author: Pozdnyakov Dmitry, pozdniackow.dmitry@yandex.ru*

Introduction

White mistletoe (*Viscum album* L.) the belt (*Loranthaceae* Yuss.) or mistletoe (*Viscaceae* Miers.) family is one of the types of parasitic plants – «hemiparasite» with haustorial roots. Usually various species grow on deciduous trees, more often representatives of the *Rosaceae* family (apple, pear), as well as on willows, poplars. *Viscum album* consumes water and minerals, salts of the host tree, belonging to special organs - haustorium, but its chloroplasts, produced organic substances during photosynthesis (Plant resources of USSR, 1988, Eddouks M, 2017).

The main biologically active compounds of mistletoe are: lectins, viscotoxins, nitrogen-containing substances, amino acids, polysaccharides, triterpenoids (ursolic acid), tannins, flavonoids, phospholipids (Korman, 2011; Tomkevich, 2005; Bavashcheva and Turyanov, 2004)

The largest therapeutic group of compounds of nitrogenous origin, with a high spectrum of biological activity. Choline is involved in the formation of the myelin in the nervous system and is an integral part of phospholipids (lecithins and sphingomyelins), a biosynthetically active neurotransmitter of acetylcholine, which transmits impulses between nerve cells and from nerve cells to muscles, which is necessary for the activity of autonomic nerve ganglions, motor nerves and parasympathetic endings. The breakdown product of choline, betaine, regulates intracellular pressure (Pilat and Ivanov, 2003; White, 1981; Kuksis and Mukherjea, 1978).

Choline is a substrate of betaine-homocysteine-S-methyltransferase and thus takes part in the elimination of hyperhomocysteinemia. Its high content in blood plasma is detrimental to the vascular walls and at times increases the risk of cardiovascular pathologies, Alzheimer's disease, Parkinson's disease, ischemic stroke, and osteoporosis. Choline-rich food is very important not only for the

developing, but also for the mature brain (Fioravanti and Yanagi, 2005; Velasquez et al.2019). Choline deficiency, first of all, reveals disorders of cholinergic innervation, the most important manifestations of which are disorders of mnestic disease. *Velazquez et al, 2019* demonstrated that the inclusion of choline in the diet restores cognitive functions in Alzheimer's disease (Velasquez et al.2019). It has also been shown that systemic choline intake causes neurodegeneration in a high-fat diet (Prabhu et al. 2021). An important aspect of the central pharmacological effects of choline is the reduction of the toxic effect of ethanol on the brain (Bearer et al. 2015).

The purpose of this work was to determine the content of choline in mistletoe leaves, as well as to study effect of mistletoe leaves exctract during the sporadic form of Alzheimer's disease in rats.

Materials and methods

Test-objects

The test-object was white mistletoe leaves (*Viscum album* L.), growing on apple tree (*Malus homemade Borch*.), collected in the vicinity of the Stavropol city. Raw materials - leaves were collected in autumn (09/25/2021) and winter (01/21/2022) periods. Identification of raw materials was carried out by the professor of the Department of Pharmacognosy, Botany with the course of technologies of phytomedicines , Ph.D. O.I. Popova.

The mass of the analytical sample to determine the choline excess concentration was 3.0 g (State Pharmacopoeia of Russian Federation, 2018).

Quantitative choline determination

At the base of the method for choline determining by the detection of optical density of colored complexes of nitrogenous bases with Reinecke's salt.

The analytical sample (leaves) was crushed to the size of particles passing through a sieve with holes with a 3 mm diameter.

About 3.0 g of raw materials are placed in a round-bottom flask with a capacity of 200 ml, pour 30 ml of purified water, weigh with an error of ± 0.01, attach to a reflux condenser, heat on a water bath for 2 hours and bring the purified water to the original weight. The hot extract is then filtered through a 10 cm paper filter into a 50 ml flask. When cooled to 0˚C, a 2 M solution of hydrochloric acid pH = 3 (according to the universal indicator) is added dropwise to the solution. The resulting solution is cooled to 0°C, 15 ml of Reinecke's salt solution is added and left in the refrigerator for 18 hours. The precipitate is filtered off through a glass filter and washed at 0°C with n-butyl alcohol 3 times with 5 ml portions. Then, at the same temperature, the precipitate is dissolved on the filter with acetone, and the volume of the resulting filtrate is adjusted to a pycnometer with acetone to 10 ml.

The optical density of the obtained solution is measured not much more than after 5 min on an SF-102 spectrophotometer (Acvilon, Russia) at 400 nm. Acetone is used as a control solution. In parallel, under the same conditions, the optical density of a standard solution of choline chloride with Reinecke's salt is measured (Muravyova, 1991; Popova and Muravyova, 1996; Pozdnyakova and Bubenchikov, 2015).

The content of choline in terms of choline chloride and absolutely dry raw materials in percent (X) is calculated by the formula:

$$X_1 = \frac{A \cdot m_0 \cdot 30 \cdot 100 \cdot 100}{A_0 \cdot m \cdot 10 \cdot (100 - W)};$$

where: A – sample optical density; A_0 is the optical density of the standard sample of choline chloride; m - the mass of raw materials, g; m_0 - a sample of the working sample of the choline chloride standard; W – humidity of raw material (8.34%).

