Journal of Medicinal Plants and By-Products (2024) 3: 637 – 643
https://doi.org/10.22034/jmpb.2023.362255.1558

Original Article

Total Phenolic and Flavonoid Contents and Hydro Alcoholic Extract of *Polygonum aviculare* L. Effects on Learning and Memory in a Rat Model of Alzheimer Disease

Mozhgan Shabani[1], Hasan Fallah Huseini[2], Majid Ghorbani Nohooji[2], Mehrdad Roghani[3] and Sima Nasri[1*]

[1]*Department of Biology, Payame Noor University, Tehran, Iran*
[2]*Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran*
[3]*Neurophysiology Research Center, Shahed University, Tehran, Iran*

Article History

Received: 05 May 2023
Accepted: 18 September 2023

Keywords
Polygonum aviculare
Learning
Memory
Alzheimer's rat

***Corresponding author**
s_nasri1@pnu.ac.ir

ABSTRACT

Alzheimer's disease via various pathways, including augmented oxidative stress accompanies disturbances in learning, memory and cognitive skills in the human society. The present study was conducted to evaluate the effect of *Polygonum aviculare* (*P. aviculare*) on learning and memory in streptozotocin induced Alzheimer in rats. Aerial parts of P.*aviculare* extracted by percolation method. Total phenolic and flavonoid contents were measured by colorimetric method. Fifty male wistar rats were divided into 5 groups: control, Alzheimer's group, and three groups Alzheimer's induced and *P. aviculare* treated. In order to evaluate of learning and memory, initial (IL) and step-through latencies (STL) tests were determined at the end of study using passive avoidance test and alternation behavior percentage was obtained using Y maze. Total phenolic contents of extract were 67.7 ± 6.3 mg/g and the total flavonoid content was 12.7 ± 1.3 mg/g. In behaviors test, there was no significant change in IL in Alzheimer's and *P. aviculare* treated Alzheimer's group as compared to control group. Meanwhile, STL significantly decreased in Alzheimer's group as compared to control group (p<0.005). The existing difference between Alzheimer's and *P. aviculare* Treated-Alzheimer's groups was statistically significant (p<0.01). Intraperitoneal injection of *P. aviculare* could enhance the capability of consolidation and recall in Alzheimer's animals treated, and improve spatial memory in Alzheimer's animals treated group using Y maze.

INTRODUCTION

Alzheimer's disease (AD) is a kind of progressive neurodegenerative disease, which is not reversible and is gradual progressive that causing impaired memory, loss of intellectual abilities and behavioral changes and loss of cognitive functions [1]. Almost 90 to 95 percent of cases of Alzheimer's disease in the case of late- onset Alzheimer's type (sporadic) usually is specified in the sixth decade of life. This type of the Alzheimer cellular utilization of glucose in the brain significant reduced with a steady decline in energy available to the brain. This anomaly is caused due to dysfunction of neural signals [2].

Studies showed that intracerebral injection of Streptozotocin (STZ) by inhibiting the ATP and Acetyl coenzyme-A synthesis of Acetylcholine, resulting in damage to cognitive functions and acetylcholine transferase enzyme will reduce activity in the hippocampus of rats with Alzheimer. STZ chronically produces multiple effects that resemble molecular, pathological, and behavioral features of Alzheimer's disease [3].

There may be a connection between life style and the risk of developing AD. In addition to the living conditions; age provides grounds for Alzheimer's disease [4]. Although there is no cure for this disease, it may be possible to slow the onset and reduced the progression by suppressing free radicals [5].

Analysis of antioxidant, anti-inflammatory, and neuroprotective phytochemicals used in various traditional medicines around the world reveal potential to ameliorate and prevent the devastating neurodegeneration observed in AD [6].

Studies have shown antioxidant such as flavonoids can be effective in alleviating Alzheimer's disease [7].

P. aviculare (Knotweed), is utilized as medicinal plant in Europe [8], and known plant in traditional Iranian medicine [9].

