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# CORRECTION WITH POLYPHENOL PC-7 ON CHANGES IN COGNITIVE STATES AND CALCIUM DYNAMICS IN BRAIN SYNAPTOSOMES IN RATS UNDER CONDITIONS OF MODELED ALZHEIMER'S DISEASE IN VIVO

**Kozokov Islom Bakhtiyarovich**
Institute of Biophysics and Biochemistry at the National University of Uzbekistan named after M. Ulugbek, Tashkent, Uzbekistan

**Dedaboev Jobir Ismoil ugli**
Institute of Biophysics and Biochemistry at the National University of Uzbekistan named after M. Ulugbek, Tashkent, Uzbekistan

**Khoshimov Nozim Numonjonovich**
Institute of Biophysics and Biochemistry at the National University of Uzbekistan named after M. Ulugbek, Tashkent, Uzbekistan

**Asatillayev Joxongir Ne'matjon og'li**
Uzbekistan State University of Physical Education and Sports, Uzbekistan

**Saidmurodov Samandar Abdumalik o'g'li**
Alfraganus university. Tashken, Uzbekistan

**Rakhimov Rakhmatilla Nurillaevich**
Institute of Bioorganic Chemistry named after Acad. A.S. Sadykova ASRUz Tashkent, Uzbekistan

**Nasirov Kabil Erkinovich**
Institute of Biophysics and Biochemistry at the National University of Uzbekistan named after M. Ulugbek, Tashkent, Uzbekistan

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**Abstract**
Alzheimer's disease (AD) is one of the most common manifestations of neurodegenerative dementia, characterized by changes in brain tissue long before the appearance of the first clinical symptoms. Animal models induced by AD are the cornerstone of any drug development program and offer the opportunity to make a significant and meaningful contribution to successful therapeutic development. We conducted experiments to determine whether this polyphenol crosses the blood-brain barrier by administering in vivo the polyphenol PC-7, which has a good effect on infection of these disease models, in a liposomal form based on soy phospholipids, in different ways. rats. In our experiments, when studying the behavior of rats modeled by AD, the behavior of rats initially selected in the tests "Open field", "Conditioned reflexes of passive escape" and "Conditioned reflexes of active escape" was studied. McGraw scale. It was found that the used polyphenol PC-7 has a positive effect on changes in cognitive states and the level of synaptosomal calcium that occur in AD states.

**Keywords** Synaptosome, cognitive impairment, Alzheimer's disease, calcium channels, polyphenols.

## INTRODUCTION

The World Health Organization has recognized the treatment and prevention of Alzheimer's disease (AD) as a global health priority. AD is one of the leading neurological diseases diagnosed in older people and is the most common form of dementia [1].Scientists estimate that the prevalence of Alzheimer's disease worldwide will increase from 84 million by 2040 to over 100 million by 2050 [2, 3]. Although significant progress has been made in understanding the pathogenesis of the disease since Alois Alzheimer reported the first case in 1906, there are still no effective methods to prevent the onset of the disease or simple, safe, specific preventive methods and effective treatments that could stop its progression [4-7].

Objective of the work: In our experiments, the reliable influence of polyphenols on changes in AD in our previous experiments was determined by in vitro methods [8-13], in this work, the influence of polyphenol PC-7 on changes in AD of individual parameters of the behavior of model rats and calcium transport through the synaptosomal membrane of the brain were determined in vivo.

## METHODS

### Experimental models of AD.

### “Animals”

The experiments were carried out on outbred white male rats kept on a standard vivarium diet. All experiments model experience in these 10 groups performed comply with the requirements of the World Society for the Protection of Animals and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes 1986) and American Psychological Association. (2017). Ethical principles of psychologists and code of conduct (2002, amended effective June 1, 2010, and January 1, 2017) [14].