Preparation of a choline chloride standard sample solution.

0.2 g (accurately weighed) of the working sample of choline chloride (Sigma-Aldrich) is dissolved in 10 ml of purified water, a 2 M solution (2 mol/l) of hydrochloric acid is added dropwise to pH = 3 (according to the universal indicator). The resulting solution is cooled to 0°C, 15 ml salt solution is added and left in the refrigerator for 18 hours. The precipitate is separated and at the same temperature dissolved in acetone. The resulting solution is adjusted with acetone to a depth of 10 ml with acetone. Optical evaluation of the solution no later than 5 min (Muravyova, 1991)

In vitro evaluation of anti- amyloid activity of extracts from mistletoe leaves

During the evaluation of anti- amyloid activity, water extracts from white mistletoe leaves (from choline determination) were evaporated in a water bath to a dry residue, 20 mg of residue were dissolved in 10 ml of dimethyl sulfoxide with the resulting working solution, after which a series of two-fold dilutions was prepared with a concentration of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.625 mg/ml. β - amyloid (fragments $Aß_{1-42}$) dissolve in phosphate-buffer (PBS) solution within 24 hours with constant recovery. Next, to 25 µl of the 2-fold diluted solution was added 225 µl of 20 mM Congo red solution in PBS and 50 µl of Aβ solution. The resulting mixture was incubated at ambient temperature. Next, the optical density of the samples was recorded at wavelengths of 540 and 405 nm through the 9 days of incubation. The number of aggregates Aβ, calculated by the formula

$$Aβ = \frac{A540}{4780} - \frac{A405}{6830} - A405CR/8620, \text{ where}$$

where ACR405 is the optical density of the Congo red at 405 nm; A540 and A405 - optical density of sample at 540 and 405 nm, respectively. As a reference,

choline alfoscerate is used in analogues concentrations. All tests were performed six times (Wang et al. 2017).

Experimental animals

The work was performed on 50 mature male Wistar rats weighing 220-240 grams. Animals were obtained from the nursery of laboratory animals Rappolovo (Russia, Leningrad region) and for the duration of the experiment were kept in controlled conditions of Laboratory of living systems of the Pyatigorsk Medical and Pharmaceutical Institute at an ambient temperature of 22 ± 2 ^{0} C, relative humidity - 60± 5%, and 12-hour change of the daily cycle. Animals were kept in groups by 5 rats in macrolon cages with granular hardwood bedding freely available for water and complete food. Content and all manipulations carried out with animals , applications recommendations Directive 2010/63/EC of the European Parliament and of the Council on the protection of animals used for scientific purposes, September 22, 2010 and ARRIVE 2.0 recommendations (Percie du Sert et al. 2020).

Experimental model of the sporadic form of Alzheimer's disease (AD)

AD was reproduced the pathway of direct injection of Aß$_{1-42}$ aggregates to the hippocampus of animals. Prior to inclusion, fragments Aß$_{1-42}$ were solubilized in cold PBS with pH=7.4 for 36 hours while constantly changing the top-drive receiving mechanical mixing unit to Aß$_{1-42}$ aggregates. Next, the rats were anesthetized with an intraperitoneal injection of chloral hydrate at a dose of 350 mg/kg, the parietal area was scalped and animal was fixed in a stereotaxic setup. Aggregates Aß$_{1-42}$ were injected at a final concentration of 1 mmol /l in a volume of 2 µl using a microdoser and a G 30 needle (anterior-posterior = -3.8 mm, medial-lateral = 2.0 mm, dorsal-ventral = 2.6 mm from bregma). Stereotaxic coordinates were estimated according to Paxinos G., 2007 (Paxinos and Watson,

2007). The needle was left in injection region for 5 min. If possible, restore the topography of tissues and sutured the wound. The suture was treated with 10% povidone-iodine solution (Rosales-Corral et al. 2012)

Study Design

Experimental animals were divided into 5 groups of 10 animals each: sham-operated animals (SO) - rats without Alzheimer's; negative control (NC) - animals with experimental AD, but without pharmacological correction; CHA - a group of animals with AD and treated with the reference drug choline alfoscerate; ALE - a group of rats with experimental AD, which was treated with aqueous extract from the mistletoe leaves harvested in autumn; WLE - a group of rats with experimental AD, which was treated with aqueous extract from the mistletoe leaves collected in winter. Referents and tested extracts was administered orally within 30 days from the injection of $Aß_{1-42}$ at doses of 50 mg/kg and 100 mg/kg, respectively. Before the administration, the extracts were evaporated to a dry residue, which was dissolved in purified water. The change in cognitive deficit in rats in the Y - maze test is assessed after 30 days. Next, the animals were anesthetized and the level of cerebral blood flow was detected, after which the rats were decapitated and the brain was removed, the hippocampus was isolated. In the tissues of the hippocampus, the content of Aß and tau protein is determined.