In folkloric medicine of Egypt, *P. aviculare* was used to arrest bleeding, laxative, diuretic, defluxions. In Algeria, it was used as febrifuge, remedy chronic diarrhea and urinary bladder stones. In Chinese medicine was applied for hemorrhoids, diuretic, anthelmintic and antidiarrheal. In Swedish and Polish traditional medicine, it is used to treat inflammatory and wounds [10].

It has anticancer [9], antiobesity [11], Antioxidant and anti-inflammatory [12], α-glucosidase inhibitors [13] property.

As reported in many research studies, the *P. aviculare* is rich in phenolic and flavonoids compounds. *P. aviculare* is an annual plant, consisting in *Polygonaceae* family of dicotyledonous plants[14]. It is a weedy species, which has been known for its antioxidant effects. The basic compound of *P. aviculare* are flavonoids [15].

This study was conducted to evaluate the effect of *P. aviculare* extract on learning and memory in Alzheimer's rats.

MATERIALS AND METHODS

Plant Collection and Extraction

The aerial part of medicinal plant was collected in October 2020 from natural habitat in North of Tehran City with N: 35° 44′ 54 " and E: 51° 20′ 3" geographical coordinates and 1350 m in elevation. Determination of plant species and the voucher specimen (No: IMPH-7000) has been deposited at the Herbarium of the Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran (IMPH).

The aerial parts of the plant were dried at shade and ground to fine powder using a grinder. 500 grams of the powdered material was extracted with 80% methanol by percolation method with 72h extraction time. The extract was filtered and concentrated in next steps and finally was dried with a freeze-drying apparatus [16]. For the preparation of different doses (50, 100, 200 mg /kg) of *p. aviculare* extract was dissolved in normal saline.

Determination of Total Phenol and Flavonoid

The concentration of total phenolic compounds (TPC) was measured by the method described by Kim *et al*. with some modification [17]. An aliquot (1 ml) of appropriately diluted extracts (2000, 1000 and 500 mg/l) and standard solutions of gallic acid (200, 100, 50, 25 and 12.5 mg/l) was added to a 10 ml volumetric flask containing 5 ml of distilled water. Also a reagent blank using distilled water was prepared. 0.5 mililiter of Folin & Ciocalteu's phenol reagent was added to the mixture and shaken. After 3 min, 1 ml of 20% Na2CO3 solution was added with mixing. The solution was then immediately diluted to volume (10 ml) with distilled water and mixed thoroughly. After incubation for 60 min, the absorbance versus prepared blank was read at 725 nm. Total phenolic contents were expressed as mg gallic acid equivalents (GAE)/100 g dry extract. All samples were analyzed in triplicates.

Also the concentration of total flavonoid (TFC) content was measured using a colorimetric assay developed previously Yoo *et al*. with some modification [18]. One milliliter of the extracts or standard solutions of rutin in methanol (1200, 600, 300, 150 and 75 µg/ml) was added to a 10 ml volumetric flask. 4 ml of distilled water was added. At first, 0.3 ml of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.3 ml of 10% (w/v) AlCl3 was added and, then 6 min, 2 ml of 1M NaOH were also added to the mixture, followed by the addition of 3.4 ml distilled water. The absorbance of the pink colour mixture was read at 510 nm against prepared water blank and flavonoid content was expressed as mg rutin equivalents per g of dry extract. All samples were analyzed in triplicates.

Animals

In this study, fifty male Wistar rats (Pasteur's Institute, Iran) (200– 250 g), kept in a temperature-controlled animal room (21–23 °C) under 12:12 light/dark cycle, with free access to food and water. Animals allowed habituating to their environment for one week prior to experiment. All behavioral experiments carried out between 10 a.m. and 4 p.m. This study was conducted in accordance with the policies stipulated in the Guide for the Care and Use of Laboratory Animals [19].

The study was approved by the Ethics Committee of Payame Noor University, with the ethical code IR.REC.1400.185 (Tehran, Iran).