For the modeling of the AD, laboratory rats of males were used, weighing 200-300 gr. First of all, weighing and selection of animals for experiments were carried out. Then, behavioral tests are carried out: an open field, a conditioned response of passive avoidance (CRPA) and active avoidance (ACRA), swimming on the pool (Morris test). We feed animals with a standard diet with add-on for a month or two. After a week, we repeat behavioral tests. Analyzing the data, depending on the test results, enter neurotoxin. After playing the model AD, we score the animal and take biological materials for further research.

The results of behavioral tests showed that in control groups, experimental animals on the “open field” tests were very active and overexcited, quickly moved and practical did not stand in one place. At the same time, the AD groups are very passive, the nervous system inhibited and the animals were delayed for a long time in one place.

This slows down that after the introduction of neurotoxin into the animal's body, the normal functioning of the nervous system is violated, the destruction of the transmission of inpuls in neurons and the death of the cell.

At the tests of CRPA and ACRA, the obtained experts showed that in the control group the animals were in the bright phase quickly sought to go to the dark phase, after they received fragmentation quickly moved to the bright phase. When this test was repeatedly carried out by an ec -permanent animal did not pass into the dark phase from the light. When the model AD groups of animals were placed in the bright phase, she did not strive to go to the dark phase, after she went in the graternation.

When this test is repeated, the experimental animal, as last time, slowly went into the dark phase and again received gratification. The data obtained witnesses that under the control group, experimental animals quickly moved into a dark phase that resembled the mink of animals, but after receiving irritation when repeated the same test did not go into the dark phase. From this we can conclude that the animals have a reaction to the gratification in the dark phase and this remained unih in memory. In the model group, the initially animals were very passive and slowly perpeted in the languid phase. When the test is repeated, the animals again went into the dark phase and received gratification. This suggests that in the model group of animals, cognitive functions, the reaction to the environment and memory, which are symptoms of AD, are greatly impaired.

Studies of the connection with this task set at various stages were carried out on an experimental modeling of AD - aluminum neurotoxicity (ANT) in rats. The models were used in white outbred rats (280-300 g). The animals were carefully weighed and various behavioral tests were performed: open field n=3, CPPA and ACRA active avoidance n=3, pool swimming (Morris test) n=3.

The animals were fed a standard diet and sugar was added to the drinking water. Cholesterol and margarine in the amount of 0.4% of the total food. Mercazalol in an amount of 0.04-0.06 mg per rat.

Acute aluminum neurotoxic effect (AAN) was caused by subcutaneous administration to white rats (two groups of 12 animals each): the first group was the control (0.9% NaCl solution was administered), the second group was administered 0.2 ml of 10% aluminum chloride solution for 5 days.

Experimental animals were sacrificed under light ether anesthesia. Blood and internal organs were collected into different vessels and processed simultaneously.

After modeling AD, behavioral tests were repeated: Open field n=3, CRPA and ACRA n=3, swimming in the pool (Morris test) n=3 [15].

**Isolation of synaptosomes**: Synaptosomes are obtained by two-stage centrifugation Centrifuge K-24 (ELN13893354.Veb MLV Zenrifugenbau Engelsdorf. Germany) [16]. The entire isolation procedure is carried out at -40C. After decapitation, the brain is removed as quickly as possible and crushed on ice. The crushed tissue is homogenized at a ratio of 1:10 in the isolation medium - 0.32 M sucrose solution in 0.01 M Tris-HCl buffer with the addition of 0.5 mM EDTA (pH 7.4). The obtained homogenate is exposed to a 4-stage centrifugation. The supernatant after the first centrifugation (10 min, 4500 rpm) is carefully removed without capturing the myelin layer and exposed to further centrifugation for 20 min at 14000 rpm. The obtained dense precipitate P2 is resuspended in the isolation medium. The obtained suspension is used further in the experiment as a coarse synaptosomal fraction (synaptosomal-mitochondrial). In the case of 4-stage isolation, the second centrifugation is carried out at 11,000 rpm for 20 minutes. The dense pellet of P2 is resuspended in 0.32 M sucrose solution (pH 7.4) and then carefully layered on 0.8 M sucrose solution (pH 8.0), after which it is centrifuged for 25 minutes at 11,000 rpm. As a result of centrifugation in a sucrose gradient, factions are separated - mitochondria settle tightly at the bottom of the tube, and synaptosomes remain in suspension in a layer of 0.8 M sucrose. This layer is carefully removed, mixed with an equal amount of isolation medium and left for 15 minutes to restore the ultrastructure of synaptosomal particles, after