Y- maze test

30 days after surgery, rats were subjected to reverse cognitive functions in the Y-maze test. The device consisted of three sleeves combined at an angle of 120^0 . The animal was placed in the center of the setup and the number of animals moved between the sleeves was recorded for 8 minutes. At the same time, spontaneous alternating entries are recorded in the sleeve (1-2-3, 3-1-2, 2-3-1). Based on the

initial data, the percentages of spontaneous alternation are calculated, which provide a change in the cognitive abilities of animals (Amani et al. 2019).

$$\text{Percentage of spontaneous alternation} = \frac{\text{Number of subsequent entries in the arms}}{\text{Total number of entries}} * 100$$

Determination of the cerebral blood flow

Changes in cerebral hemodynamics are observed in the basin of the middle cerebral artery. In rats, a parietal area was scalped and a burr hole 1 mm in diameter was made with a burr, 0.3 ml of a sound-conducting gel was placed, after which a probe of an ultrasonic dopplerograph UZOP -01-10 with a frequency of 25 MHz was installed. Analytical Signal processing by using Minimax Software Doppler v.2.0. Calculate the estimate of systolic blood flow velocity in cm/sec.

Preparation of biomaterial for determination of the content of Aß and tau protein

The hippocampus was homogenized in PBS with pH 7.4 in a ratio of 1:7. The resulting homogenate was centrifuged at 10,000 g for 10 minutes to obtain a supernatant , which was used for analysis.

Detection detection of A ß and tau protein

The content of Aß and tau protein in the hippocampus is assessed by enzyme-linked immunosorbent assay. Species-specific reagent kits manufactured by Cloud Clone corp were used in the work. The analysis was made in accordance with the manufacturer's recommendations. The optical density is recorded at 450 nm on microplates. ELISA reader Infinite F50 (Tecan , Austria).

Statistical analysis

MS Excel 2013 and STATISTICA 6.0 software packages (StatSoft , USA) for Windows OS was used in statistical analysis. Data a given as M (mean) ± SEM (standard error of the mean). The normality of the distribution as estimated by the Shapiro-Wilk test , the uniformity of variances by the Levene test. Statistically significant differences between groups measured by one-way analysis of variance (ANOVA) with Newman-Keuls post-processing under normal data distribution and Kruskal-Wallis post-processing under non- normally distributed data. The critical level of significance was $p<0.05$.

Results

Choline determination in mistletoe leaves extracts

Spectrophotometrically determined the amount of choline in the leaves of mistletoe. During the study it was found that in white mistletoe leaves collected in autumn and winter, 2.88±1.03% and 3.07±1.84% of choline.

Figure 1

Table 1

Influence of aqueous extracts of mistletoe on the aggregation of amyloid particles in vitro

Analysis of changes in the aggregation of fragments Aß *in vitro* it was found that both tested objects show decrease an accumulation of amyloid particles by 70.2±1.3% and 79.3±2.8%, respectively, while the choline alfoscerate reduces Aß aggregation by 69.8±2.5%. It should be noted that no statistically significant deviations of Aß aggregation obtained by taking extracts of mistletoe leaves and choline with alfoscerate were found (Fig. 2).

Influence of course administration of water extracts of mistletoe on changes in cognitive functions of animals with sporadic form of AD

Figure 2

The study of changes in cognitive functions of animals in the sporadic form of AD showed that in rats of the NC group, the number of spontaneous alternations in the Y - maze test decreased by 51.3% in relation to SO animals ($p < 0.05$). The administration of choline alfoscerate causes the appearance of cognitive functions in rats by 57.6% ($p < 0.05$) relative to NC rats (Fig. 3). The use of extracts from the leaves of mistletoe, collected in autumn and winter, an increase in the number of spontaneous movements between the arms of the labyrinth by 38.0% ($p < 0.05$) and 51.1% ($p < 0.05$), respectively, that involvement did not differ on the indicators of the group of animals treated with choline alfoscerate (Fig. 3).

Figure 3

Influence of course administration of water extracts of mistletoe on changes in cerebral blood flow in animals with sporadic AD

In rats of the NC group, the increase in cerebral blood flow by 26.8% ($p < 0.05$) relative to the SO animals (Fig. 4). The use of choline alfoscerate causes an increase in the rate of cerebral blood flow in rats by 16.8% ($p < 0.05$), while the gradual administration of extracts from mistletoe leaves collected in autumn or winter, an increase in this indicator is 12.6% ($p < 0.05$) and 19.3% ($p < 0.05$), respectively. It should be noted that no significant differences were found between the f rats that received the reference and the investigated extracts (Fig. 4).

