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Short Communication

Research on the influence of Siberian fir polyprenols on learning and memory of mice with an experimental model of Alzheimer's disease

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Abstract

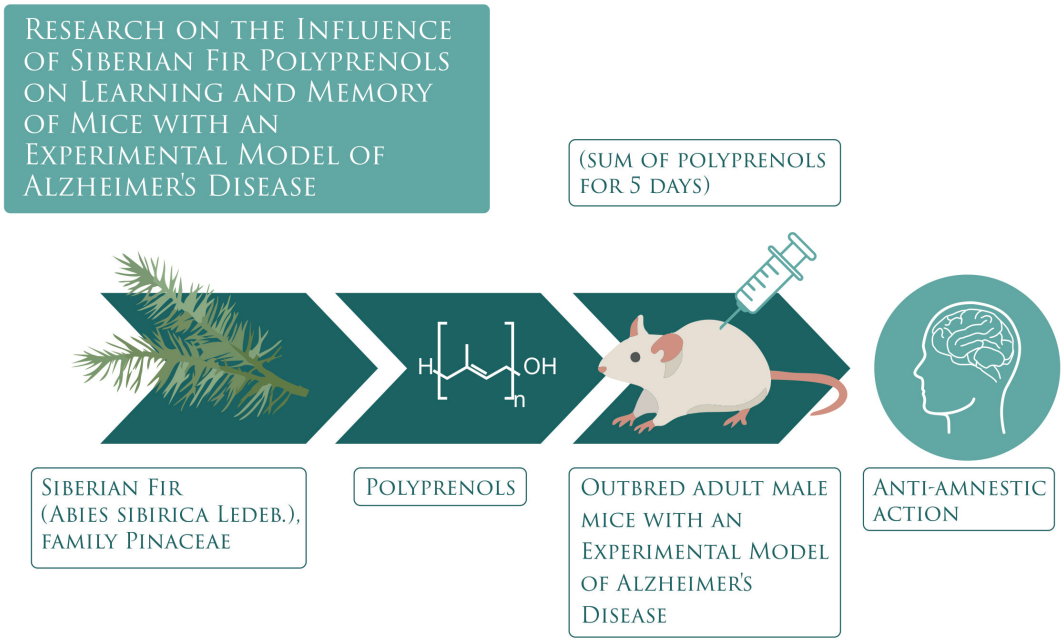
Introduction: Alzheimer's disease is increasingly becoming a cause of early disability and death. Attempts to create a pharmacotherapeutic agent that provides an effective result in treating this pathology have so far been unsuccessful. This article presents the results of experimental studies aimed at creating a more effective treatment of Alzheimer's disease.

Material and Methods: The object of the study was polyisoprenoids isolated from Siberian fir (*Abies sibirica* Ledeb.). Experiments were conducted on 108 outbred adult male mice from the CD1 (cluster of differentiation 1) stock, weighing 28-30 g (at 5 weeks old). The effect of the sum of polyisoprenoids on learning and memory was studied in a dose range of 5, 20, 50, 100, 200, and 500 mg/kg. Gliatilin was used as a reference drug at a dose of 90 mg/kg. The experimental model of cognitive dysfunction was created by chronic (for 20 days) intraperitoneal injection of scopolamine at a dose of 2 mg/kg. Cognitive dysfunction was assessed by changes in the indicators of conditioned passive avoidance reflex (CPAR).

Results and Discussion: The results of the experiments showed that chronic administration of scopolamine caused a pronounced decrease in the reproducibility of CPAR. Simultaneously, the studied substance at doses of 20 and 50 mg/kg restored the reproducibility of the reflex to a level close to the values of intact control. Gliatilin showed a similar effect. The authors believe that cholinergic receptors are involved in the development of the anti-amnestic effect.

Conclusion: The presented study demonstrates the discovery of a new agent with anti-amnestic action based on the sum of polyprenols from Siberian Fir (*Abies sibirica* Ledeb.), family Pinaceae. This agent can be used for the treatment of neurodegenerative pathologies, particularly Alzheimer's disease.