Study Protocol

50 healthy rats were randomly divided into 5 groups: Control group, Alzheimer's group and 3 Alzheimer's extracts treated groups. The Alzheimer's group intracerebral injection of STZ (for each rat ventricle, 3 mg / kg) was dissolved in 25 µl of ACSF and injected at 5 µL with a Hamilton syringe for each rat ventricle and Passive avoidance and Y Maze were also applied to them [20]. In Alzheimer's groups treated with *P.aviculare* extract with doses 50, 100, 200 mg/kg after injection of STZ, for 21 days was received intraperitoneal extract of *P. aviculare*. After the end of the treatment, Y and passive avoidance tests were performed.

Surgical Procedure

The rat was anesthetized by intraperitoneal injection (I.P), of 40 mg / kg ketamine and 5 mg/kg xylazine. The rat was placed in a stereotaxic device and fixed on the surgical table. Head was antisepticised by alcohol, and a longitudinal cut was made between the eyes and the ears by the cutter. Bergma point specified, set the pointer device on it. Then, according to the coordinates extracted from the Atlas Watson (0.8 for Bergeman, 1.2 and 1.4 for each side of the Bergma for the ventricular, 3.2 and 3.4 the depth), according to the weight of the rat and specify the parts of the bones of the skull was pierced by the dental drill. Streptozotocin (3mg/kg) was dissolved in ACSF and injected intracerebral 5µL per ventricle by Hamilton syringe [21]. In control group, intracerebral saline (0.9%) 5µL per ventricle was injected. The surgical section was disinfected and sutured. The procedure was repeated after three days.

Y-Maze Task

This device has three arms, 30 cm lengths, 30 cm height and 15 cm widths perpendicular to each, specified by letters A, B and C. All three arms are connected by a triangular plate with sides equal to 15 cm. Measurement of the memory process by Y maze for each rat was performed only once. At the start of the experiment, each rat was placed in the beginning of the arm A with no previous acquaintance with the device. The test started with the opening of the chamber door. Within eight minutes, the arms that the mouse enters (with the criterion that the tail of the animal is in the arm) was recorded in sequence. At the end of eight minutes, the rats left the device. The arms that the animal entered was categorized in triple sequences (excluding the starting arm), the group in which there was a repeating arm was removed. The following formula was calculated based on the percentage of frequency:

$$Alteration\ percentage = \frac{ACTUAL\ ALTERATION}{MAXIMAL\ ALTERATION - 2} \times 100$$

In addition, the number of arms that each animal entered during the experiment was also compared. The Y-maze experiment will measure the animal's performance in terms of working memory by observing and measuring periodic behavior. Periodic behavior determines the amount of spatial memory [22].

Passive Avoidance Task

To investigate the passive avoidance behavior, shuttle box device with a bright chamber and a dark chamber was used. The metal bars in the dark chamber floor were used to shock the animal's foot. For this purpose, single electrical shock (50 Hz square wave, 1 mA for 1 sec) was applied.

The method for analyzing passive avoidance behavior was as follows:

Habituation

At this stage, before starting the experiment, each animal was placed inside the device for at least 5 minutes for 2 consecutive days to get familiar with the environment. The animal moves freely between the two chambers.

Acquisition

At this stage (third day), each animal was placed in a bright chamber for 10 minutes. During this time the door between the dark and bright chamber was closed. At the end of the period, the chamber light was turned on and the door was opened. Once door's opened, the stopwatch was turned on and the length of time spend for the animal to travel from the light chamber to the dark chamber (initial delay) was noted. This initial delay, called the Initial Latency or IL (The criterion for entering the animal from bright to dark chamber). Then, the door was closed and a single shock hit the animal. The rats with an Initial Latency of more than 60 seconds were excluded from the experiments.

Retention

This stage was performed 24 hours after the second stage on the fourth day. This stage was similar to the previous one with the difference that it did not receive any shock when the animal entered the dark chamber. At this stage, the Step-Through Latency or STL was measured. The Step-Through Latency is the delay time that animal spend in bright chamber before

enters the dark chamber. The cut-off time was considered 600 seconds [23].

Statistical Analysis

The SPSS software was used to examine the results of behavioral tests. The parametric test of one-way ANOVA was used for data analysis and difference at ($P<0.05$) was regarded as significant in all presented results.