which it is exposed to further centrifugation at 14,000 rpm for 30 minutes. The dense final precipitate P4 is resuspended in the isolation medium and then used in the experiment as a synaptosomal faction.

The amount of cytosolic Ca2+ [Ca2+]in was calculated using the Grinkevich equation [17] in synaptosomes isolated from rat brains. To measure free cytosolic Ca2+, synaptosomes (1x108 cells/ml) were loaded with 4 μM Fura-2AM acetoxymethyl ester for 40 min at 37□C. At the same time, in the dye molecules that have penetrated into the cytoplasm, under the action of intracellular esterases, the ester group is cleaved off, resulting in the Fura-2 anion that binds Ca2+. After completion of the loading, the dye remaining in the medium was removed by double washing and centrifugation in standard medium. In the experiments, the cell concentration in the cell was 5x106 cells/ml. Fluorescence excitation was induced at 337 nm and fluorescence registration at 496 nm. Ca2+ saturated dye fluorescence (Fmax) was determined by adding 50 μM digitonin to cells loaded with Fura-2AM. Fmin was determined by measuring the fluorescence intensity in a calcium-free medium, Fmin = [(Fmax – Faf)/3]+ Faf, where Faf is cell autofluorescence determined by adding 0.1 mM MnCl2 to thymocytes loaded with Fura-2AM and processed with digitonin [18].

**Statistical analysis**: The measurements were carried out on a universal spectrometer USB-2000 (USB2E7916.OceanOptics.USA.2010). Statistical significance of differences between control and experimental values, determined for a data series using a paired t-test, where control and experimental values are taken together, and an unpaired t-test, when taken separately. A P value <0.05 indicates a statistically significant difference. The results obtained are statistically processed in Origin 7.5 (Origin Lab Corporation, USA).

## RESULTS AND DISCUSSION

During the experiments to induce the Alzheimer's disease model, male rats weighing 200-300 g were selected and behavioral tests were performed using the McGraw scale to determine their cognitive functions: (Open field), conditioned reflex of passive avoidance (CRPA) and active avoidance (CAA), swimming in the pool (Morris test). After that, when studying the effects of polyphenols in vivo, polyphenol PC-7 was administered in the AK state on Alzheimer's disease models in rats using various methods currently used [19-24].

The correcting effect of polyphenol PC-7 encapsulation in liposomes based on brain and soy phospholipids (intraperitoneal, intranasal and oral) was studied in the model AD.

The operation of polyphenol encapsulation in liposomes was carried out by the staff of the Institute of Chemical Plant Substances Laboratory of Molecular Genetics of ASRUz.

In these experiments, we first divided the rats into 10 groups. We administered 2 different liposomes to the rats in these groups by different routes. These rats and the groups used for encapsulation of polyphenol PC-7 in liposomes derived from brain and soy phospholipids are described below.

