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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

NEUROPHYSIOLOGICAL STUDY OF RETINAL FUNCTION IN NEURODEGENERATIVE BRAIN DISORDERS AND THE EFFECT OF PLANT EXTRACTS ON COMPENSATORY PROCESSES

Spesiality: 2411.01–Human and Animal Physiology

Field of science: Biology

Applicant: **Rubaba Nazim Quliyeva**

Baku -2025

The work was performed at the "Physiology of Vision and Neurodegenerative Processes" laboratory of the Institute of Physiology named after Academician Abdulla Garayev, under the Ministry of Science and Education of the Republic of Azerbaijan.

Scientific supervisor: Doctor of Biological Sciences, professor
Ulduz Faizi Hashimova

Official opponents: Doctor of Biological Sciences, associate professor
Arif-Ala Ali-Ovsad Mekhtiev

Doctor of phylosophy in biology,
Sevidj Ibrahimkhalil Mamedova

Doctor of phylosophy in biology, associate professor
Nazaket Naib Aliyeva

Dissertation Council FD1.08 of Supreme Attestation Commission under the President of the Republic of Azerbaijan, operating on the basis of the Institute of Physiology named after Academician Abdulla Garayev of the Ministry of Science and Education of the Republic of Azerbaijan

Chairman of the Dissertation council:

Doctor of Biological Sciences, professor
Ulduz Faizi Hashimova

Scientific secretary of the Dissertation council:

Doctor of phylosophy in biology, associate professor
Yegana Ogtay Bayramova

Chairman of the scientific seminar:

Doctor of Physical and Mathematical Sciences, professor
Ahmad Mahammad Hajıyev

GENERAL DESCRIPTION OF THE WORK

The relevance of the theme: The increase in life expectancy in developed countries has highlighted the importance of diagnosing and treating neurodegenerative diseases (NDDs). These conditions are the most common cause of memory impairment. Each pathological case may present with various memory-related symptoms. Currently, the most frequent cause of dementia in the elderly (accounting for up to 80%) is Alzheimer's disease (AD), the incidence of which continues to rise each year[1]. According to WHO data, by 2025, approximately three-quarters of the 1.2 billion people aged 60 and over will be living in developing countries[2].

NDDs begin long before the appearance of obvious signs of cognitive impairment, which is why early diagnosis can contribute to more effective treatment. Changes in the brain are also reflected in the retina, as its condition is closely linked to the function of the central nervous system. [3,4,5,6]. Recent scientific literature increasingly reports the detection of visual disorders in AD, some of which are identified even at the prodromal (pre-dementia) stage.

[1]Barnes, D.E., Yaffe, K. The projected effect of risk factor reduction on Alzheimer's disease prevalence // Lancet Neurol., – 2011. –№ 10, – p. 819-828.
[2]Kalaria, R.N. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors / R.N. Kalaria, G.E. Maestre, R. Arizaga [et al.] // Lancet Neurol., – 2008. – № 7, – p. 812-825.
[3]Gupta, V.B. Retinal changes in Alzheimer's disease—Integrated prospects of imaging, functional and molecular advances / V.B. Gupta, N. Chitranshi, J. den Haan [et al.] // Prog. Retin. Eye Res., – 2021. –№82, – p. 100899.[4] Hussain, A. The Eye as a Diagnostic Tool for Alzheimer's Disease / A. Hussain, Z. Sheikh, M. Subramanian [et al.] // Life., – 2023. – № 13, – p. 726.
[5]Panakhova, E.N. Alzheimer disease Vision. Amygdala. Epilepsy. Neurophysiological, Neurological and Biochemical Mechanisms/ U.

Hashimova, Kh. Miryusivova, A. Allahverdiyeva, N. Huseynova, K. Javadova –New York: – 2022. –214 p.
[6]Zhang, Y. Advances in retina imaging as potential biomarkers for early diagnosis of Alzheimer's disease / Y. Zhang, Y. Wang, C. Shi [et al.] // Transl Neurodegener, – 2021. – №10, – p. 6.

These include color vision and contrast sensitivity impairments, alterations in electrophysiological parameters, retinal thinning, and changes in peripapillary nerve fiber layers[7]. The debate about the nature of visual impairment in AD continues, as morphological studies reveal markers of neurodegeneration in various parts of the visual pathway [18,9,10].

The strategy for developing models for studying memory dysfunction was to reproduce the pathological features of the disease. Removal of the olfactory bulbs (olfactory bulbectomy-OBX) in

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[7]Gao, R. Retina as a potential biomarker for the early stage of Alzheimer's disease spectrum / R. Gao, H. Luo, S. Yan [et al.] // Ann. Clin. Transl. Neurol., – 2024. – № 11, – p. 2583–2596.

[8]Garcia-Martin, E. Ganglion cell layer measurements correlate with disease severity in patients with Alzheimer's disease / E. Garcia-Martin, M.P. Bambo, M.L. Marques [et al.] // Acta Ophthalmol., – 2016. –№ 96(6), – p. e454–459.

[9]Ikram, M.K. Retinal pathology as biomarker for cognitive impairment and Alzheimer's disease / M.K. Ikram, C.Y. Cheung, T.Y. Wong [et al.] // J Neurol Neurosurg Psychiatry., – 2012. – № 83, – p. 917e922. doi:10.1136/jnnp-2011-301628

[10] Panakhova E., Mustafayeva N. Visual Uncertainty, Amygdala and Control of Behavior / XVI European Congress of Psychology., – 2019, – p. 361-362.

[11]Bobkova, N.V. Morphofunctional changes in neurons in the temporal cortex of the brain in relation to spatial memory in bulbectomized mice after treatment with mineral ascorbates / N.V Bobkova, I.V Nesterova, R. Dana [et al.] // Neurosci Behav Physiol., – 2004. – № 34, – p. 671–676.

[12]Bobkova, N.V. Therapeutic effects of exogenous HSP70 in mouse models of Alzheimer's disease / N.V. Bobkova, D.G. Garbuz, I. Nesterova [et al.] // J Alzheimer Dis., – 2014. – № 38, – p. 425–435.

[13]Borre, Y.E. Neurodegeneration and depression in an animal model of multi-target food intervention: neuroprotective and cognitive-enhancing effects / Y.E. Borre, T. Panagaki, P.J. Koelink [et al.] // Neuropharmacology, – 2014. – № 79, – p. 738-749.

rodents results in symptoms that resemble those seen in the progression of Alzheimer-type neurodegeneration [11,12,13]. This allows the use of OBX for modeling neurodegenerative processes.

Recently, the polyphenol curcumin has been shown to be a promising anti-amyloid, anti-inflammatory, and neuroprotective agent in the treatment of NDDs.

The antioxidant effect is associated with the neutralization of free radicals and the reduction of oxidative stress[14, 15, 16].

The aim of the study. The main aim of this dissertation s to study the changes occurring in the brain and retina following olfactory bulb damage, as well as to investigate the associated memory impairments. Additionally, the study examines the effect of curcumin on the development and progression of this pathological process.

Objectives of the study:

1. To study the behavior of control and experimental animals in the Morris Water Maze (MWM).
2. To determine the effect of turmeric on cognitive functions in rats with OBX
3. To identify histological changes in the brain and retina of rats with OBX
4. To study the effect of turmeric on histological changes in the brain and retina of rats with OBX

Key theses to be defended:

1. Animals with experimental damage to the OB showed disturbances in spatial behavior and decreased memory performance in the MWM test
2. Oral administration of turmeric extract to animals with the OBX model contributed to the improvement of cognitive functions, including spatial learning and memory.

