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EFFECT OF QUERCETIN AND NERVE GROWTH FACTOR ON BIOCHEMICAL PARAMETERS OF THE BRAIN TISSUE IN RATS WITH EXPERIMENTAL NEURODEGENERATIVE DISORDER

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ABSTRACT: The work was initiated to induce a toxic model of a neurodegenerative disorder with Alzheimer's disease symptoms in rats and to study the effect of quercetin, an anti-oxidant, and nerve growth factor on some biochemical parameters and parameters of behavioral performance of the animals. It was established that upon induction of the neurodegenerative disorder by an intranasal administration of aluminum chloride and lypo-polysaccharide, there was a decline in motor-sensor, emotional/motivation and cognitive parameters of the animals' behavioral performance. An increase in the neutral and some fractions of glycolipids, as well as changes in phospholipid profile, were found; the activity of the antioxidant system enzymes with the MDA increase in the hippocampus of the animals was found to decrease. The deviations above in the biochemical parameters of the animals' hippocampus seem to be a cause for the changes in the animals' behavioral performance. Intranasal administration of the anti-oxidant and NGF in the liposomal form partially restored the parameters of the animals' behavioral performance and the biochemical parameters to the control values. Thus, quercetin and NGF seem to prevent further damage of cells in the hippocampus of animals under study and stimulate the undamaged nerve cells.

INTRODUCTION: Degenerative nerve diseases, also called neurodegenerative diseases, are figuring larger among underlying causes of work decrement and mortality increase. Aggregation of data on the factors underlying the onset of the degenerative nerve diseases is enormous.

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Aggregation of the amyloid-beta protein, hyper-phosphorylation of the tau protein, free radical activation and changes in the sphingomyelin cycle ultimately resulting in the death of the nerve cells are thought to underlie the onset of neurodegenerative diseases [1, 2, 3, 4].

Meanwhile, today, there are few works on the role of lipids in the neuron membranes underlying the onset of neurodegenerative diseases. In the view of the aforesaid, the work was initiated to study lipid composition, lipid peroxidation and antioxidant system enzymes in the hippocampus of rats with the experimentally induced neurodegenerative

disorder, as well as the effect of quercetin, an antioxidant, and nerve growth factor (NGF) on biochemical and behavioral parameters of animals.

MATERIALS AND METHODS: The experiment was conducted on the outbreed rats weighing 250-300 g kept on a standard diet with the addition of cholesterol, making up 2% of the food volume. The experiments with animals were approved by the Bioethical Commission at the Institute of Biophysics and Biochemistry under the Mirzo Ulugbek National University of Uzbekistan to be conducted in accordance with the Animal Testing Regulations. The animals were kept in the properly ventilated, illuminated and heated space with well-timed clean-up by 5 in one cage and with free access to the food and water. Prior to the administration of aluminum chloride, all rats were subjected to the cognitive tests to be chosen for experiment [5]. For the purposes of the experiment, 25 animals were divided into two groups, to name the active group including 5 animals receiving normal saline intranasally, and the exposure group consisting of 20 animals intranasally receiving aluminum chloride in the dose of 50 mg per kg of weight for 7 days; *E. coli* as bacterial lipopolysaccharide (LPS) was added on the 14th day in the dose of 200 mg/kg of weight [6]. On the 3rd, 7th, 14th, 17th, 21st and 25th day after administration of aluminum chloride + LPS the animals were subjected to the behavioral tests, such as the open field test, the elevated cross maze test [7, 8], the passive avoidance test and the active avoidance test [9] to monitor neurodegenerative disorder model. Starting from the 16th day of the experiment, for 7 days 15 animals were intranasally administered with quercetin, an antioxidant isolated from the local raw material, in the dose of 5 mg/kg of weight and NGF-B (Sigma, USA) in the liposomal form in the dose of 60 μg/kg of weight.