RESULTS AND DISCUSSION

Total Phenolic and Flavonoid Content

Total phenolic contents of the *P. aviculare* dry extract expressed as mg gallic acid equivalents/g of the extract was 67.7 ± 6.3 mg/g. The total flavonoid content of the *P. aviculare* extract expressed as mg rutin equivalents/g of the extract was 12.7 ± 1.3 mg/g

Passive Avoidance

The intracerebral injection of streptozotocin with a dose of 3 mg / kg significantly reduced the Step-through latency, which means memory degradation (Fig. 1).

In Alzheimer's rats, initial latency was not significantly different compared with the control group (Fig. 1). In Alzheimer's treated groups with doses of 50, 100, and 200 *P. aviculare* extracts, initial latency was not significantly different with control and Alzheimer's group (Fig. 1).

In Alzheimer's group and *p. aviculare* extracts 50 and 100 mg/kg treated Alzheimer's groups the Step-through latency was significantly decrease compared with the control group ($p <0.005$ and $p<0.01$) (Fig. 1). In *P. aviculare* extracts 100 and 200 mg/kg treated Alzheimer's groups the Step-through latency was significantly increase compared with the Alzheimer's group ($P<0.005$ and $P<0.01$) (Fig. 1).

Y-maze Test on Spatial Memory

In Alzheimer's group and Alzheimer's *P. aviculare* extracts (50,100mg/kg) treated groups, the percentage of alternation behavior (Y Maze test) was significantly decreased compared with control group ($P<0.05$ and $P <0.01$) (Fig. 2).

The percentage of alternation behavior in the Alzheimer treated extract (200 mg/kg) group significantly increase compared to the Alzheimer's group ($P<0.05$) (Fig. 2).

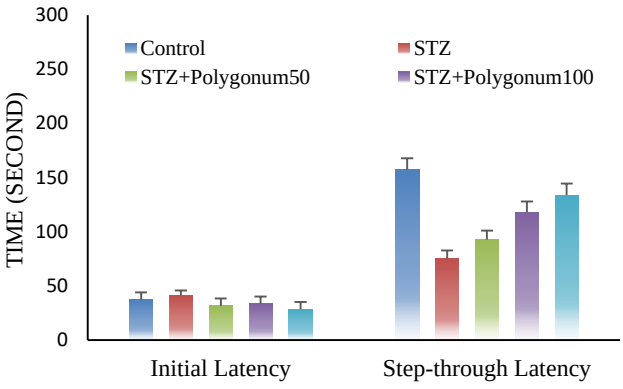

Fig. 1 Initial latency and Step-Through Latency during passive avoidance testing in control groups, STZ, and STZ -treated with hydroalcoholic extract of *P. aviculare* at doses of 50, 100 and 200 mg / kg

*** p <0.005, ** p <0.01, * p<0.05 compared to the control group. ## p <0.01, # p <0.05, Compared to the STZ group.

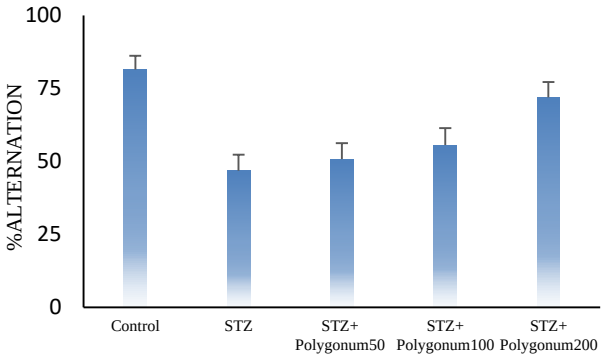

Fig. 2 The rate of frequency in the Y-Maze test in control groups, STZ, and STZ -treated with hydroalcoholic extract of *P. aviculare* in doses of 50, 100 and 200 mg / kg