Graphical abstract



Keywords

Siberian fir, polyprenols, dementia, neurodegenerative diseases, cognitive functions disorders therapy, memory enhance, Alzheimer's disease

Introduction

Alzheimer's disease is an incurable neurodegenerative disorder that ranks seventh in terms of mortality in the United States. Its global economic burden, along with other dementias, amounts to approximately 1 trillion dollars.

The amyloid hypothesis plays a central role in our current understanding of the pathogenesis of Alzheimer's disease (AD). This hypothesis suggests that amyloid-β is synthesized and aggregated in the bodies of individuals with AD. Aggregation of amyloid-β (Aβ) is the main cause of developing disorder in AD patients. Aβ is synthesized from amyloid precursor protein (APP). Aβ aggregates bind to the cell surface of neurons and glial cells, causing synaptic dysfunction and neuroinflammation. Additionally, disturbances in the higher nervous activity (HNA) processes, particularly pathologies of the cholinergic and glutamatergic systems of the brain, play a major role (Pfundstein et al. 2022).

Dysfunctions of the nervous system are observed in the early stages of AD, starting from minimal deviations in the regulation of regulatory system functions at the level of signaling processes. Subsequently, these changes in central nervous system function manifest as extensive dysfunctions, particularly evident in memory processes, behavior, and personality changes. Synaptic transmission impairment may be due to dysregulation of calcium exchange, induced by overstimulation of glutamate receptors, specifically NMDARs (Liu et al. 2019).

The treatment of Alzheimer's disease poses a serious challenge. The main reason for this is the lack of understanding of the etiological factors that trigger and sustain the pathogenetic mechanisms of the disease. There is also a lack of necessary knowledge regarding the pathogenetic mechanisms of dementia and the specific mechanisms of their action (Tyuvina and Balabanova 2015).

Alzheimer's disease is a predominant neurodegenerative disorder affecting the aging population worldwide. The late stage of dementia in Alzheimer's disease is a multifaceted disease characterized by progressive decline in cognitive functions such as memory, learning, mental acuity, and behavioral disorders.

The neuropathological features of AD at the morphological level are characterized by the accumulation of beta-amyloid plaques and hyperphosphorylated tau in associated microtubules. Other neuropathological features of AD include changes in synaptic signaling, synapse loss, and in some cases, neuronal loss or degeneration. Some studies indicate signs of dysregulation in the cholinergic system in the brain with AD, as well as in the noradrenergic system in the locus coeruleus.

This nucleus is responsible for the physiological response to stress and anxiety (Beckdash 2021).

Since cholinergic synapses are ubiquitous in the human central nervous system, the involvement of synapses and neurons of this system in the development of Alzheimer's disease is reasonable to assume (Hampel et al. 2018).

Several neuronal processes, including neuroinflammation and oxidative stress, are also involved in the pathogenesis of Alzheimer's disease. Therefore, new therapeutic agents capable of modulating both of these processes could be highly useful in the treatment regimens of neurodegenerative pathology (Companys-Alemany et al. 2022). For this reason, a significant group of plant-derived compounds that show a pronounced effect on oxidative stress and neuroinflammation could be promising agents in combating neuroinflammation process.

Due to the multifaceted nature of the pathogenesis and the unclear role of individual functional systems, the treatment of AD should be multifactorial. The use of agents that have a complex effect on several mediator systems, including anti-inflammatory and anti-stress actions, is justified, and the search for such multipotent drugs is highly relevant (Shi et al. 2022).

It is also worth noting that attempts to create a novel drug have been unsuccessful for the past 20 years. Attempts have been made to create a medicinal product with a pathogenetically justified mechanism of action based on antibodies to Aβ; however, they have not led to the desired result (Huang et al. 2023).

Thus, currently, there are no completely safe drugs for the treatment of AD, making the search for this group of substances among plant remedies highly relevant, as they may serve as a source of new original medicinal products.