[14] Dong, S. Curcumin Enhances Neurogenesis and Cognition in Aged Rats: Implications for Transcriptional Interactions Related to Growth and Synaptic Plasticity / S. Dong, Q. Zeng, E.S. Mitchell [et al.] // PLoS ONE., – 2012. – № 7(2): e31211.

[15] Sharman, J. The Efficacy of Curcumin on Cognition, Depression, and Agitation in Older Adults with Alzheimer's Disease / J. Sharman, R. Galeshi, L. Onega [et al.] // The Open Nutrition Journal, – 2017. – Vol. 11, – p. 11-16.

[16]Lopresti, A.L. Curcumin for the treatment of major depression: a randomised, double-blind, placebo-controlled study / A.L. Lopresti, G.L. Maker, S.D. Hood [et al.] // J Affect Disord., – 2014. – № 167, – p. 368–375.

3. Rats with OBX showed morphological changes in the structures of the brain and retina, indicating neurodegenerative processes.
4. Turmeric has a positive effect on the condition of the retina, promoting the restoration of its structure in animals that have underwent bulbectomy.

Scientific novelty of the study.

In this study, the behavior of rats with a model of olfactory bulb (OB) damage in the MWM test was analyzed. The data obtained indicate that cognitive impairment caused by OB damage is accompanied by a significant decrease in spatial memory. Along with memory impairment, animals with the OBX model showed pronounced destructive changes in the structure of the retina. The use of turmeric contributed to a partial restoration of the retinal architecture, which correlated with an improvement in spatial orientation in experimental animals.

Practical significance of the study.

The practical significance of the study is the experimental substantiation of the neuroprotective effect of turmeric extract in neurodegenerative changes affecting the structures of the brain and retina. The results obtained can be used to optimize experimental models of neurodegeneration, as well as for a preliminary assessment of the effectiveness of natural compounds with potential therapeutic activity. The diagnostic value of morphological changes in the retina as a possible reflection of pathological processes occurring in the central nervous system has been established. In addition, the research materials can be introduced into the educational process when teaching disciplines related to physiology, neurophysiology and histology.

Approbation of dissertation.

The results of the dissertation work were presented in the form of oral reports at the following conferences:

1. International AEGEAN conferences on social sciences & humanities. Turkey, Izmir, September 23-25, 2023;
2. 6th International Antalya Scientific Research and Innovative Studies Congress. Antalya, December 2-4, 2023;
3. Biomüxtəlifliyin dayanıqlılığının təmin edilməsində yeni çağırışlar (COP29) Yaşıl dünya və insan sağlamlığı". Beynəlxalq elmi-praktiki konfrans. Баку, 3-4 июн 2024;
4. Международная научно-практическая конференция Физиология и здоровье в современное время: научные приоритеты и перспективы, посвященная. Году солидарности зеленого мира. Баку, 19-20 декабря 2024;
5. Qarabağ ekesistemlırinə antropogen faktorlarının və iqlim dəyişikliklərinin təsiri. Баку, 30 сентября, 2024.

Scientific publications. Based on the materials of the dissertation, 12 scientific works have been published, including 5 conference abstracts and 7 articles, 4 of which were published in foreign scientific journals with an impact factor.

Volume and structure of the dissertation.

The dissertation is presented on 135 pages of computer text (161902 characters) and consists of an introduction (11912 characters), chapters of the literature review (47781 characters), materials and methods of research (13464 characters), research results (62408 characters), discussion (27839 characters), conclusions (1069 characters), practical recommendations (600 characters).

The work is illustrated with 8 diagrams, 15 figures, 3 tables, 1 graph. The list of references includes 169 sources, of which 12 are in Russian and 157 in English.

MATERIALS AND METHODS OF THE RESEARCH

The experiments were conducted on white rats weighing 250-300 g, in accordance with the NIH Guide for the Care and Use of Laboratory Animals [Guide for the Care and Use of Laboratory Animals, 2017]. Training, histological, and pharmacological methods were used to conduct the studies. The MWM was used as the main test for assessing spatial learning and memory in animals. The MWM is a cylindrical pool filled with colored water and divided into four sectors. During the training, the rats are taught to find the rescue platform using near and far landmarks, memorizing the sequence of movements. The time spent finding the platform, the distance from the start to the platform, and the total distance were measured to assess the effectiveness of the training. The success of the training was determined by a decrease in the time of rescue on the platform and the distance traveled.

To create a model of memory impairment, OBX was used, which made it possible to study changes in spatial memory in animals [17].

Histological sections of the eyes and brain were analyzed using light and electron microscopes (Primo Star (Zeiss) and JEM 1400 (Joel Japan) Transmission Electron Microscope (TEM).

Aqueous extract of turmeric (Curcuma Longa) (250mq/kq) was used as an antioxidant.

The obtained statistical results were processed using the EXEL program (ANOVA).

RESULTS OF RESEARCH

Results of the study of rat behavior in the MWM

Before the OBX, the animals underwent a training course, according to which they found the platform in 15-38 seconds

[17] Bobkova, N.V. Morphofunctional changes in neurons in the temporal cortex of the brain in relation to spatial memory in bulbectomized mice after treatment with mineral ascorbates / N.V Bobkova, I.V Nesterova, R. Dana [et al.] // Neurosci Behav Physiol., – 2004. – № 34, – p. 671–676.

(average time 27.8 ± 10.24 sec). After the OBX and rehabilitation, repeated tests were conducted.

On the first day of testing, the rats started from the position (X), on the second - from the opposite (Y). All animals had 120 seconds to find the platform. Intact rats found the platform faster than rats with OBX, which indicates a deterioration in cognitive functions in animals with damage. On the second day, intact rats significantly reduced the search time, in contrast to rats with OBX.

Additionally, the time spent near the walls of the pool and in the target quadrant were assessed. Rats with OBX spent 3.4 times more time near the walls and 2.4 times more in the target segment than intact rats. In the study of delayed recall, rats with OBX spent less time in the target quadrant, which indicates a violation of spatial memory. In terms of the time spent in the different quadrants of the pool, intact animals did not show significant differences, with the maximum time being in the right quadrant. Rats with OBX showed more time in the left and right quadrants and a decrease in time in the target quadrant, which confirms damage to spatial memory.

Table 1

Behavior of groups of rats in the Morris water maze

Animal groups	Platform location time, sec			
	Learning		Playback	
	Position X	Position Y	Position X	Position Y
Intact	74,33±8,16 [66,2; 83,0]	33,55±6,79 [22,4; 38,2]	34,7±6,15* [22,4; 38,2]	6,28±2,05* [22,4; 38,2]
Bulbectomized animals	98,10±14,24 [79,6; 118,8]	84,92±13,74 [77,8; 91,0]	87,56±11,4 [79,8; 96,5]	77,07±10,1 [70,4; 92,7]

Note: * - statistical significance of the differences between learning and recall (p<0,05-0,001)

The percentage distribution of time in intact rats was 41.5% in the target quadrant, while in animals with OBX it was 30.1%. In addition, rats with OBX swam more slowly, indicating a deterioration in the ability to remember the location of the platform, with a decrease in the average swimming speed in the target quadrant by 1.7 times compared to the control group.