Figure 4

Influence of course administration of water extracts of mistletoe on changes in the concentration of Aß and tau protein in the hippocampus of animals with sporadic AD

Analysis of changes in the content of Aß and tau protein in the hippocampus of rats (Fig. 5) showed that in the NC group of animals relative to the SO rats, the content of Aß and tau protein increased 2.9 times ($p < 0.05$) and 5.8 times ($p < 0.05$), respectively (Fig. 5). Against the background of the administration of choline alfoscerate, the concentration of Aß decreased by 27.0% ($p < 0.05$), with a decrease in the content of tau protein by 29.6% ($p < 0.05$). In animals treated with white mistletoe leaf extract, collected in autumn, the concentration of Aß and tau protein decreased in relation with NC group by 24.2% ($p < 0.05$) and 32.9% ($p < 0.05$) respectively. At the same time, when extracting from the leaves of white mistletoe, collected in winter, the content of Aβ and tau protein was less than assimilated in the group of animals by 35.5% ($p < 0.05$) and 36.8% ($p < 0.05$).

Figure 5

Discussion

Choline is a vitamin-like compound synthesized endogenously in the human body. Choline is a vital factor involved in the synthesis of membrane phospholipids (e.g., phosphatidylcholine), the neurotransmitters acetylcholine, and in betaine-dependent observations methyl group S -adenosylmethionine. Choline deficiency is one of the predictors of the development of cognitive deficits. It has been noted that insufficient intake of dietary choline leads to variable neurodegenerative dependence, which includes AD, Parkinson's disease, amyotrophic lateral sclerosis. In this regard, the replenishment of choline intake is an actual direction in the prevention and treatment of diseases of the central nervous system. *Velazquez et al, 2020* demonstrated that lifelong intake of choline at doses 4.5 times the daily dietary allowance leads to a significant regression of

tense microglial activity, thereby reducing neuroinflammation and stabilizing the cognitive background of animals (Velazquez et al. 2020*)*. Long-term gradual administration (9-12 months) of choline to reduce astrogliosis and proteins-marker of cholinergic dysfunction – CHAT (Blastein et al.2017). Thus, in order to prevent neurodegenerative diseases of the CNS and their successful pharmacotherapy, it is important to take into account the amount of choline that enters the human body with food. To achieve the daily recommended intake of choline consumption of foods rich in edible food: eggs, red meat and poultry. However, the consumption of meat by the population tends to decrease, which may be one of the reasons for the dietary deficiency of choline. Decreasing the share of choline-fortified foods in the diet requires finding alternative sources of choline, for example, plant-based products that exclude food sources of choline may also be the price of drug sources. High choline content has been found in soybeans, Brussels sprouts, and cereals (Caudill et al. 2018). Nevertheless, the search for new natural sources of choline may become an important trend in modern phytochemistry and ethnopharmacology (Hananea, et.al., 2021). During the study it was found that in white mistletoe leaves collected in autumn and winter, 2.88±1.03% and 3.07±1.84% of choline. At the same time, at the level of choline in winter mistletoe leaves were higher than those in leaves harvested in autumn, which revealed the promising phytochemical research of mistletoe leaves harvested in summer and spring for the presence of choline content. The block of pharmacological manifestations reveals that the dry residues of mistletoe leaves, collected in autumn and winter, *in vitro* reduce the aggregation of Aβ plaques, probably due to the hydrotropic properties of choline (Gainanova et al. 2018). Also, the potential anti-amyloid properties of other pharmacological compounds that find in mistletoe, such as flavonoids or specific lectins, cannot be denied (Nazaruk et al. 2016).

Results of *in vivo* analysis of indicators of improvement in cognitive functions of animals with experimental sporadic AD in the conditions of the Y - maze test. It should be revealed that there were no significant differences between the groups of animals that was treated by obtained extracts of white mistletoe leaves collected in winter and autumn. The analyzed detections also reveal an equivalent reference drug (choline-alfoscerate) level of pharmacological activity. An important aspect of the neurotropic properties of the exceptional mistletoe extracts is the restoration of cerebral hemodynamics, the reduction of Aβ and tau protein in the hippocampus tissue of animals. It is known that mistletoe finds wide ethno -medical application as an effective remedy for correcting CNS disorders. In particular, the range of neurotropic properties of mistletoe includes: antiepileptic, sedative, neuroleptic, anxiolytic, antidepressant and antinociceptive action. In addition, the probability of extracting from mistletoe the capture of apoptotic neurons caused by Aβ (Szurpnicka et al. 2019). Under various AD conditions, mistletoe extracts demonstrated a high level of pharmacological efficacy. *Ekpenyong et al, 2016* that a oral 21-day administration of an aqueous extract of *Viscum album* leaves growing on an orange tree (*Rutaceae* : *Citrus sinensis* (L.) Osbeck), at a dose of 100 mg/kg, the level of BDNF increases in animals with Alzheimer's disease caused by aluminum chloride (Ekpenyong et al. 2016*)*. Methanol and ethanol extracts of *Viscum album* subspecies *coloratum* Com. at concentrations of 10, 30 and 50 µg/ml prevent the death of a neuron culture under conditions of neurotoxic exposure to Aβ and hydrogen peroxide. In an experiment *in vivo* administration of a methanol extract decrease Aβ -induced mental disorders in mice (Jang et al. 2015). Thus, the conducted study expands the understanding of the neurotropic activity of white mistletoe extract, in particular, focusing on pharmacological effects and the analysis of biologically active substances (choline), which are the most therapeutically attractive in AD.