Biological materials from the hippocampus for biochemical investigation were taken on the 26th day after aluminum chloride administration. Small single lamellar liposomes were prepared from egg phosphatidylcholine (0.053 M) and cholesterol (0.024 M) with the molecular ratio of 7:3 and the total concentration of lipids 50 mg/ml by ultrasound degeneration of the lipid film at 22 kHz for 10 minutes with primarily dispersed NGF in 0.9% NaCl at low temperatures [10]. The thin-layer chromatography was used for total lipid extraction, and fractionation of lipids was performed [11]. Lipid and total protein quantification were performed as described elsewhere [12]. Basal levels of the substrates reactive with the thiobarbituric acid (MDA) were measured as described elsewhere [13]; the activity of antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase was estimated as described elsewhere, respectively [14, 15]. Cary 60 spectrophotometer (Agilent Technologies, USA) was used to make optical measurements. The data were processed by means of the Student's t-test and the origin 6.1 programs (Origin Lab, Northampton, MA, USA).

RESULTS AND DISCUSSION: Changes in the behavioral performance of rats in the open field test before and after induction of neurodegenerative disorder, as well as after administration of quercetin + NGF, can be seen in **Table 1**.

TABLE 1: BEHAVIORAL PERFORMANCE OF ANIMALS IN THE OPEN FIELD TEST

Behavioral activity	Groups of animals (n = 25)		
	Active control (n=5)	Exposure group (n = 20)	Exposure group administered with quercetin + NGF (n = 15)
Latent period **(s)**	42.5 ± 9.1	84.3 ± 13.6*	63.3 ± 11.1
Distance traveled **(м)**	11.6 ± 3.1	4.2 ± 1.6	7.6 ± 2.5
Line crossings (n)	16.3 ± 1.5	4.5 ± 1.1*	12.2 ± 1.5
Squares transversed (n)	40.3 ± 11.4	9.5 ± 4.2*	29.1 ± 10.4
Groomings (n)	2.3 ± 0.3	1.8 ± 0.2	2.1 ± 0.3
Frequency of head-dipping (n)	15.3 ± 2.3	7.0 ± 2.1*	11.1 ± 2.3

Note: *statistically significant differences, significance level $p<0.05$. Active control group-a group of animals intranasally administered with normal saline, exposure group-a group of animals intranasally administered with aluminum chloride + LPS,

The animals demonstrated the deferred reactions in the open field test; elongation of the latent period, a reduction in the number of line crossings, of the squares transversed, and in the frequency of hear-dipping could be seen. The Mc Graw Stroke Index Scale was used to evaluate the neurological status

of the animals, the total score being 2.5 in the exposure group. Administration of quercetin + NGF to the exposure group animals demonstrated partial restoration of the behavioral performance parameters in the open field test coming close to those in the control animals, but the changes are insignificant. The findings from the study on behavioral performance (motivation and emotional tests) in the elevated cross maze test can be seen in **Table 2**; those from the passive avoidance test and the active avoidance test can be seen in **Fig. 1.**

TABLE 2: BEHAVIORAL PERFORMANCE OF RATS IN THE ELEVATED CROSS MAZE TEST

Groups of animals	Latent period (s)	Time spent in a maze arm (s)		
		Arm A	Arm B (closed)	Arm C
Active control (n=5)	8.4 ± 2.5	156.3 ± 23.2		151 ± 20.1
Exposure group (n=20)	11.2 ± 3.7	139.1 ± 25.6		145.6 ± 25.2
Exposure group administered with quercetin + NGF (n=15)	9.6 ± 2.7	148.2 ± 20.5		154.7 ± 21.1
The test repeated in 24 h				
Active control (n=5)	8.6 ± 2.6	126.2 ± 21.2	90,8 ± 12.3	101.5 ± 17.2
Exposure group (n=20)	6.4 ± 1.8	62.5 ± 15.2	58.5 ± 8.5*	185.2 ± 25.1
Exposure group quercetin + NGF (n=15)	8.0 ± 2.1	88.3 ± 17.3	95.3 ± 13.5**	91.2 ± 15.2

Note: *the differences are significant between active control and exposure groups, significance level at $p<0.05$, **- the differences are significant between exposure group and exposure group administered with quercetin + NGF, significance level at $p<0.05$.