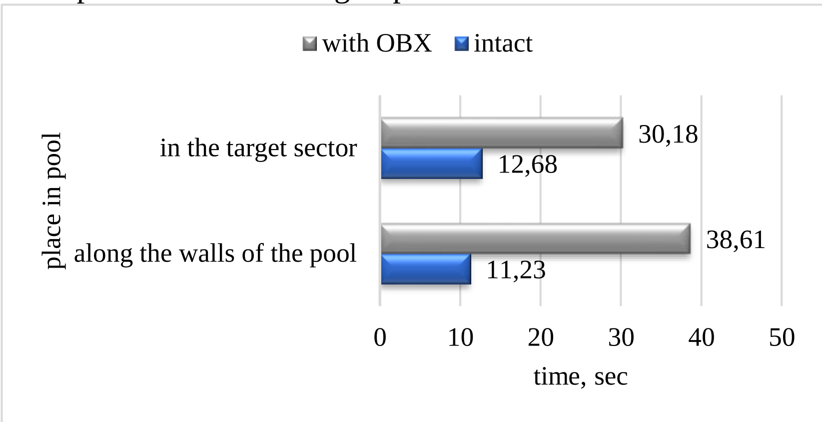
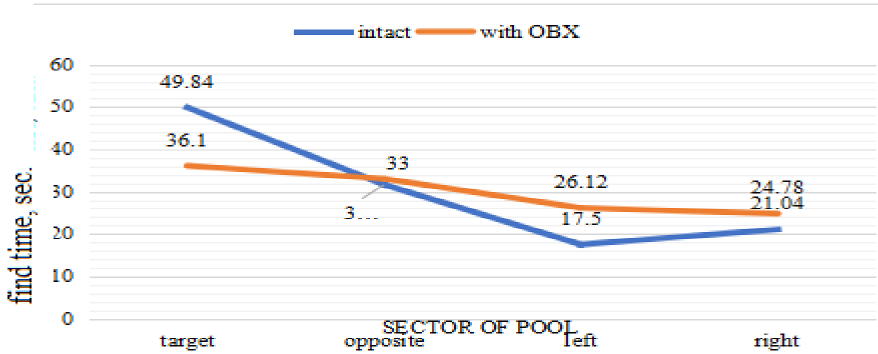


Diagram 1. Time spent by animals at the pool walls and in the target sector on the second day of testing



Graph 1. Time spent by rats in the target quadrant in the absence of an invisible platform

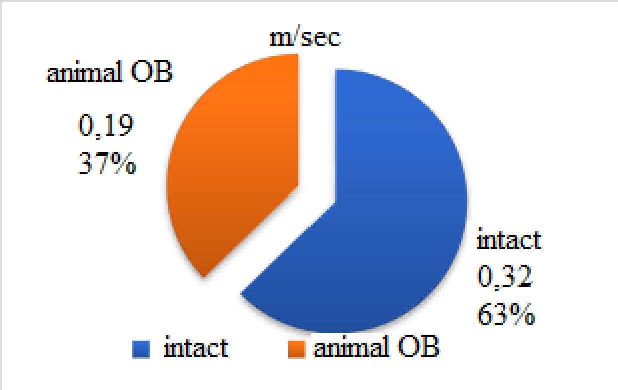


Figure 1. Average swimming speed of animals in the target quadrant

Animals with OBX had virtually lost their orientation memory. If before OBX they found the hidden platform in an average of 27.8±10.24 sec, then after the operation the time to search for the platform increased to an average of 81.0±10.86 sec.

Based on the obtained results, it was revealed that animals with OBX had statistically significant differences in the reproduction of skills, time spent in the target quadrant, as well as the average swimming speed in the target sector.

Results of the study of the effect of turmeric on spatial behavior and memory of rats in the MWM

The animals under study were divided into three groups: I — intact, II — with OBX, III — with OBX and turmeric administration. Rats of group III were administered turmeric for one month, after which testing of all three groups was continued. After 2.5 months, intact rats, as before, found the hidden platform in 20–30 seconds. After turmeric administration to animals with OBX, a comparative assessment of the platform search time was conducted between all groups (Fig. 2).

In group II rats, the average platform search time was 80.2±9.74 sec on the first day and decreased to 69.5±9.11 sec on the second day (1.1 times). In group III animals, the time decreased from 84.7±8.92 to 56.3±9.0 sec (1.5 times; $p < 0.05$) (Fig. 2). Rats that received turmeric spent significantly more time in the target quadrant of the VLM than intact animals, 1.7 times longer than the control (Fig. 2). A comparative analysis of all 7 days showed that the positive effect of turmeric became clearly evident from the third day of testing (Fig. 3).

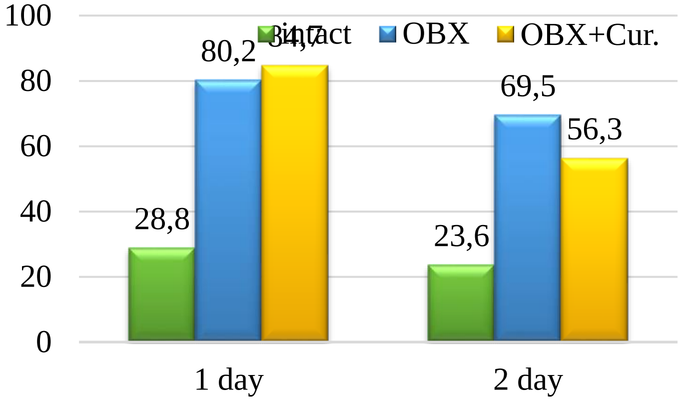


Diagram 2. Time (sec) of platform search by rats in MWM after 2 days of training

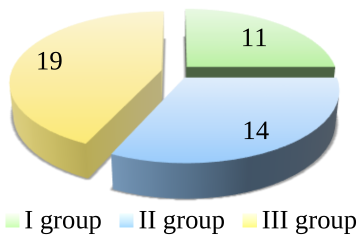


Figure 2. Time spent by rats in the target segment on the day of reproduction

On the first day, the longest search time for the hidden platform was observed in rats of group III: 83.3±10.52 sec, which exceeded the similar indicator in intact animals (28.8±6.82 sec) by 2.9 times ($p < 0.01$). In rats of group II, the time was 79.9±9.01 sec (2.8 times more, $p < 0.01$). On the second day, the indicators decreased in all groups: in intact animals - 23.4±5.44 sec, in groups II and III - 68.8±8.17 sec and 56.1±6.12 sec, respectively. In animals of groups II and III, the search time remained higher than the control by 2.9 ($p < 0.01$) and 2.4 times ($p < 0.05$), while the introduction of turmeric reduced the search time by 22.6%.

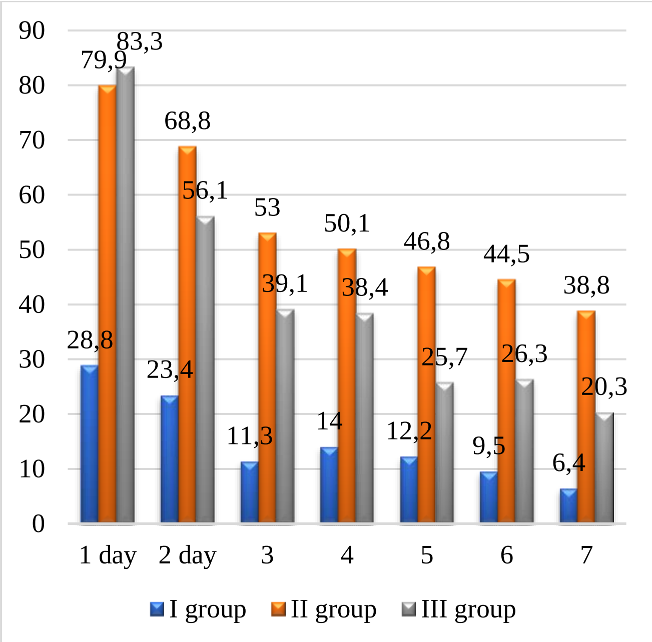


Diagram 3. The effect of turmeric on the spatial orientation of rats in the MWM in intact (I), in OBX (II), and in animals receiving turmeric after OBX (III).