Conclusion

In the leaves of white mistletoe, collected in autumn and winter, a high content of choline was found, amounting to 2.88±1.03% and 3.07±1.84%, respectively. In the course of a pharmacological experiment, anti- amyloid properties were demonstrated , analyzed extractable, restored with those of choline alfoscerate. The course of the study showed an improvement in cognitive functions and cerebral circulation in rats with an experimental sporadic AD treated with mistletoe leaf extracts. The use of the studied extracts also led to a decrease in the concentration of Aβ and tau protein in the hippocampus tissue of animals. In accordance with the purpose of the amount of accumulation, the relevance of the phytochemical and pharmacological study of the leaves of mistletoe and extracts on their basis as adjuvant agents for the treatment of AD is assumed.

Conflict of interests

The authors declare no conflict of interest

Financing

The work is carried out without financial financial support.

Acknowledgments

The authors are grateful to the staff of the Laboratory of Living Systems for their help in handling animals.

Scientific contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Pozdnyakov Dmitry, Adzhiakhmetova Simila, Popova Olga and Oganesyan Eduard. Pozdnyakov Dmitry and Adzhiakhmetova Simila wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Table 1. Choline content in *Viscum leaves album* L.

Raw material	Mistletoe leaves, white, collected	
	autumn	winter
Choline content, % (n =6)	2.88±1.03	3.07±1.84

Note: The optical density of the standard solution of choline chloride with Reinecke's salt at 400.00 nm is 1.735.

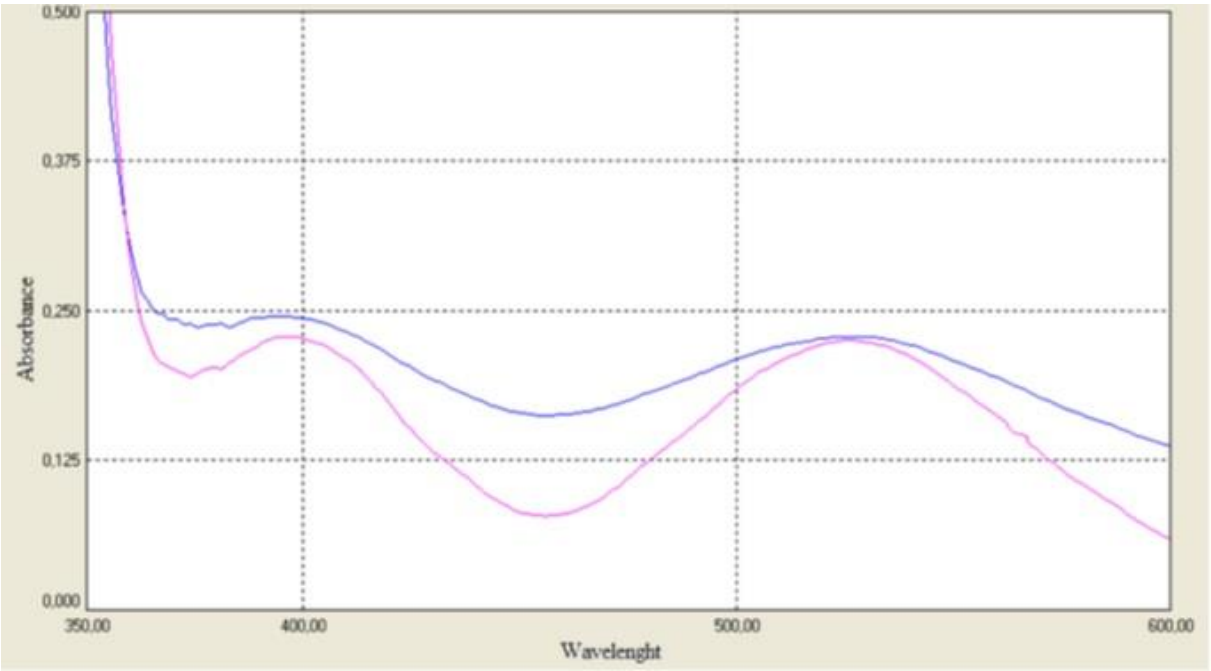


Figure 1. UV spectra of an acetone solution of choline rheinecate isolated from white mistletoe leaves collected in autumn (pink) and winter (blue)