Correction of AD conditions by administration of polyphenol PC-7 by different routes (intraperitoneal, intranasal and oral).

| Number of groups. № | Description of groups | Number of rats |
|---|---|---|
| 1 | It was administered intraperitoneal (0.2 ml saline) daily for 7 days. | 6 |
| 2 | $AlCl_3$ (10 mg/kg) was administered intraperitoneal daily for 7 days. | 6 |
| 3 | $AlCl_3$ was administered intranasal (in the nose) at a constant dose of 50 mg/kg body weight for 7 days. | 6 |
| 4 | $AlCl_3$ was administered orally at a dose of 50 mg/kg once daily for 6 weeks | 6 |

| | | |
|---|---|---|
| | (Singh and Goel, 2015). | |
| **5** | $AlCl_3$ (10 mg/kg intraperitoneal) was administered daily for 7 days and polyphenol PC-7 encapsulated in liposomes prepared from BRAIN phospholipids was administered at a dose of 50 mg/kg (daily) from the 3rd day of treatment. | 6 |
| **6** | $AlCl_3$ (10 mg/kg intraperitoneal) was administered daily for 7 days, and polyphenol PC-7 encapsulated in soybean phospholipid-derived liposomes was administered at doses of 50 mg/kg (daily) from the 3rd day of treatment. | 6 |
| **7** | After intranasal (i.n.) administration of $AlCl_3$ at a dose of 50 mg/kg body weight for 7 days, polyphenol PC-7 wrapped in brain phospholipid-based liposomes was administered intranasal at a dose of 50 mg/kg. | 6 |
| **8** | After intranasal (i.n.) administration of $AlCl_3$ at a dose of 50 mg/kg body weight for 7 days, polyphenol PC-7 wrapped in soybean phospholipid-based liposomes was administered intranasal at a dose of 50 mg/kg. | 6 |
| **9** | Polyphenol PC-7 encapsulated in liposomes based on soybean phospholipids at doses of 50 mg/kg (Akisu et al., 2002) and $AlCl_3$ at doses of 50 mg/kg (Singh, Goel, 2015) were administered orally once a day for 6 weeks. | 6 |
| **10** | Polyphenol PC-7 encapsulated in liposomes based on brain phospholipids at doses of 50 mg/kg (Akisu et al., 2002) and $AlCl_3$ at doses of 50 mg/kg (Singh, Goel, 2015) were administered orally once a day for 6 weeks. | 6 |
| **TOTAL** | | 60 |

To determine the condition and cognitive functions of the model rats in the groups presented in the table above, behavioral tests were carried out using the McGraw scale: open field tests (Fig. 1ABS), conditioned reflex of passive avoidance (CRPA) and active avoidance (CRPA).

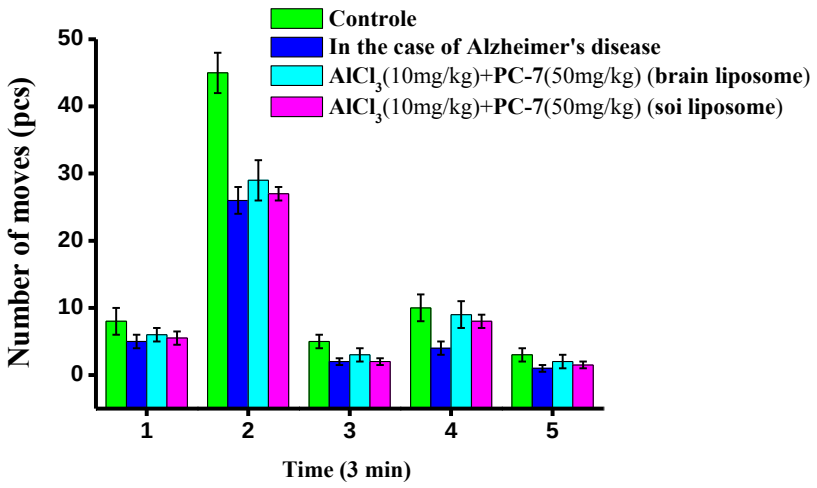


**Figure 1A. When inducing the AD model, $AlCl_3$ (10 mg/kg, intraperitoneal) of the polyphenol PC-7 encapsulated in liposomes obtained on the basis of phospholipids of the brain and SOI [15] was injected**

**into the abdominal cavity daily for 7 days, and when injected at a dose of 50 mg/kg (daily) from the 3rd day of treatment in the cognitive behavior test of model rats for 3 minutes in the "Open Field" test.** 1. Vertical movement, 2. Horizontal movement, 3. Washing, 4. Mink. 5. Garbage. n=6.