** p <0.01, * p <0.05 compared to the control group. # p <0.05, Compared to the STZ group.

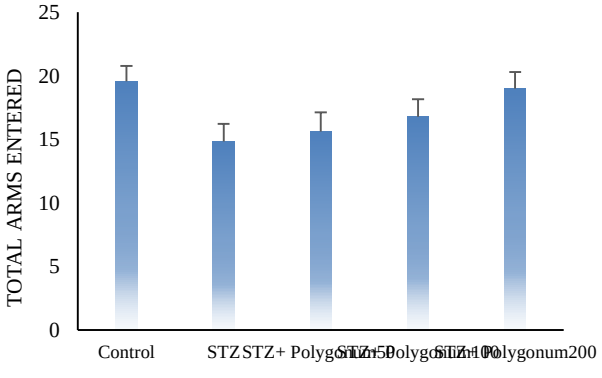

Fig. 3 The total number of arm entries in the Y-Maze test in the control groups, STZ, and STZ -treated with hydroalcoholic extract of *P. aviculare* in doses of 50, 100 and 200 mg/ Kg ($p > 0.05$)

Animal Mobility "Y Maze" Test

The animal mobility in the "Y maze" test in Alzheimer's group and Alzheimer's *P. aviculare* extracts (50,100,200 mg/kg) treated groups were not significantly different compared with control groups, which itself clearly demonstrates that the actions performed on them did not significantly affect the behavior of the animal (Fig. 3).

DISCUSSION

Alzheimer's disease, with learning and memory impairment, causes severe damage to brain glucose metabolism and brain energy. Any disorder in energy metabolism and glucose is associated with damage to cognitive and memory processes [24]. Inside Cerebral Ventricle-Streptozotocin (ICV-STZ) by inhibiting brain insulin receptors, glucose utilization and activity of glycolytic enzymes reduced. Due to above metabolic disorder, energy formation and acetylcholine transferase activity decreased in the favors of, cholinergic anuric neurons damage and decreasing the activity of aminergic catechol, which ultimately leads to learning disorders and Memory capacity [25]. Streptozotocin, on the one hand, reduces the activity of protein kinase C and induces oxidative stress and, on the other hand, increases the level of intracellular calcium. Under such conditions, caspase 3 levels in the cortex and the hippocampus increase and neoplastic cell apoptosis occurs [21].

In the present study, using an inhibitory avoidance method, which is an acceptable method for long-term memory evaluation, as well as a Y-shaped maze test that shows the performance of animals in terms of working memory, the effect of injection of *P. aviculare* extract in treatment the withering effects of intracerebral streptozotocin injection on male rats' memory were investigated. The Y Maze test is an indicator of space memory in rodents, such as rats. The rate of animal mobility is shown in different groups. There was practically no significant difference between the groups, which clearly indicates that the actions performed on them, did not significantly affect the behavior of the animal. Also, no significant difference was detected in the initial Latency between groups. This suggests that intraperitoneal injection of the *P. aviculare* in rats was not able to produce new information. Furthermore, Step-through latency, which is an indicator of the ability of the animal to store information in memory warehouses and to remind them, revealed that intraperitoneal injection of the *P. aviculare* at higher doses (200 mg/kg) was able to increase Step-through latency and thus increase the power of reminding information in treated rats.

Flavonoids, important herbal components, seem to protect neurons against damage from neurotoxins and inflammatory neurotransmitters, supporting vein and neurogenesis, especially in the hippocampus and inhibit apoptosis induced by neurotoxic species [24, 25]. Flavonoids also have an effect on protein kinases, lipid kinases, and the pathway of MAP kinases to improve neuronal communication in dentate gyrus and CA3 layer of the hippocampus, improve neuronal communication and subsequently improve LTP [26].

The chemical analysis of the *P. aviculare* indicates the presence of high levels of phenolic compounds and flavonoids in this plant [9] that is in line with study *P. aviculare* contains myricetin, kampferol, isorhamnetin, kaempferide glucuronides, taxifolin, luteolin, quercetin [10]. Myricetin increased the number of CA3 pyramidal neuron in hippocampus of Alzheimer's rat. In addition, this flavonoid improves learning and memory in Alzheimer's rat [27]. In transgenic Drosophila model of Alzheimer's disease, Kampferol delayed the loss of memory, decreased the oxidative stress and acetylcholinesterase (AChE) activity [28].