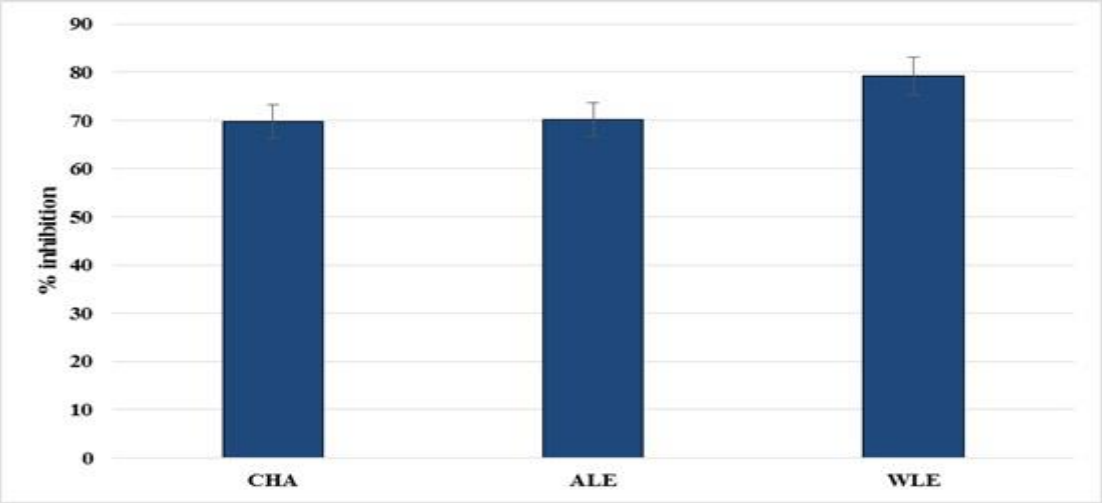
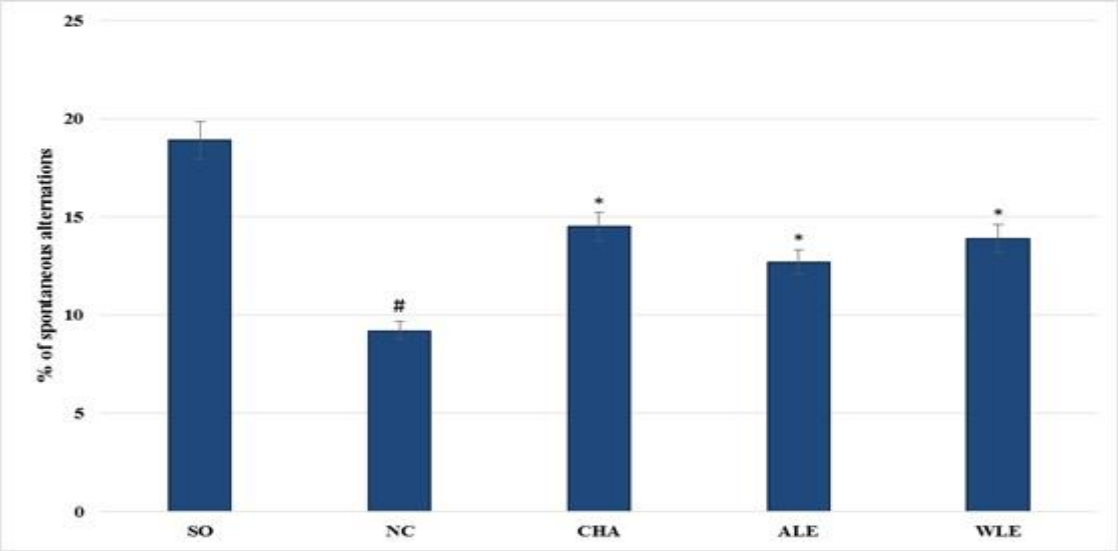
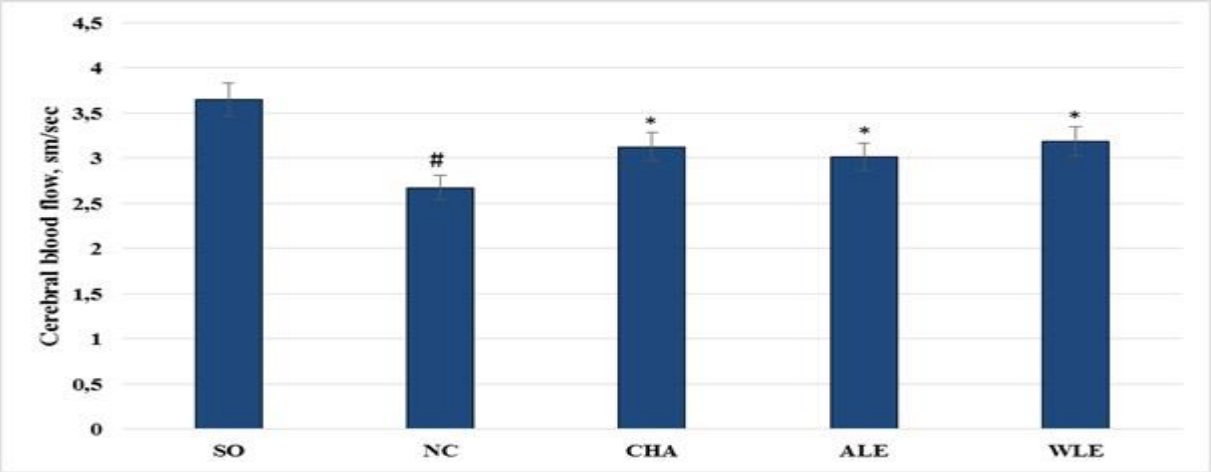