On the third day, the differences remained: Group I - 11.3 ± 5.88 sec, Group II - 53.0 ± 7.72 sec, Group III - 39.1 ± 7.08 sec, which exceeded the control by 4.7 ($p < 0.01$) and 3.5 times ($p < 0.01$), respectively. On the fourth day, intact rats searched for the platform in 14.0 ± 7.34 sec, Groups II and III - in 50.1 ± 5.27 and 38.4 ± 7.24 sec. Compared with the previous day, intact animals showed an increase in time by 19.3%, while Groups II and III showed a decrease of 5.8% and 1.8%, respectively. Compared with the control, the differences were 3.6 ($p < 0.01$) and 2.7 times ($p < 0.05$). On the fifth day: I — 12.2±6.04 sec, II — 46.8±5.62 sec, III — 25.7±4.88 sec; the excess of control values was 3.8 ($p<0.01$) and 2.1 times ($p<0.05$). On the sixth day: I — 9.5±2.66 sec, II — 44.5±6.08 sec, III — 26.3±5.17 sec; the difference compared to the control was 4.7 ($p<0.01$) and 2.8 times ($p<0.01$). On the seventh day: I - 6.4±2.02 sec, II - 38.8±8.24 sec, III - 20.3±6.33 sec, which exceeds the control values by 6.1 ($p<0.001$) and 3.2 times ($p<0.01$), respectively (diagram 3).

Evaluation of the effect of turmeric on spatial learning and memory in rats with OBX

The obtained data show that rats with OB spent more time searching for the hidden platform and stayed in the target segment longer than intact animals. However, there were significant differences between groups II and III.

On the first day, rats of group III spent 4.1% more time searching for the platform than group II. Already on the second day, the search time in animals of group III decreased by 22.6% compared to group II. From the third day, the effect became more pronounced: the search time in group III was 35.6% less ($p < 0.05$), and on the fourth and fifth days the reduction was 30.5% and 82.1%, respectively ($p < 0.05$).

On the sixth day, the search time in group III increased by 2.3%, while in group II it decreased by 5.2%. However, group III completed the task 66.9% faster ($p < 0.05$). The maximum effect was achieved on the seventh day; the search time in group III was 91.1% less compared to group II ($p <0.001$).

Analysis of the time spent near the pool walls showed similar trends. On the seventh day, the latent time spent near the walls decreased by 55.58% ($p < 0.05$) in Group II and by 60.46% ($p < 0.05$) in Group III compared to the third day.

When the platform was hidden and delayed recall was assessed (120 seconds of observation), rats in group III spent more time in the target quadrant — 50.65±9.17 sec, although the difference between the groups was not statistically significant. The time spent in the target quadrant was: group I — 48.14±7.14 sec (40.1%), group II — 40.6±10.22 sec (33.8%), group III — 50.65±9.17 sec (42.2%) (Fig. 4).

The swimming speed in group III was significantly higher compared to group II — 1.6 times ($p < 0.05$), which confirms the improvement of cognitive and motor functions in rats treated with turmeric (Fig. 3). As can be seen from Table 2, animals of groups II and III reduced the latent time spent near the walls of the tank. Thus, the time spent near the walls of the tank on the 7th day of testing compared to the 3rd day in groups II and III decreased by 55.58% ($p<0.05$) and 60.46% ($p<0.05$), respectively.

Thus, the introduction of turmeric to rats with OBX improved their spatial learning and memory, which was manifested both in a reduction in the time spent finding the hidden platform and in an increase in the time spent in the target quadrant. These improvements became statistically significant from the third day and reached a maximum on the seventh day, confirming the potential of turmeric as a means of correcting cognitive impairment caused by bulbectomy.

Table 2

Time (sec) spent by animals at the pool walls and in the target sector during the testing period

Location in MWM	Testing days	Animal groups		
		I group (n=10)	II group (n=10)	III group (n=10)
in the target quadrant/ at the pool walls	3	13,44±3,65 12,0±2,84	27,84±7,35 37,51±9,18	27,25±7,15 36,65±8,61
	4	11,58±3,07 10,14±2,69	28,22±7,56 30,27±7,10	28,40±7,08 29,83±8,15
	5	11,05±2,16 9,78±2,05	28,06±7,21 27,31±7,02	28,71±6,54 26,70±6,76
	6	11,34±2,24 9,03±1,88	27,56±6,72 24,68±7,70	27,62±7,02 23,15±6,43
	7	10,61±1,88 7,92±1,57	27,30±6,58 24,11±7,62	27,43±7,17 22,84±6,12

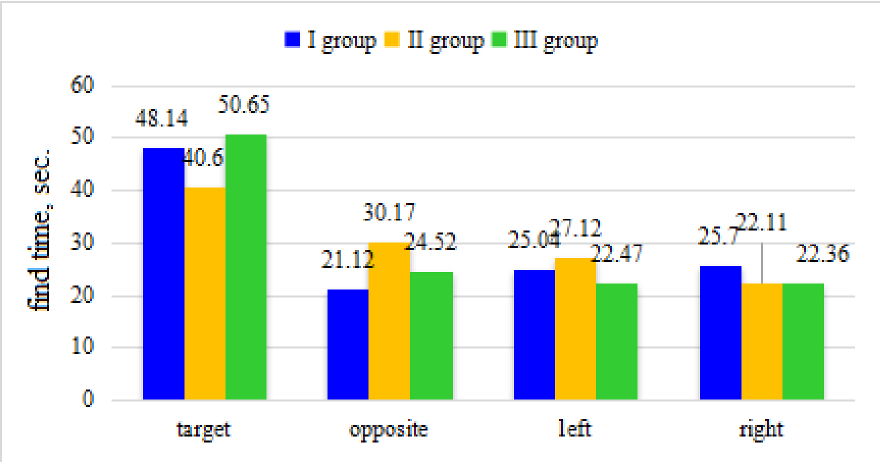


Diagram 4. Time spent by rats in tank sectors without an invisible platform

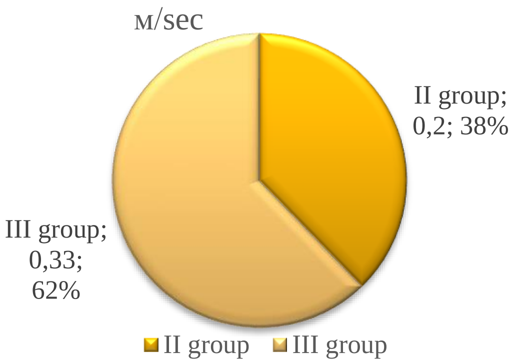


Figure 3. Average swimming speed of experimental animals of groups II and III in the target quadrant

It was also found that the percentage of time spent in the target quadrant decreased with each testing day. At the same time, rats spent significantly more time in the target quadrant than in the others. Intact rats (Group I) spent 40.1% of the time in the target quadrant, rats with OB (Group II) - 33.8%, and rats with OB (Group III) - 42.2% (Fig. 4).

In addition, during training, rats that received turmeric showed faster results compared to animals that did not receive turmeric. On the 1st and 2nd days of the experiment, animals with OB needed more time to find the platform, but starting from the 3rd day, a significant reduction in search time was noted. Also, over the following days, statistically significant differences were observed between the groups in the time spent finding the platform. Thus, turmeric had a positive effect on spatial memory, helping to improve the rats' ability to quickly and accurately locate the hidden platform.

Results of the effect of turmeric on histological changes in the brain and retina in rats with experimentally induced memory impairment

The animals were divided into 5 groups of 10 rats each:

• Group I: intact animals.

• Group II: animals with the olfactory bulb (OB) injury model; histological examination was performed on the 7th day after surgery.

• Group III: animals with OBX that received turmeric extract orally for 21 days; the analysis was performed on the 21st day.

• Group IV: animals with OBX that received turmeric for 90 days; the study was performed on the 90th day.