AChE hydrolyses acetylcholine that impacts on neurons involved in memory formation. Some flavonoids are AChE inhibitors [29]. Quercetin antagonizes AChE by increasing acetylcholine concentration at the cholinergic synapses [30].

It proposed that anti-obesity effect of *P. Aviculare* in high-fat diet-induced obesity in rat may be due to its antioxidative property [31]. Therefore, it seems that the effect of *P.aviculare* extract on memory and learning may be due to its polyphenols and flavonoids content.

CONCLUSION

The results of an inhibitory avoidance study indicated that intraperitoneal injection of *P. aviculare* in a dose (200 mg / kg) for 21 days significantly increased Step-through latency compared with the STZ-induced Alzheimer group. The flavonoids of the

hydroalcoholic extract of *P.aviculare* can increase acetylcholine concentration.

It would be suggested to investigate further the effect of *P. Aviculare* for preclinical treating of Alzheimer's disease

Conflicts of Interest

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

This article is the result of the first author M.Sc. student thesis Payame Noor University, which was conducted in collaboration with the Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran.

REFERENCES

1. Hassanpour H,Pouyan A.A., Dehghan H. Detection of Alzheimer's Disease Using Multitracer Positron. Emission Tomography Imaging.International J. Engineering2014;27(1): 51-56.
2. Nagasako-Akazome Y., Kanda T., Ohtake Y., Shimasaki H., Kobayashi T. Apple polyphenols influence cholesterol metabolism in healthy subjects with relatively high body mass index. J Oleo Sci. 2007; 56(8):417-28.
3. Zhi-Ru C., Jia-Bao H., Shu-Long Y., Fen-Fang H. Role of cholinergic signaling in Alzheimer's disease. Molecules 2022; 27(6): 1816.
4. Cooper E.L., Melissa J.M. Alzheimer Disease: Clues from traditional and complementary medicine.J. Tradit Complement Med. 2017;7(4):380–385.
5. Arab L., Sabbagh M.N. Are certain life style habits associated with lower Alzheimer disease risk? J.Alzheimers Dis. 2010;20(3):785–794.
6. Bushra S., Wei D., .Hussain A., Yeong S.K., Khan S. Anti-neuroinflammatory Potential of Natural Products in Attenuation of Alzheimer's Disease. Front Pharmacol. 2018;9:548.
7. FengY, Wang X. Antioxidant therapies for Alzheimer's disease. Oxid. Med. Cell Longev. 2012; 2012: 472932.
8. Pawlowska K.A., Kryzman M., Zidorn C., Pagitz K., Popowski D., Granica S. HPLC-DAD-MS3 fingerprints of phenolics of selected Polygonum taxa and their chemometric analysis. Phytochemistry. 2023;208:113605.
9. Habibi R.M., Mohammadi R.A., Delazar A, *et al*. Effects of *polygonum aviculare* herbal extract on proliferation and apoptotic gene expression of MCF-7. *Daru*. 2011;19(5):326-331.
10. Akbar S. *Polygonum avicular* L. (Polygonaceae). In: handbook of 200 medicinal plants. Springer. Cham. https://doi.org/10.1007/978-3-030-16807-0_153.
11. Sung Y.Y., Yoon T., Yang W.K., Kim S.J., Kim D.S., Kim H.K. The antiobesity effect of *Polygonum aviculare* L. ethanol extract in high-fat diet-induced obese mice. Evid. Based Complement. Alternat. Med. 2013, 2013, 626397.
12. Granica S., Piwowarski J.P., Popławska M., Jakubowska M., Borzym J., Kiss A.K. Novel insight into qualitative standardization of *Polygonia vicularis* herba (Ph. Eur.). J. Pharm. Biomed. Anal. 2013;*72*:216–222.
13. Cai Y., Wu L., Lin X., Hu X., Wang L. Phenolic profiles and screening of potential α-glucosidase inhibitors from *Polygonum aviculare* L. leaves using ultra-filtration combined with HPLC-ESI-qTOF-MS/MS and molecular docking analysis. Ind. Crop. Prod. 2020; 154, 112673
14. Chin-Yuan H. Antioxidant activity of extract from *Polygonum aviculare,* L. *Biol Res*2006; 39(2): 281-288.
15. Ghahreman, A. *Colored Flora Iranica*. Tehran: Tehran University. 1979; 87-89. [persian]
16. Fu-Quan X., Yuan-Yuan F., Li-Yun G. Optimization of Extraction Process for Flavonoids from *Polygonum aviculare*L. Based on HPLC Quantitation. Food Sci. 2012; 33(14): 36-40.
17. Kim D.O., Jeong S.W., Lee C.Y. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry. 2003; 81: 321-326.
18. Kyung Mi Y., Choong H.L., Hyungjae L., BoKyung M., Chang Yong L. Relative antioxidant and cytoprotective activities of common herbs. Food Chemistry. 2008; 106: 929-936.
19. Nazari L., Komaki S., Salehi I., Raoufi S., Golipoor Z., Kourosh-Arami M., Komaki A. Investigation of the protective effects of lutein on memory and learning using behavioral methods in a male rat model of Alzheimer's disease. J Functional Foods. 2022; 99:105319.
20. Tayanloo-Beik A., Kiasalari Z., Roghani M. Paeonol ameliorates cognitive deficits in streptozotocin murine model of sporadic Alzheimer's disease via attenuation of oxidative stress, inflammation, and mitochondrial dysfunction. J. Mol Neurosci. 2022; 72(2): 336-348.
21. Grieb P. Intracerebroventricular Streptozotocin Injections as a Model of Alzheimer's disease: in Search of a Relevant Mechanism. Mol.Neurobiol. 2016; 53(3):1741–1752.
22. Roghani M., Joghataie M.T., Jalili M.R., BaluchnejadmojaradT. Timecourse of changes in passive avoidance and Y-maze performance in male diabetic rats. IBJ. 2006; 10 (2):99-104.
23. Ahshin-Majd S., Zamani S., Kiamari T., Kiasalari Z., Baluchnejadmojarad T., Roghani M. Carnosine ameliorates cognitive deficits in streptozotocin-induced diabetic rats: possible involved mechanisms. Peptides. 2016; 86:102-111.