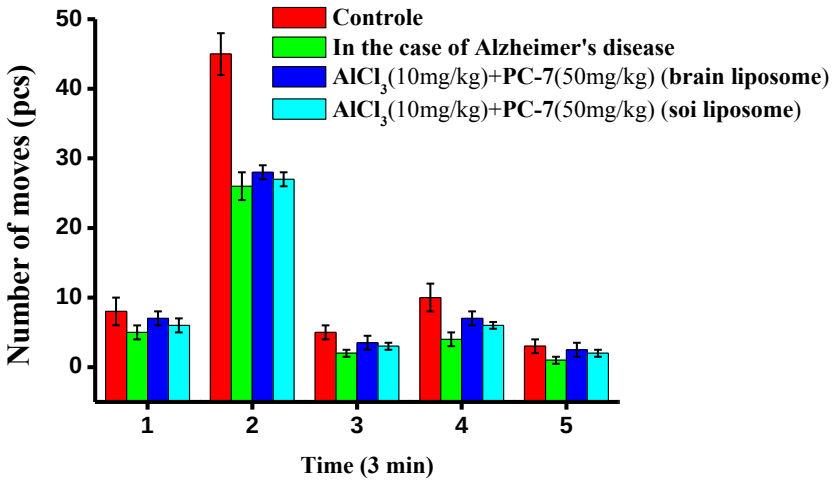


**Figure 1B. The AD model was created by chronic (i.n.) administration of AlCl3 at a dose of 50 mg/kg body weight for 7 days of the polyphenol PC-7 wrapped in liposomes obtained on the basis of phospholipids of the brain and COI; Two hours after the administration of $AlCl_3$, the polyphenols encapsulated in liposomes were similarly administered intranasally at a dose of 50 mg/kg in a 3-minute open field test to model rats.** 1. Vertical movement, 2. Horizontal movement, 3. Washing, 4. Mink. 5. Garbage. n=6.

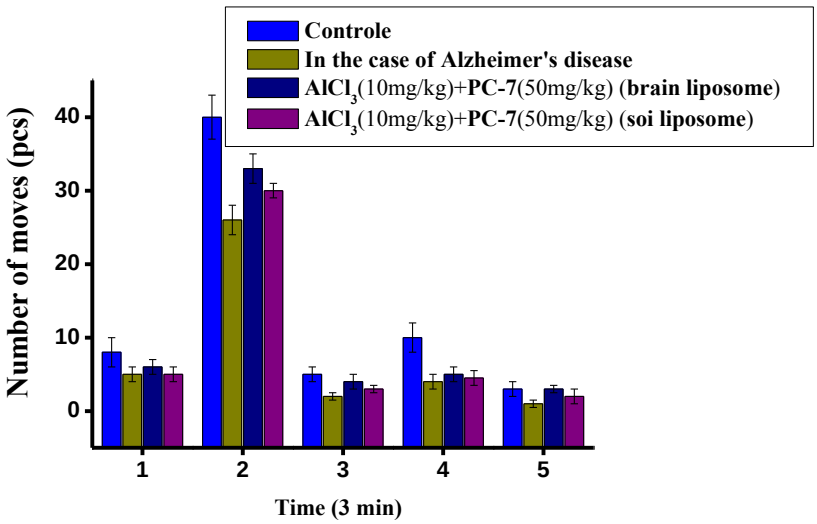


**Figure 1S. In the induction of AD model, liposome-wrapped polyphenol PC-7 obtained from brain and COI phospholipids was administered orally at a dose of 50 mg/kg once a day for 6 weeks [22] to determine the cognitive behavior of model rats. in the Open Field test for 3 minutes with the**

**introduction of $AlCl_3$ at a dose of 50 mg/kg of the test.** 1. Vertical movement, 2. Horizontal movement, 3. Washing, 4. Mink. 5. Garbage. n=6.