The aim of this work is preclinical investigation of a new drug that is highly effective in the treatment of AD.

Materials and Methods

The object of the study

It was the sum of polyprenols isolated from the needles of Siberian Fir (*Abies sibirica* Ledeb.), Pinaceae family as an oil solution containing 74% of active substances (polyprenols).

Experimental animals

Experiments were conducted on 108 outbred adult male mice from the CD1 stock, weighing 28-30 g (at 5 weeks of age), conventionally bred, category 1, obtained from the Department of Experimental Biomodeling of E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center (Russia).

Animal husbandry conditions

The animal husbandry conditions were approved by the Ethics Committee of E.D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center (Minutes No 191112021 of 26 November 2021).

Studied doses and substances

The anti-amnestic activity of the substance, the sum of polyprenols, was studied in a dose range of 5, 20, 50, 100, 200, and 500 mg/kg. The introduction of a solution of polyprenols was carried out in the form of an emulsion, which was prepared immediately before administration, by mixing 676 mg of the initial oil solution and saline solution, with the addition of 1 drop of emulsifier (Twin-80), bringing the total volume of the mixture to 10 mL. Thus, when administered to an animal weighing 20 g, 0.2 mL of the resulting solution was administered at a dose of 500 mg/kg. Further dosages were obtained by sequentially diluting the previous dose with a sequential series of dilutions in accordance with the dose change of the test substance. The experimental model of Alzheimer's disease was created by intraperitoneal administration of scopolamine at a dose of 2 mg/kg (Mironov 2012) for 20 days. Choline alphoscerate (Gliatilin – Catalent Italia S.p.A.) was used as a reference drug (Kim et al. 2017).

The experimental design

All animals were divided into 9 groups of 12 individuals each. The first group (intact control) received intraperitoneal saline throughout the experiment. The remaining groups were also administered intraperitoneal scopolamine for 20 days, and from day 21 to day 30, intraperitoneal saline was administered. In addition to saline, the second group (negative control) received water orally in a volume of 0.4 mL. The third group received the reference drug Gliatilin at a dose of 90 mg/kg (positive control) from day 21 to day 30. Groups four through nine received the claimed substance at the specified doses. On the 31st day of the experiment, one hour after substance administration, all animals underwent conditioned passive avoidance reflex (CPAR) training. Reflex testing was performed 24 hours and 7 days after conditioning.

Experimental technique

The method of conditioned passive avoidance reflex (CPAR) is based on the suppression of the innate preference reflex for dark spaces in rodents (Buresh et al. 1991). The experimental setup included a chamber consisting of two compartments: a large, illuminated one, and a small, dark one. The animal was placed in the light compartment and soon (within 10-20 seconds), due to the innate preference reflex for dark space, it would move to the small compartment. Subsequently, the door connecting the compartments was closed, and an electric current was applied to the floor of the dark compartment, which consisted of parallel alternating electrodes, with pulses of 50 ms duration, frequency of 5 Hz, and amplitude of 50 mA. After 10 seconds, the door was opened, allowing the animal to escape to the illuminated compartment with a normal floor. As a result of this procedure, the animals developed a conditioned avoidance reflex of the dark space. To test the reproducibility of the reflex, the animals were placed in the light compartment corner farthest from the entrance to the dark compartment and observed for 3 minutes. The time of the first entry into the dark compartment (latent entry time) and the total time spent in the dark compartment were recorded.

Statistical analysis

Statistical analysis of the obtained results was performed using by the method of Student Fischer Statistica 6.0 software package for Windows.

Results and Discussion

During the conducted studies, it was established that scopolamine, administered once at a dose of 2 mg/kg for 20 days, caused a significant impairment in the acquisition and reproducibility of CPAR. At the same time, the course administration of the test article – sum of polyprenols – for 5 days had an enhancing effect on the reproducibility of the reflex, exceeding the effect of the reference drug Gliatilin (see Table 1). However, a decrease in the effect of the substance at higher doses compared to its effect at lower doses, is a common phenomenon in pharmacology of receptor drugs (Kenakin 2008).