24. Kumar A., ArtiS Kavali E. A review on Alzheimer's disease pathophysiology and its management: an update. PharmacolRep. 2015; 67 (2): 195-203.
25. Smith M.A., Perry G., Richey P.L., Sayre L.M., Anderson V.E., Beal M.F., Kowall N. Oxidative damage in Alzheimer's. Nature. 1996; 382 (6587): 120-121.
26. Vauzour D. Dietary Polyphenols as Modulators of Brain Functions: Biological Actions and Molecular Mechanisms Underpinning Their Beneficial Effects. Oxid Med Cell Longev. 2012; 2012: 914273.
27. Ramezani M., Darbandi N., Khodagholi F., Hashemi A. Myricetin protects hippocampal CA3 pyramidal neurons and improves learning and memory impairments in rats with Alzheimer's disease. Neural Regen Res. 2016;11(12): 1976-1980.
28. Beg T., Jyoti S., Naz F., Ali F., Kashif Ali S., Reyad A.M., Siddique Y.H. Protective effect of kaempferol on the transgenic Drosophila model of Alzheimer's disease. CNS Neurol Disord Drug Targets. 2018;17(6): 421-429. doi: 10.2174/1871527317666180508123050
29. Khan H., Amin M.S., Ajmal Kamal M., Patel S. Flavonoids as acetycholiesterase inhibitors: Current therapeutic standing and future poospects. Biomedicine & pharmacotherapy. 2018; 101: 860-870. https://doi.org/10.1016/j.biopha.2018.03.007
30. Jung M., Park M. acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*. Molecules. 2007; 12(9), 2130-2139.
31. Yoon-Young S., TaesookY., Yang W., SeungJu K., Dong-Seon K., Ho Kyoung K. The Antiobesity Effect of *PolygonumAviculare* L. Ethanol Extract in High-Fat Diet-Induced Obese Mic. Evidence-Based complementary and alternative medicine. 2013;2013:626397.