• Group V: animals with OBX that were administered turmeric for 180 days; histological analysis was performed on the 180th day.

Macroscopic examination revealed that the brain tissue was grayish-white, with a soft consistency and noticeable boundaries between the right and left parts.

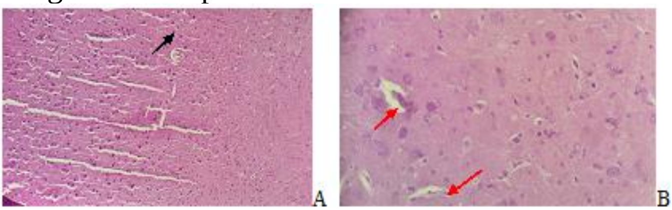


Figure 4. Microscopic image of the bulbar part of intact animals (A - magnification x40, B - magnification x200) Stained with hematoxylin and eosin.

The histological structure of the brain tissue in Group I retains normal histoarchitecture. The number of nerve cells is within normal limits, no damage is detected. No cells with signs of karyopyknosis are found. The cytoplasm of neurons is eosinophilic stained, a large nucleus is in the center of each neuron (Fig. 4A, black arrow). Capillaries covered with endothelium with a thin basement membrane are visible around the neurons (Fig. 4B, red arrow).

Astrocytes and oligodendrocytes are within normal limits, gliosis and edema are absent. Olfactory bulb cells demonstrate a preserved structure. Myelination is provided by oligodendrocytes, astrocytic glia are normal.

Microscopy of the retina showed the preservation of the structure of the cellular layers: ganglion, inner and outer layers, outer nuclear layer and pigment epithelial cells. The morphology of the cells and vascular structures is normal, with clearly visible cell nuclei (Fig. 5.C, black arrow). and healthy endothelial cells (Fig. 5.C, red arrow).

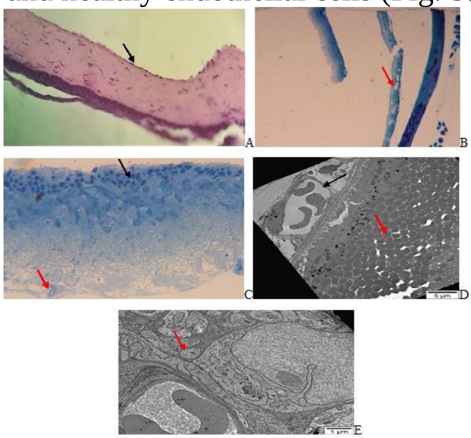


Figure 5. Retina sections of intact animals obtained using light (A) and electron microscopy (B, C, D, E).

B, C — staining with methylene blue, magnification x100.

D — staining with uranyl acetate and pure lead citrate, scale bar — x 5 µm.

E — staining with uranyl acetate and pure lead citrate, scale bar — x 1 µm.

In ultrathin sections of the retina, normal blood circulation in the capillary vessels is confirmed by the presence of erythrocytes at their mouth (Fig. 5.D, black arrow). There are no degenerative processes in the retinal cells, mitochondria have normal volume and activity, and the structures of the endoplasmic reticulum and lysosomes are preserved.

In animals of the first group, the morphological structure of the retina is completely preserved. All cellular layers — including the outer nuclear layer with photoreceptors, ganglion layer and pigment epithelium — have clear boundaries and normal structure. The absence of vacuoles and degenerative signs, the preservation of mitochondria, microfibrils, microtubules and glycogen in the cytoplasm confirm stable metabolism and normal functional activity. The vascular endothelium and blood flow in the capillaries correspond to the physiological norm (Fig. 5. E, red arrow).

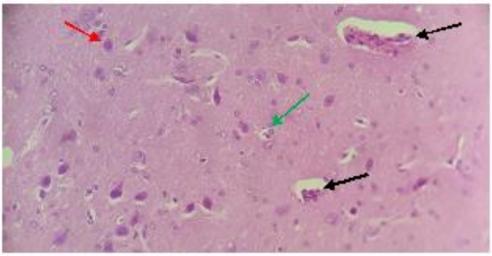


Figure 6. Neuronal and glial cells 7 days after OBX. Staining. Hematoxilin-eosin: magnification X200.

In the second group, 7 days after OBX, pronounced morphological changes were recorded. Macroscopically, the brain tissue acquired a pale grayish tint, with areas of necrosis and hemorrhage. Edema caused an increase in the volume of the brain and an expansion of the damage zone. Microscopically, hyperchromia of neurons was noted (Fig. 6, red arrow), due to the destruction of nuclei, proliferation of glial elements. Inflammatory changes with an increase in the number of plasma cells, vasodilation and signs of hemorrhage were detected in the necrosis zone (Fig. 6, black arrow). Proliferating oligodendrioglyocytes are noted, restoring the myelin layer and protecting the vessels (Fig. 6, green arrow).

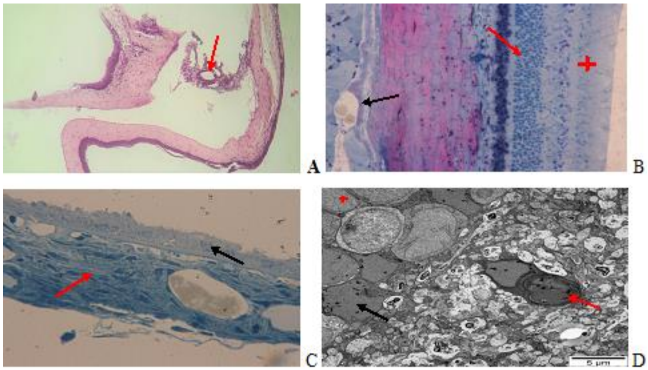


Figure 7. Retinal sections of animals 7 days after OBX under light (A) and electron microscopy (B,C,D).

A - Hematoxilin-eosin staining: 40x magnification.

B,C - Methylene blue staining. Magnification x100.

D - Uranyl acetate and pure lead citrate staining. Scale x5µm.

In the retina of rats after OBX, microscopy revealed edematous areas, hemorrhages and congested capillaries (Fig. 8.A, red arrow). The basal layer and collagen fibers are edematous, neutrophilic leukocytes and endothelial cells with enlarged nuclei are present (Fig. 7.B, black arrow). The amount of glycogen in the cytoplasm is reduced, the staining is weak, the stroma is edematous (Fig. 7.C, red arrow) and there are signs of karyopyknosis in the nuclei of endothelial cells (Fig. 7.C, black arrow). On the 7th day, the retinal ganglion cells are edematous, the cytoplasm is pale (Fig. 7.B, red star), dystrophic changes are revealed: vacuolar degeneration, damage to the endothelial cells of the capillaries, degeneration of the cytoplasm of neurons, swelling of the mitochondria with fuzzy cristae (Fig. 7.D, red asterisk). Lysosomes are vacuolated, signs of karyolysis are noted, microfibrils and microtubules are absent, myelin bodies are present (Fig. 7.D, red arrow).

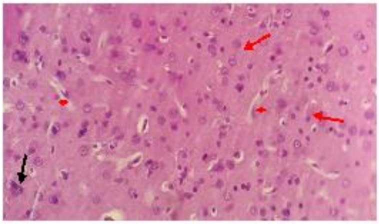


Figure 8. Microscopic image of glial cells and capillaries in the brain tissue of rats treated with turmeric extract (21 days after bulbar injury). Stain: hematoxylin and eosin. Magnification x200.