The substance – sum of polyprenols (C6-C22) from Siberian Fir (*Abies sibirica* Ledeb.), family Pinaceae – exhibits an anti-amnestic action in an experimental model of Alzheimer's disease induced by chronic (for 20 days) administration of scopolamine (scopolamine amnesia). The most pronounced effect was observed at doses of 20 and 50 mg/kg.

According to the experimental findings, the drug freely crosses the blood-brain barrier (BBB) and serves as a direct donor of the neurotransmitter acetylcholine in the presynaptic membranes of cholinergic neurons. Upon entering the body, under the action of enzymes, it undergoes cleavage into choline and glycerophosphate. Choline, due to its electrical neutrality, penetrates through the blood-brain barrier and enters the brain, where it serves as a basis for the formation of acetylcholine. In addition, glycerophosphate, being a precursor of neuron membrane phospholipids, stimulates the formation of phosphatidylcholine, which restores the phospholipid composition of neuron membranes and improves their plasticity, positively affecting the functional state of cell microstructures, enhancing neuron cytoskeleton, increasing the mass of mitochondria and ribosomes. The drug normalizes nerve impulse transmission, potentiates anabolic processes in neurons responsible for membrane phospholipid and glycerolipid synthesis, improving cognitive and behavioral functions (Pfundstein et al. 2022).

Conclusion

The presented study demonstrates the discovery of a new agent with anti-amnestic action based on the sum of polyprenols from Siberian Fir (*Abies sibirica* Ledeb.), family Pinaceae. This agent can be used for the treatment of neurodegenerative pathologies, particularly Alzheimer's disease in doses 20-50 mg. But this fact requires additional research in clinical trials.

Conflict of interests

The authors declare no conflict of interests.

Data availability

All of the data that support the findings of this study are available in the main text.

Table 1. The effect of chronic administration of sum of polyprenols on the development of CPAR according to the results of reflex reproducibility testing at 24 hours and 7 days after training in CD1 mice with cognitive and amnestic disorders induced by chronic (20 Days) administration of scopolamine at a dose of 2 mg/kg (X±m; n=12)

Experimental groups	After 24 hours of development			After 7 days of development	
	Latent entry time into the dark compartment during reflex development, sec	Latency to enter the dark compartment during reflex testing, sec	Percentage of animals with developed reflex	Latent time of entry into the dark compartment during reflex testing, sec	Percentage of animals with developed reflex
1. Intact control	11.4±15.4*	164.6±16.9	83.3*	128.3±12.7	66.7*
2. Control + SC	78.4±18.1	45.2±19.8	8.3	88.6±11.5	8.3
3. Sum of polyprenols, 5 mg/kg + SC	83.8±26.5	112.3±11.5*	25.0 *	128.3±14.8	16.7
4. Sum of polyprenols, 20 mg/kg + SC	36.0±13.6	132.2±0.6*	58.3 *	145.3±29.1*	42.7*
5. Sum of polyprenols, 50 mg/kg + SC	29.6±9.1	144.8±23.4*	66.7 *	89.3±14.5	33.3 *
6. Sum of polyprenols, 100 mg/kg + SC	49.8±10.9	109.5±9.1	16.7	75.3±12.9	8.3
7. Sum of polyprenols, 200 mg/kg + SC	79.1±6.2	128.1±13.9	25.0*	118.6±16.8	16.7
8. Sum of polyprenols, 500 mg/kg + SC	96.5±11.4	83.4±12.7	8.3	122.4±18.9	16.7
9. Gliatilin 90 mg/kg + SC	41.5±11.8	142.8±14.5*	50.0*	129.8±19.1	33.3*

Note: * – probability of null hypothesis less that 0.05 compared to group 2 (scopolamine control); SC – scopolamine control.

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