As compared to the exposure group animals, in the repeated test, those administered with normal saline demonstrated a significant reduction in the time spent in the maze arm previously closed, to be the evidence for memory disturbance and disorientation in the former. As compared to the exposure group animals, in rats receiving quercetin + NGF intranasally, a significant elongation of time for exploration of new arms could be seen, to be the evidence for a reduction of the effect the neurodegenerative disorder produces on the animals' memory **Table 2**. The findings from the passive avoidance test and the active avoidance test in the control and exposure groups can be seen in **Fig. 1.**

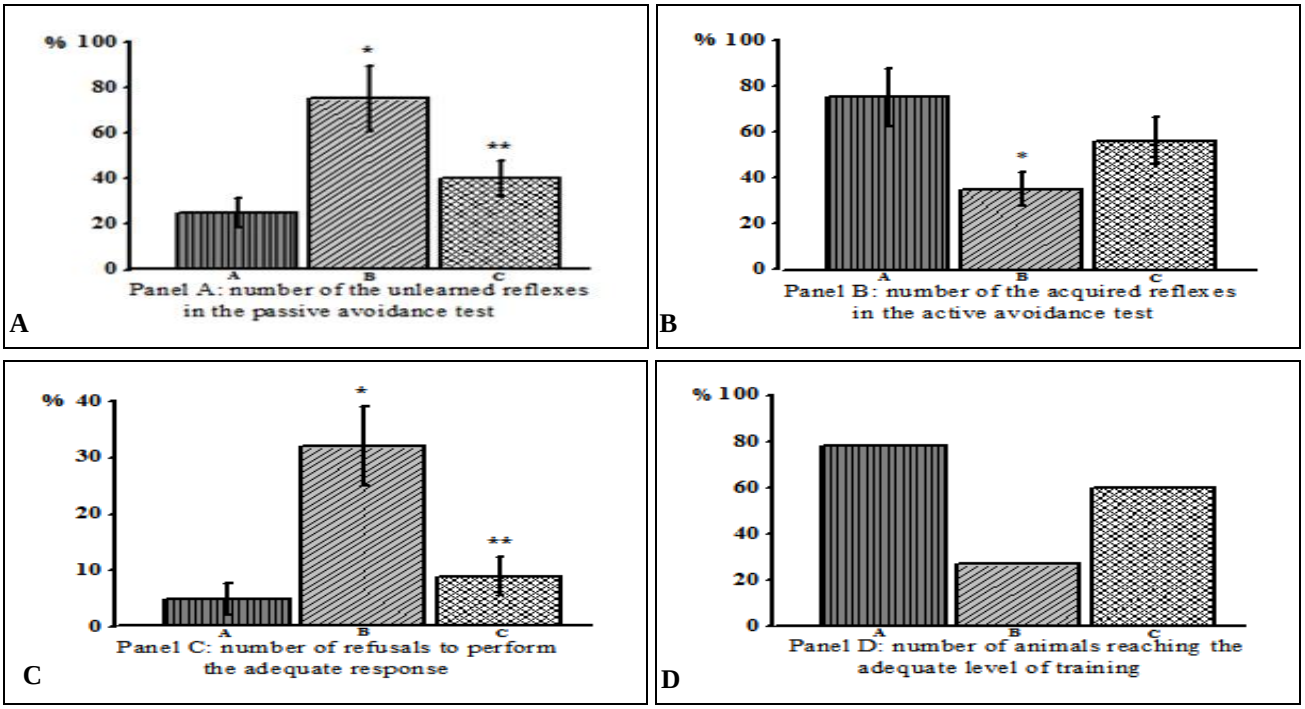

FIG. 1: RESULTS OF THE PASSIVE AND ACTIVE AVOIDANCE TESTS IN THE A: ACTIVE CONTROL GROUP, B: EXPOSURE GROUP AND C: EXPOSURE GROUP ADMINISTERED WITH QUERCETIN + NGF. Note: *the differences are significant between active control and exposure groups, significance level at $p<0.05$, **- the differences are significant between exposure group and exposure group administered with quercetin + NGF, significance level at $p<0.05$.

In the rats receiving normal saline intranasally for the whole period of experiment for passive avoidance, after 8 repeated placements in the undimmed part of the chamber with subsequent pain stimulation in the dimmed part of the chamber the animals entered the dimmed part of the chamber in 20% only; in 80% of cases the memory of the pain stimulation was preserved. In the exposure group, the rats entered the dimmed part of the chamber in 73.3% of cases, to be the evidence for preservation of the acquired reflex to the pain stimulation in 27% of animals only. 7-day intranasal administration of quercetin + NGF resulted in preservation of the acquired reflex to the pain stimulation in 60% of cases; in 40% of cases the rats entered the dimmed part of the chamber.