The results of the study showed that in the group of animals with the Alzheimer's disease model (active control), changes in sensory and motor-sensory activity, postural instability, distances traveled, squares, burrow reflexes and exploration time were observed. The percentage of execution of the conditioned reflex reaction of passive escape decreases and the coefficient of learning of the conditioned reflex decreases. In animals of this group, a decrease in calcium transport through the membranes of the synaptosomes of the brain is observed.

Open field test results show that brain phospholipid-based liposomes are more effective than SOI phospholipid-based liposomes when polyphenols are loaded into brain-soy phospholipid-based liposomes. To summarize these results, first of all, brain phospholipid-based liposomes can facilitate the transport of polyphenols across the blood-brain barrier (BBB) due to their biochemical similarity to neuronal membranes. Liposomes containing brain phospholipids may interact more with nerve cells, causing polyphenols to accumulate around the membrane. Typically, the composition of liposomes derived from SOI phospholipids is considered less specific to brain tissue. Less penetration of polyphenols across the BBB may be associated with the observed weaker effects on cognitive function in open field tests.

A change in the percentage of the conditioned reflex passive avoidance reaction (CRPR) and a relative increase in the conditioned reflex learning coefficient were also observed

The observed results show that polyphenols, especially those encapsulated in liposomes derived from brain phospholipids, enhance the formation, retention and acquisition of conditioned reflexes, indicating improved cognitive function in treated rats.

The significant results observed with brain phospholipid-based liposomes indicate high permeability of the BBB barrier and significant effects on the central nervous system. This is likely a result of their structural compatibility with neuronal membranes, which may lead to better delivery and absorption of polyphenols into nerve tissues.

The results support the use of brain phospholipid-based liposomal encapsulation methods as a promising strategy for delivering neuroprotective drugs in the treatment of Alzheimer's disease. The results highlight the importance of permeable transport systems in enhancing the therapeutic efficacy of polyphenols in AD. Liposomes based on brain phospholipids demonstrate a clear advantage in restoring cognitive functions, making them a promising direction for further development of neuroprotective therapy.

Calcium transport through the membranes of synaptosomes in the brain of animals in this group changed differently in different groups (Fig. 2ABS).

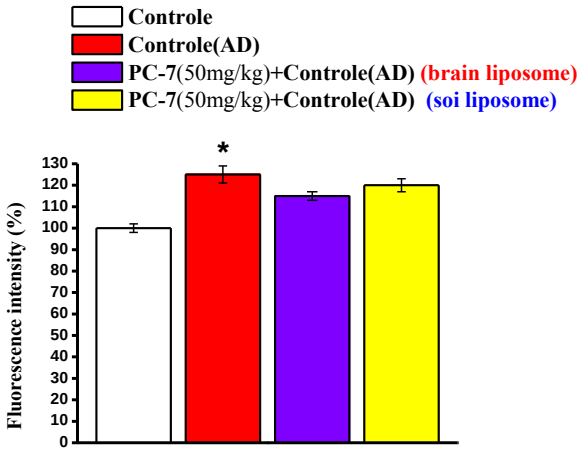


**Figure 2A. When testing the AD model, $AlCl_3$ (10 mg/kg intraperitoneal) was administered intraperitoneally for 7 days of polyphenol PC-7 encapsulated in liposomes obtained from phospholipids of the brain and SOI, and 50 mg/kg daily from the 3rd day of treatment. The changes in calcium content in the synaptosomes of the brain in model rats were determined.** Confidence level: * - $p<0.05$; ($n = 6$).