Figure 2. Effect of white mistletoe leaf extracts collected in autumn or winter and choline alfoscerate on the process of aggregation of Aß particles *in test tube*



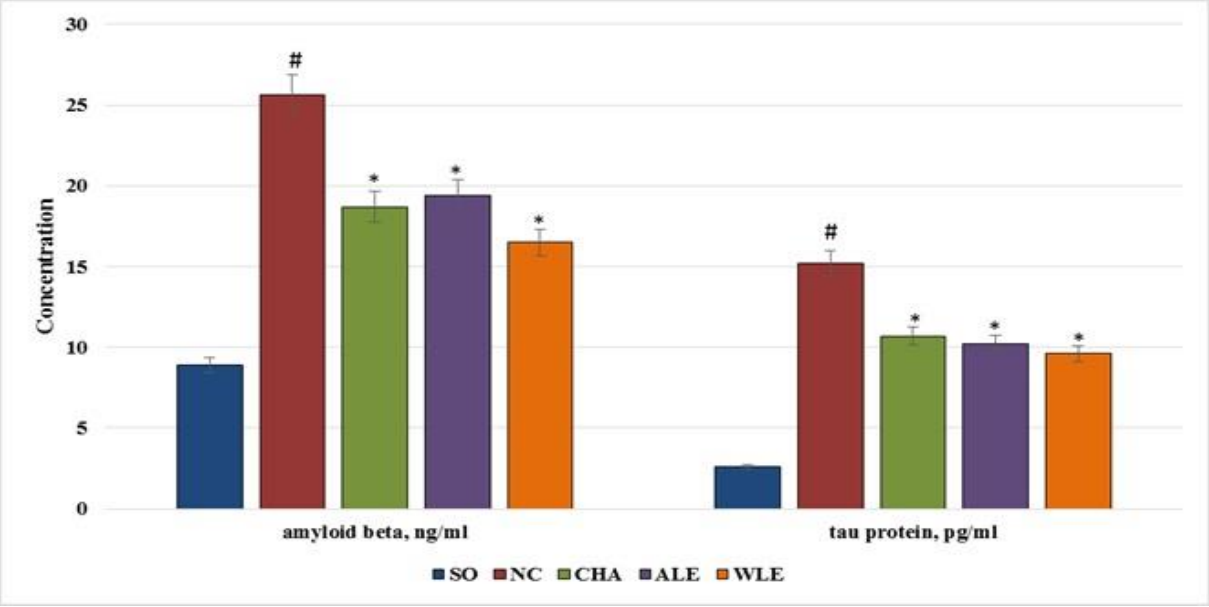
Note: # - significant relative to SO group (Newman-Keuls test , p <0.05); * - significant relative to NC group (Newman-Keuls test , p <0.05).

Figure 3. Effect of white mistletoe leaf extracts collected in autumn or winter and choline alfoscerate on changes in cognitive functions of animals with sporadic AD



Note: # - significant relative to SO group (Newman-Keuls test , p <0.05); * - significant relative to NC group (Newman-Keuls test , p <0.05).

Figure 4. Effect of white mistletoe leaf extracts collected in autumn or winter and choline alfoscerate on changes in cerebral blood flow in animals with sporadic AD



Note: # - significant relative to SO group (Newman-Keuls test, p <0.05); * - significant relative to NC group (Newman-Keuls test , p <0.05).

Figure 5. Effect of extracts of white mistletoe leaves collected in autumn or winter and choline alfoscerate on changes in the concentration of A ß and tau protein in the hippocampus of animals with sporadic AD

References

Amani M, Zolgadrnasab M, Salary AA (2019). The NMDA receptor in the hippocampus alters neurobehavioral phenotypes through inflammatory cytokines in rats with sporadic Alzheimer's disease. PhysiolBehavior. 202: 52-61. 10.1016/j.physbeh.2019.01.005.

Bavashcheva EV, Turyanov MKh (2004). Domestic experience in the treatment of chronic viral hepatitis using the drug Iskador. XI Rus. National congress Man and medicine. 1: 420.

Bearer CF, Wellmann KA, Tang N, He M, Mooney SM. (2015). Choline reverses the balance deficit caused by acute exposure to ethanol in newborns. Cerebellum. 14 (4):413-420. 10.1007/s12311-015-0691-7

Blastein JK, Slack BE, Mellott TJ (2017). Neuroprotective action of dietary choline. Nutrients. 9 (8):815. 10.3390/nu9080815.

Caudill MA, Strupp BJ, Muscalu L, Nevins JEH, Canfield RL (2018). Maternal choline supplementation in the third trimester of pregnancy improves infant processing speed: a randomized, double-blind, controlled feeding trial. FASEB J. 32(4):2172-2180. 10.1096/fj.201700692RR.

Ekpenyong EE, Ayodele OA, Linus E (2016). *Viscum album* increased serum levels of brain-derived neurotrophic factor (BDNF) in an experimental model of Alzheimer's disease. World J. Pharm.Scientific. 5:157-171.

Eddouks M. (2017). Contribution to the study of medicinal plants used in the treatment of diabetes, obesity and hypertension in Tafilalet region (Morocco). Arabian Journal of Medicinal and Aromatic Plants, 3(2), 124-161.