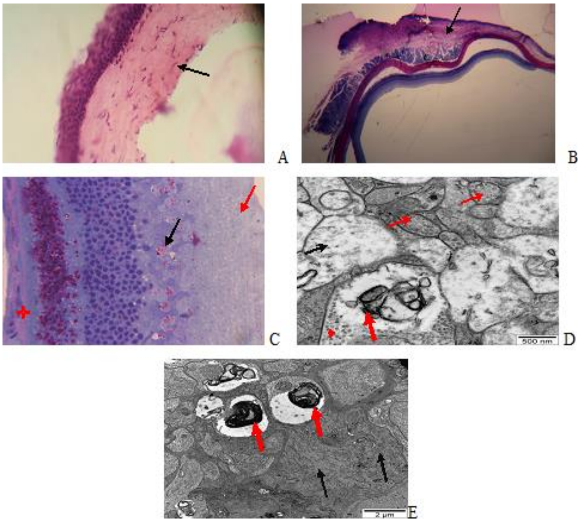


Figure 9. Microscopic image of the retinal epithelium of rats treated with turmeric (21 days) after BO.

A — under a light microscope, dye — hematoxylin and eosin, magnification x100.

B, C — under an electron microscope, dye — methylene blue, magnification x100.

D — under an electron microscope, dye — uranyl acetate and pure lead citrate, scale bar x500 μm.

E — under an electron microscope, dye — uranyl acetate and pure lead citrate, scale bar x2 μm.

On the 21st day after injury, rats treated with turmeric showed an increase in the number of neurons and glial cells in the brain. An infiltrate of inflammatory cells and plasma cells formed around the cysts, the size of the cysts remained small. Some neurons increased compensatory (Fig. 8, black arrow), new astrocytes accumulated in the surrounding tissue (Fig. 8, red arrow). New capillaries were also detected (Fig. 8, asterisk).

Microscopy showed capillaries filled with blood, a fibrous structure of collagen fibers in the basal layer (Fig. 9A, black arrow). At 21 days post-injury, turmeric-treated rats showed edema between cell layers in the retina (Fig. 9B, black arrow), the cytoplasm of the inner layer cells was pale, the membrane was indistinct, and the nuclei were weakly stained (Fig. 9C, red arrow). The capillaries were filled with blood (Fig. 9C, red asterisk).

In ultrathin section preparations from rats, the following changes are observed: capillary endothelial cells undergo vacuolization (Fig. 9.D, black arrow), neuronal cytoplasm undergoes degeneration, glycogen is not detected in the cytoplasm (Fig. 9.D, red arrow). Mitochondria are swollen, with smoothed ridges, the number of lysosomes is increased. Ganglion cells are edematous, with pale cytoplasm, myelin bodies in the cells of the inner retinal layer (Fig. 9.D, thick red arrow). Microfibrils and microtubules (Fig. 9.E black arrow), myelin bodies (Fig. 9.E red arrow) are detected in the cytoplasm.

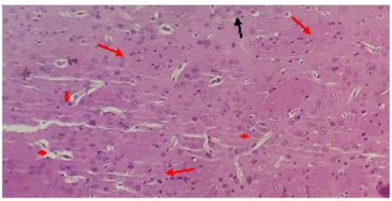


Figure 10. Microscopic image of brain tissue from rats treated with turmeric (90 days) after BO. Hematoxilin-eosin dye. Magnification x100.

Rats treated with turmeric (90 days) showed neuronal hypertrophy and glial cell proliferation (Fig. 10, red arrow). The number of plasma cells, astrocytes, oligodendrocytes (Fig. 10, black arrow), and newly formed vessels (Fig. 10, asterisk) was increased.

Microscopically, mild retinal edema was detected (Fig. 11.A, black arrow), and glycogen supplements accumulated in the cytoplasm. The epithelial layer of the retina was thickened, with lymphocytic infiltration, the cells were pink, with centrally located nuclei (Fig. 11.A, asterisk).

In semi-thin sections of the retina of rats treated with turmeric for 90 days, the capillary walls are thin, endothelial cells with normal nuclei proliferate and surround the retina. The nuclear layer of the retina is clearly visible (Fig. 11, B red arrow).

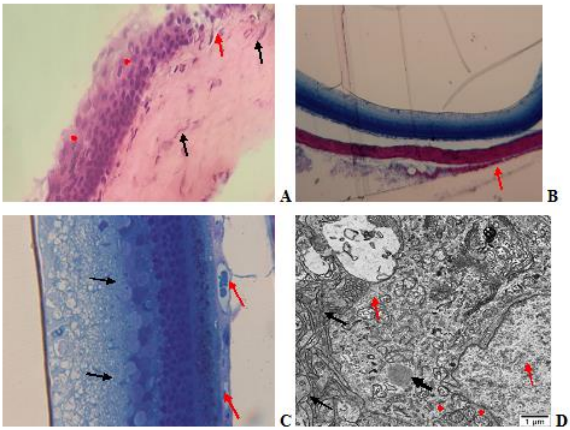


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Figure 11. Microscopic image of the retinal epithelium of rats treated with turmeric (90 days) after BO.

A-Hematoxilin-eosin dye. Magnification x200.

B,C- Dye: Methylene blue. Magnification x200.

D- Dye: uranyl acetate and pure lead citrate. Scale x1µm.

In rats treated with turmeric extract for 90 days, microscopic examinations showed normalization of intracellular structures. Retinal capillaries retained physiological sizes, and the reticular layer thickened due to cellular proliferation (Fig. 11.C, black arrow). Capillary endothelial cells remained morphologically intact (Fig. 11.D, black arrow). Evidence of restoration of ganglion cells and cytoskeletal structure was also observed (Fig. 11.D, thin black arrow).

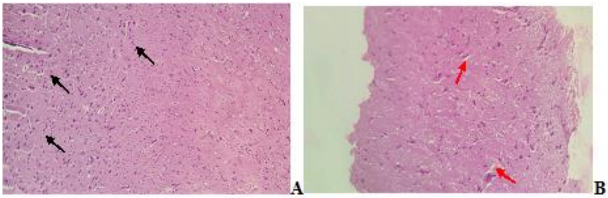

Figure 12. Microscopic image of bulbar tissue in rats treated with turmeric for 180 days after BO. Stain: hemotoxylin and eosin. Magnification x 100.

Microscopic examination of the ocular tissues of rats treated with turmeric extract for 180 days showed significant improvements. Hypertrophy of neurons and proliferation of glial cells were observed (Figs. 12.A and 12.B, arrows).

Microscopic examination of the retinal epithelial layer showed that its structure remained ordered, the cells were uniformly distributed, and the proliferation processes occurred within physiological limits (Fig. 13.A, marked with arrows).

Intracellular fibers maintained a stable organization, which confirms the integrity of the cytoskeleton. The endothelial cells of the retinal capillaries were normal in size, showed no signs of hyperplasia or destruction (Fig. 13 B, black arrow), and were evenly distributed around the vessels to form organized capillary networks (Fig. 13 B, red arrow). The retinal layers had clear boundaries, and the nuclear layer contained normally functioning cells (Fig. 13 C). Ultrathin sections showed preservation of typical endothelial cell morphology (Fig. 13 D, red arrow), indicating normal blood supply and the absence of ischemia.

The number of organelles in the cell cytoplasm is proportional. The nucleus is located in the center. The number of mitochondria (Fig. 13 E, red arrow) and lysosomes is increased (Fig. 13 E, black arrow), mitochondrial ridges have a normal height (Fig. 13 E, red arrow). The cytoplasm of the cells lining the inner layer of the retina is dense, with microfibrils and microtubules visible (Fig. 13 E, red asterisk).