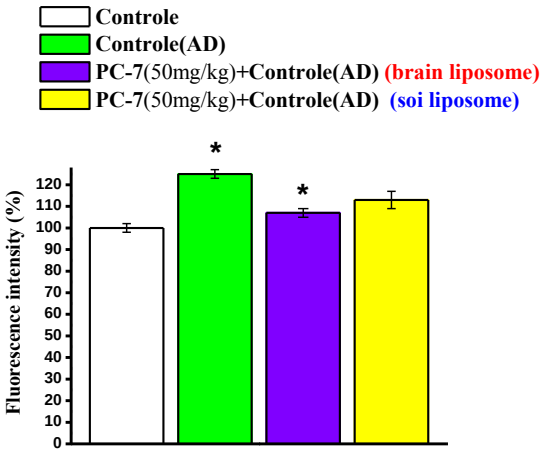


**Figure 2B. The AD model was created by intranasal (intranasal) administration of $AlCl_3$ at a chronic dose of 50 mg/kg body weight for 7 days of the polyphenol PS-7, wrapped in liposomes obtained on the basis of brain phospholipids and SOI; Changes in the content of cerebral synaptosomal calcium in model rats that received the same intranasal dose of 50 mg/kg polyphenols encapsulated in liposomes, two hours after the administration of AlCl3.** Reliability level: * - $p<0.05$; ($n = 6$).

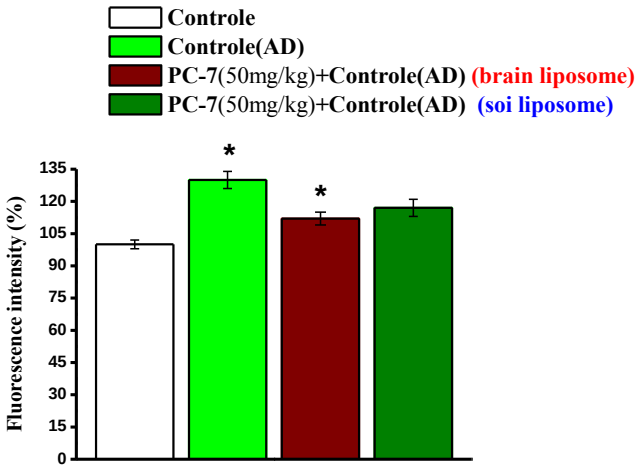


**Figure 2C. In the AD model, polyphenol PC-7 encapsulated in brain phospholipid-based liposomes and in SOI was administered orally at doses of 50 mg/kg once a day for 6 weeks (Akisu et al., 2002), and the amount of calcium in brain synaptosomes was changed in model rats when $AlCl_3$ was administered at doses of 50 mg/kg.** Significance level: * - $p<0.05$; (n = 6).

Thus, the results of the studies show that the used polyphenolic compounds weaken the neurotoxic effect of aluminum chloride in the Alzheimer's disease model and simultaneously ensure the normalization of calcium transport through the membrane of synaptosomes of nerve cells. damage and ensures their neuroplasticity and permeability (Fig. 12ABS) [25] It was found that the group of polyphenols administered intranasally had the most effective effect in the group of animals that were given the above-mentioned polyphenolic compounds together with AlCl3 in different ways (into the abdominal cavity, intranasally and orally).

## CONCLUSION

Further improvements in cognitive function, BBB permeability, and neuronal targeting in rats treated with polyphenols coated with brain phospholipid-derived liposomes suggest that these are important factors for therapeutic success in Alzheimer's disease. These results indicate that improvements in drug delivery systems may enhance the neuroprotective and cognitive benefits of polyphenols. Behavioural open field test results similar to those in healthy rats demonstrate significant improvements in cognitive function, risk perception and exploratory behaviour in Alzheimer's disease models. The high potency of brain-derived phospholipid-derived liposomes suggests that direct interaction with neural pathways may be associated with improved synaptic function or reduced neuroinflammation, thereby promoting restoration of synaptic transmission.

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