In the active avoidance test, 80% of the active control group animals reached the adequate level of training after 8 repeats by the end of the experiments, while the number of refusals to perform the adequate response could be seen in 5%. The training by the active avoidance of the exposure group animals, that is, those receiving aluminum chloride + LPS, was slower, and after 8 repeats by the end of the experiment only 27% of animals reached the adequate level of training with the refusals to perform the adequate response increased. In rats receiving quercetin + NGF the adequate level of training was reached in 60%. Nerve growth factor (NGF) is known as the main neutrophin providing support and functioning of cholinergic neurons in the brain of the adult mammalians.

The intact cells of the rat hippocampus, possibly, assume the functions of the disturbed ones to cause some restoration of the behavioral performance parameters under study. Lipid composition of the hippocampus in rats after experimentally induced neurodegenerative disorder and administration of quercetin + NGF can be seen in **Table 3**.

TABLE 3: LIPID COMPOSITION OF HIPPOCAMPUS OF RATS AFTER EXPERIMENTALLY INDUCED NEURODEGENERATIVE DISORDER AND ADMINISTRATION OF QUERCETIN + NGF

Phospholipids and lipids	Control group (n=5)	Exposure group (n=5)	Exposure group quercetin+NGF (n=5)
LP, μg of P/g of tissue	10.3 ± 0.8	14.1 ± 0.2*	11.8 ± 0.7
SPH, μg of P/g of tissue	125.2 ± 4.5	108.1 ± 4.1*	115.2 ± 5.3
PC, μg of P/g of tissue	559.8 ± 10.6	532.0 ± 9.5	550.8 ± 11.5
PS, μg of P/g of tissue	187.5 ± 9.5	147.4 ± 6.1*	167.5 ± 9.5
PI, μg of P/g of tissue	88.7 ± 4.8	83.5 ± 3.5	86.7 ± 4.8
PE, μg of P/g of tissue	491.4 ± 21.4	488.3 ± 19.1	493.4 ± 21.4
Cardiolipin, μg of P/g of tissue	59.6 ± 2.8	63.1 ± 2.1	61.1 ± 2.8
PA, μg of P/g of tissue	14.9 ± 0.8	18.2 ± 1.2*	15.9 ± 0.8
Total phospholipids, μg of P/g of tissue	1537.4 ± 31.3	1454.7 ± 25.3*	1501.9 ± 28.3
Cerebrosides, mg/g of tissue	7.8 ± 0.4	6.6 ± 0.5	7.6 ± 0.4
Sulfatides, mg/g of tissue	2.7 ± 0.2	2.5 ± 0.3	2.8 ± 0.2
Total glycolipids, mg/g of tissue	10.5 ± 0.7	9.1 ± 0.8	10.4 ± 0.9
TC, mg/g of tissue	19.0 ± 0.81	22.1 ± 0.85	20.3 ± 0.75

Abbreviations: LP-lysophosphatidylcholine, SPH-sphingomyelin, PC-phosphatidylcholine, PS-phosphatidylserine, PI-phosphatidylinositol, PE-phosphatidylethanolamine, PA-phosphatidic acid, TC-total cholesterol Note: * significant differences between active control and exposure groups, significance level $p<0.05$

As it can be seen in **Table 3**, induction of the neurodegenerative disorder model caused increase in concentrations of lysophospholipids and phosphatidic acid (PA), while concentrations of sphingomyelin (SPH) fractions, phosphatidylserine (PS) and total phospholipids appeared to decrease. Changes in PC, PE, cardiolipin and total cholesterol were less significant.

The changes in lysophospholipids and PA can be attributed to activation of phospholipases taking place upon induction of the neurodegenerative disorder model, apparently having an effect on the concentrations of total phospholipids and their fractions in the hippocampus.

Specific mention should be made of some (insignificant) increase in cholesterol (12.2%) and a reduction in glycosphingolipids upon induction of the neurodegenerative disorder. The feedback mechanism between Aβ metabolism and homeostasis of cholesterol, as well as biophysical properties of membranes and condition of cholesterol-dependent receptors is known to exist[16,17].