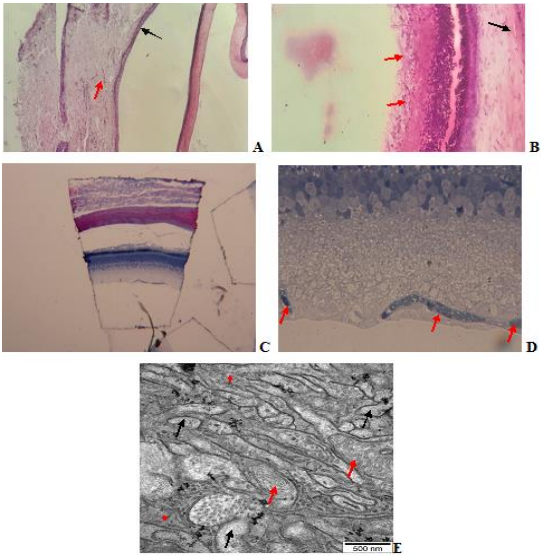

Figure 13. Retinal epithelium image in turmeric-treated rats 180 days after OBX.

A- Hematoxylin and eosin stain, magnification X 100

B- Hematoxylin and eosin stain, magnification X 200

C,D- Methylene blue stain, magnification x100.

E- Uranyl acetate and pure lead citrate stain. Scale bar x 500µm.

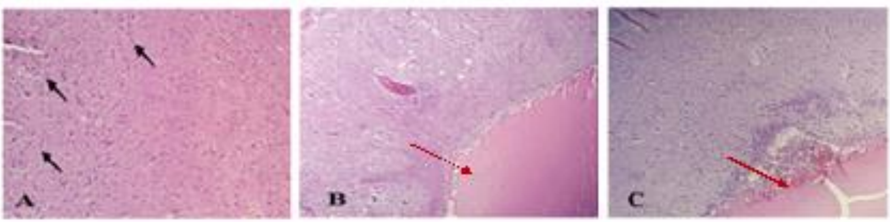

Figure 14.Microscopic image of bulbar tissue in rats treated (A) and not treated (B) with turmeric for 180 days after BO. Stain: hemotoxylin and eosin. Magnification x 100.

In the present study, a comparative histological analysis was performed between the groups of rats that underwent BO and did not receive turmeric and animals that were administered turmeric for 180 days. The obtained data indicate a marked difference in morphological changes in brain tissue between the groups.

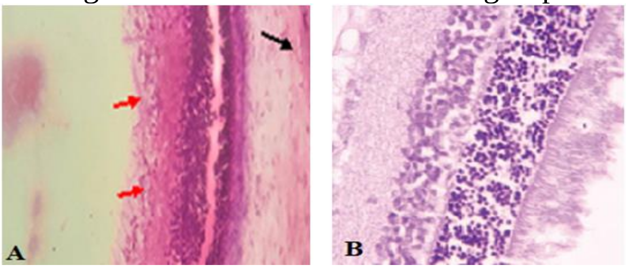

Figure 15. Retinas of rats treated (A) and not treated (B) with turmeric, 180 days after OBX.

A- Hematoxylin-eosin dye, magnification X 200, B- X 400

180 days after mechanical brain injury, animals not treated with turmeric showed persistent signs of chronic neurodegeneration. In the area of injury, pronounced reactive gliosis was formed (Fig. 14 B, C, red arrow) with proliferation of astrocytes and microglia, leading to the formation of scar tissue that disrupts neuronal conduction. Morphologically, the retina showed rarefaction of the inner cell layers, vacuolization of the cytoplasm (Fig. 15 B, red arrow).

The study compared parameters such as ganglion cell count, retinal thickness and myelin formation in different groups (intact, experimental and those treated with turmeric).

The average number of ganglion cells in group I was 1801±12 cells/mm², in group II — 1694±140 cells/mm², in group III — 1774±29 cells/mm², in group IV — 1785±21 cells/mm² and in group V — 1793±10 cells/mm². In II group, which was damaged, the number of cells decreased, which is due to degeneration. However, in group V, by the end of the study, the number of cells increased.

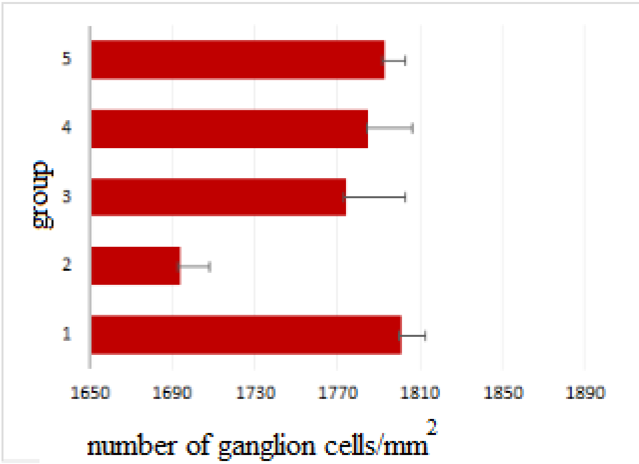

Diagram 5. Characteristics of average values of the number of ganglion cells by groups ($p<0.05$)

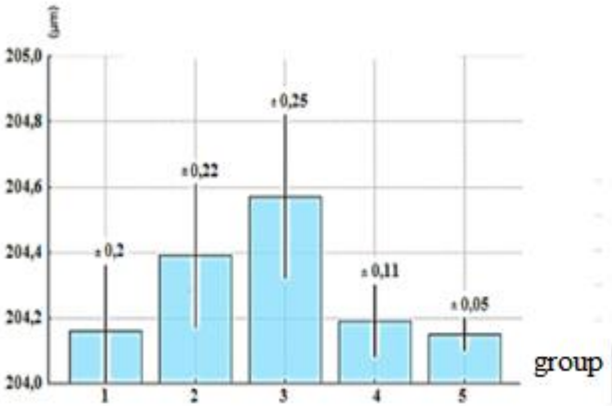

Diagram 6. Comparative characteristics of average retinal thickness values by groups ($p<0.05$)

The retinal thickness in group I was 204.16±0.20 µm. In other groups, the values were as follows: in group II — 204.39±0.22 µm, in group III — 204.57±0.25 µm, in group IV — 204.19±0.11 µm, and in group V — 204.15±0.05 µm. The greatest increase in retinal thickness was observed in group III, indicating its enhanced recovery. In group V, the increase was the smallest, but still exceeded group II, indicating the positive effect of turmeric.

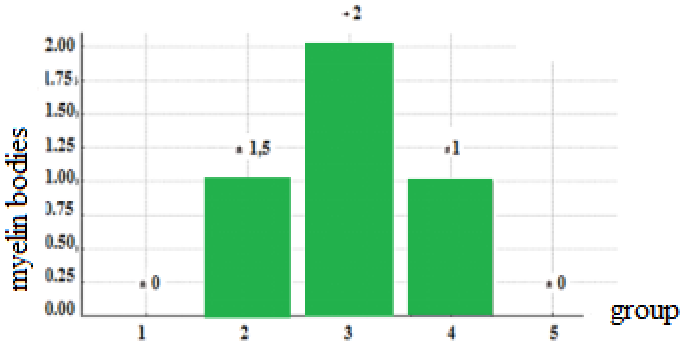

Diagram 7. Average rates of myelin body formation by groups ($p<0.05$)

Myelin body formation was most pronounced in group III, where the greatest activity in the formation of these structures was observed. In groups I and V, myelin bodies were not formed, in group II there were 1±1.5, in group III - 2±1, in group IV - 1±1, respectively. Myelin bodies are associated with cellular damage. In group V, their absence indicates a more pronounced cellular restoration.

In group III, the process of myelin body formation was more pronounced compared to other groups. This may indicate more serious cellular damage requiring the formation of myelin structures as a protective mechanism. In other groups, especially in group V, myelin bodies were not formed, which may indicate successful cellular restoration and the absence of pronounced damage.