Fioravanti M, Yanagi M (2005). Citidin diphosphocholine (CDP- choline) for cognitive and behavioral disorders associated with chronic cerebral disorders in the elderly. Cochrane Database System , eds. 2. 10.1002/14651858.

Gainanova GA, Valeeva FG, Kushnazarova RA (2018). Influence of hydrotropic compounds on self-organizing and solubilization properties of cationic surfactants. Journal of Physical Chemistry A. 92(7):1400-1405. 10.1134/S0036024418070129.

Jang JY, Kim SY, Song KS, Seong YH (2015). Korean mistletoe (*Viscum album* var. coloratum) inhibits beta- amyloid protein (25-35)-induced damage in cultured neuronal cells and memory impairment. National Prod. Scientific. 21: 134-140.

Korman DB (2011). Antitumor properties of mistletoe lectins. Issues of oncology. 6:689–698.

Kuksis A, Mukherjea S (1978). Choline. Nutr . Obr. 36:201.

Muravyova DA (1991). Nitrogenous bases of mistletoe white and simple forms. Pharmacy. 1:16-17.

Nazaruk J, Orlikowski P (2016). Phytochemical profile and therapeutic potential *of Viscum* L album. Nat Prod Res. 30 (4):373-385. 10.1080/14786419.2015.1022776

Paxinos G, Watson C (2007). The rat brain in stereotaxic coordinates. Amsterdam. Netherlands: Elsevier Inc. 446 p.

Percie du Sert N, Hirst V, Ahluwalia A, Alam S, Avey MT, Baker M, Wurbel H (2020). ARRIVE Guideline 2.0: Updated Guidelines for Reporting Animal Research. PLoS Biology. 18(7): e3000410. doi/10.1371/journal.pbio.3000410

Pilat TL, Ivanov AA (2003). Biologically active food supplements (theory, production, application). Avvalon. 710 p.

Plant resources of the USSR (1988). Flowering plants, their chemical composition, use; Families *Rutaceae – Elaegnaceae.* Nauka: 197-199.

Popova OI, Muravyova DA (1996). White mistletoe as a source of valuable pharmacologically active inclusions. Chemical-pharmaceutical production. 8:36.

Pozdnyakova TA, Bubenchikov RA (2015). The study of nitrogen-containing compounds of marsh geranium. Questions of biological, medical and pharmaceutical chemistry. 11: 37–39.

Prabhu GS, Rao M, Rai KS (2021). Hippocampal nerve cell degeneration and memory deficits in postnatal obese rats induced by a high-fat diet—exploring the comparable benefits of choline and DHA or environmental enrichment. Int J Neurosci. 131(11):1066-1077. 10.1080/00207454.2020.1773819

Putilina MV (2020). Personalized choice of drugs - defenders of choline with the positions of evidence-based medicine. Journal of Neurology and Psychiatry. S.S. Korsakov. 120(6): 144-151. 10.17116/jnevro2020120061144 .

Rosales-Corral SA, Lopez- Armas G, Cruz-Ramos J (2012). Amyloid-β-induced changes in mitochondrial membrane lipid levels: the protective role of melatonin. Int J Alzheimers Dis. 2012:459806. 10.1155/2012/459806

State Pharmacopoeia of the Russian Federation. XI V ed. M.: MZ RF. 2018; 2:3262. [Electronic resource] URL : http://http://femb.ru/femb/pharmacopea.php

Szurpnicka A, Zjawiony JK, Szterk A (2019). The therapeutic potential of mistletoe in CNS-related neurological disorders and the chemistry *of Viscum species* . J Ethnopharmacol. 231: 241-252. 10.1016/j.jep.2018.11.025.

Tomkevich MS (2005). Biological activity of mistletoe lectins and the drug Iskador KV in the practice of treating chronic viral hepatitis C. Phytopharm congress and conference of young scientists of the European phytochemical society. Plants and health.1:392-396.

Velasquez R, Ferreira E, Knowles S (2019). Lifelong choline supplementation improves Alzheimer's disease pathology and associated cognitive deficits by attenuating microglial activation. Aging cell. 18 (6):e13037. 10.1111/acceleration 13037

Velasquez R, Winslow W, Mifflin MA (2020). Choline as a Prevention of Alzheimer's Disease. Aging (Albany, New York).12 (3):2026-2027. 10.18632/aging.102849

Wang W, Zhao C, Zhu D, Gong G, Du W (2017). Inhibition of amyloid peptide fibril formation by gold-sulfur complexes. J Inorg. Biochem. 171:1-9. 10.1016/j.jinorgbio.2017.02.021

White A (1981). Fundamentals of biochemistry. Mir. 1–3: 503.

Hananea, D., Seladji, M., El-Haci, A., Benammar, C., & Belarbi Meriema, B. (2021). Phytochemical screening and Antioxidant activity of Opuntia ficus-indica seeds from Algeria. Arabian Journal of Medicinal and Aromatic Plants, 7(3), 352-366.