DISCUSSION

Spatial memory is the ability to navigate in space and remember the location of objects, such as in the Morris test [18,19,20,21] Our

[18] Mekhtiev, A.A. Impact of dihydropyrimidinase-related protein 2 in memory formation on rats and its possible role in neuronal back remodeling/ A.A.

experiments showed an improvement in spatial memory in animals after taking turmeric extract. These results support the findings that turmeric restores cognitive functions in rat animal models[22].

Curcuminoids, including curcumin, help block beta-amyloid plaque formation and nerve tissue inflammation by improving Aβ clearance in AD patients [23]. Curcumin may also increase levels of neurotransmitters such as dopamine and serotonin, which may improve cognitive function in older adults with AD.[24,25].

Our data are comparable with the results of previous studies, in which curcumin administration to rats prevented spatial memory deficits. It was shown that long-term administration of curcumin helps reduce age-related memory impairment. An improvement in

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Mekhtiev, S.M. Asadova // IBRO Neuroscience Reports, – 2024. – № 16, – p. 155-161.

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cognitive functions was observed, which is associated with the activation of antioxidant and neuroprotective mechanisms. It should be noted that curcumin administration did not affect the motor activity of animals and did not cause signs of anxiety[26]. Rats treated with curcumin showed an improvement in spatial learning and memory[27]. These data are consistent with the results of our study. Additionally, data on the protective effect of turmeric on cognitive functions in a model of neurodegenerative pathology were obtained.

The results of our experiments confirm that turmeric improves memory and behavior in rats, as evidenced by a decrease in the time to search for the platform. The time to search for the platform decreased by 1.1 times in animals with OBX, and control rats showed a significant difference ($p < 0.001$). Turmeric improved memory, reducing the time to search for the platform and increasing the swimming speed. These results confirm that turmeric has neuroprotective properties that help improve cognitive functions and slow the progression of neurodegeneration.

In addition to cognitive impairment, AD patients often have changes in the retina, including loss of ganglion cells and changes in nerve fibers[28,29] The retina shares molecular and cellular features

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with the brain, making it suitable for studying neurodegeneration in AD.

The results of our experiments showed a decrease in the number of microglial cells. This confirms the high efficiency of turmeric extract in protecting and restoring retinal cells.

The results of histological studies show the ability of turmeric to restore damaged neurons. Reduction of inflammation and restoration of mitochondrial activity confirm the neuroprotective effect of turmeric. Data on the behavior of animals, such as improved motor activity, emphasize that turmeric extract promotes cellular regeneration and improves the functional state of the body.

Comparative analysis between groups revealed significant changes at the microscopic and ultrastructural levels. In the groups receiving turmeric extract, proliferation of glial cells was observed, indicating tissue restoration. Edema was also noted, which is a response of cells to damage, and the formation of myelin bodies, indicating an attempt by cells to restore their structures. These changes confirm the positive effect of turmeric extract on cell restoration, which is visible at the end of the study - improved cellular functions and restoration of cellular structures, confirming the neuroprotective properties of turmeric. Thus, the combination of morphological and functional data allows us to conclude that turmeric is highly effective in protecting and restoring retinal cells, as well as in improving the condition of the nervous system as a whole. These results open prospects for further research into turmeric as a therapeutic agent for restoring neurons in neurodegenerative diseases.

CONCLUSIONS

1. Animals with the OB model showed statistically significant differences in skill reproduction, time spent in the target quadrant with and without the platform, and average swimming speed in the target sector.

2. The positive effect of turmeric on the spatial behavior and memory of animals was recorded from the third day of training, reaching the maximum effect on the seventh day of testing. The effect was manifested in a decrease in the time the animals spent near the walls of the tank by 60.46% ($p < 0.05$), an increase in the proportion of time spent in the target sector (by 42.2%), and an increase in swimming speed, which was 1.6 times higher than the initial values ($p < 0.05$).

3. Animals that underwent bulbectomy and received turmeric demonstrated better results in the MWM test, which was manifested in better memorization and retention of the location of the hidden platform in memory. This is also confirmed by the swimming speed indicators in animals of this group.

4. Animals that had underwent OBX showed destructive changes in the retina and brain.

5. Histological data showed that turmeric has a neuroprotective effect, helping to mitigate histological changes in the brain and retina of animals with OBX.

PRACTICAL RECOMMENDATIONS

The obtained experimental data can be used in preclinical studies to evaluate the neuroprotective efficacy of natural compounds, in particular turmeric extract, in neurodegenerative disorders. The established morphological changes in the retina during neurodegeneration allow us to consider it as an additional diagnostic marker of the state of the central nervous system, especially in the early stages of the pathological process. The developed model of OBX in rats can be recommended for further experimental studies aimed at testing substances with potential neuroprotective activity.

List of scientific works published on the topic of the dissertation

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2. Р.Н. Кулиева. Влияние куркумы на пространственное поведение и память животных Sağlamlıq jurnalı, 2019, 24(5), s.175–182
3. Р.Н. Кулиева. Экспрессия антигенов HLA II класса у пациентов с ретинопатией, страдающих болезнью Альцгеймера. Офтальмология. Восточная Европа 2019, №9(3), с..324-332
4. R.N. Guliyeva Effect of curcuma on rat retinal cells: a comprehensive analysis INTERNATIONAL AEGEAN CONFERENCES, Izmir, Turkey, 2023, s.31.
5. R.N. Guliyeva, N.Mustafayeva, A.V. Ibishova. Pathomorphohological characterstics of the effects of curcuma extract suspensions after experimental damage to the amygdala. 6th INTERNATIONAL Antalya Congress, 2023, s.774.
6. R.N. Guliyeva, U. Gashimova, K. Javadova, A. Ibishova, E. Panakhova Histological examination of retinal function and the effects of curcuma longa on memory correction in experimental olfactory bulbectomy rat models Advances in Biology & Earth Sciences, 2024, Vol.9, No.1, s.216–222
7. Р.Н. Кулиева, К.Х. Джавадова, Х.М. Мирюсифова Исследование влияния куркумы на гистологические изменения сетчатки и головного мозга крыс, перенесших обонятельную бульбэктомию Universum: Химия и биология: электрон. научн. журн., 2024, №118
8. Р.Н. Кулиева, К.Х. Джавадова, Х.М. Мирюсифова Электронно-микроскопическая характеристика ультраструктурных изменений сетчатки крыс обонятельной

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11. Р.Н. Кулиева К.Х. Джавадова Влияния куркумы на гистологические изменения сетчатки крыс с ольфакторной бульбэктомией Müasir dövrdə fiziologiya və sağlamlıq beynəlxalq konfransı, 2024, s. 61

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LIST OF ABBREVIATIONS

AD	Alzheimer's disease
WMW	Water Morris waze
NDDs	Neurodegenerative diseases
OBX	Olfactory bulbectomy
CNS	Central nervous system

The defense of the dissertation will be held on 25 June 2025 in 11.00 at the meeting of the Dissertation Council FD1.08 operating at the Institute of Physiology named after Academician Abdulla Garaev of the Ministry of Science and Education of the Republic of Azerbaijan

Address: AZ1100, Baku, Sharifzade street, 78.

The dissertation is available for review at the library of the Institute of Physiology named after Academician Abdulla Garayev under the Ministry of Science and Education of the Republic of Azerbaijan.

The electronic version of the dissertation and the abstract is available on the official website of the Institute of Physiology named after Academician Abdulla Garayev under the Ministry of Science and Education of the Republic of Azerbaijan (http://www.physiology.az).

Abstract was sent to the required addresses
on "23" May 2025

Signed for print: 20.05.2025
Paper format: 60x84 1/18
Volume: 37566
Number of hard copies: 20