All this indicates the significance of the lipids in maintaining certain microviscosity of the receptor and synaptic parts of the membrane. Changes in proportions of these components we observed seem to produce an effect on reception and neutrality resulting in abnormalities in the behavioral activity of the animals with the model of neurodegenerative disorder.

Taking into account the aforesaid, as well as the fact that quercetin and its metabolites are not only anti-oxidants, but also the agents producing effects on neurogenesis [18] and NGF stimulates the growth of receptor organs, we have managed to produce an effect on lipid composition of the brain tissues of animals with neurodegenerative disorder by the 7-day administration of quercetin, an anti-oxidant, and NGF in liposomal form. Our findings demonstrate that the administration caused some restoration of lipid ratios in the tissues under study; the reduction in total cholesterol, LP, and PA could be seen as compared to the parameters in tissues of the exposure group animals **Table 3**.

The findings from a study on the parameters of lipid peroxidation, such as malondialdehyde (MDA), and activity of the enzymes, such as catalase, superoxide dismutase (SOD) and glutathione peroxidase (GP) in the hippocampus of rats after induction of neurodegenerative disorder model and administration of quercetin + NGF to the exposure group animals can be seen in **Table 4**.

TABLE 4: MDA AND ANTI-OXIDANT SYSTEM ENZYMES IN THE HIPPOCAMPUS OF RATS WITH NEURODEGENERATIVE DISORDER MODEL AND AFTER ADMINISTRATION OF QUERCETIN AND NGF

Groups of animals	MDA µmol/mg of protein	Catalase (U/mg of protein)	SOD(U/ mg of protein)	GP nmol/min /mg of protein
Active control group (n=5)	2.60 ± 0.17	59.13 ± 2.11	65.42 ± 3.26	39.5 ± 1.13
Exposure group (n=5)	4.56 ± 0.42*	38.53 ± 3.21*	43.83 ± 2.54*	42.9 ± 2.51
Exposure group administered with quercetin + NGF(n=15)	3.05 ± 0.25**	45.49 ± 3.31	51.23 ± 3.01	40.3 ± 2.23

Note: * the differences are significant between the active control group and the exposure group, significance level $p<0.05$; ** the differences are significant between the exposure group and exposure group administered with quercetin + NGF

As it can be seen, upon induction of the neuro-degenerative disorder model levels of products reactive with thiobarbituric acid (MDA) increased by 60%, while the activity of catalase, SOD and GP reduced by 18%, 17.8% and 22.5%, respectively. Administration of quercetin + NGF to the animals with neurodegenerative disorder model was found to cause partial reduction of lipid peroxidation products and restoration of anti-oxidant system enzymes, probably producing an effect on the lipid composition and microplasticity of membranes from the brain tissue membranes under study.

Thus, increase in the LPO products and reduction in the anti-oxidant system enzymes, as well as activation of phospholipases upon induction of experimental neurodegenerative disorder is thought to result in changes observed in the lipid composition of membranes producing and effect on plasticity of the receptor and synaptic parts of the membrane and neutrality.

Changes in the parameters taking place upon induction of experimental neurodegenerative disorder seem to be a cause for changes in the behavioral performance of the animals under study.

CONCLUSION: Thus, the increase in lipid per oxidation products and the reduction in the activity of antioxidant system enzymes upon induction of the neurodegenerative disorder model were found to produce an effect on the plasticity of receptor and synaptic parts of membranes and neutrality.

The changes in the parameters upon induction of neurodegenerative disorder model are thought to be a cause for changes in the behavioral performance of the animals observed in our experiments. Administration of quercetin + NGF to the animals caused partial restoration of lipid composition and activity of antioxidant system enzymes, as well as the reduction in the activity of lipid peroxidation in the tissues of rat hippocampus, probably, being a cause of changes in parameters of animals' behavioral performance.

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REFERENCES:

1. Jack CR, Wiste HJ and Knopman DS: Rates of b-amyloid accumulation are independent of hippocampal neuro-degeneration. Neurology 2014; 82: 1605-12.
2. Overk CR and Masliah E: Pathogenesis of synaptic degeneration in Alzheimer's disease and lewy body disease. Biochem Pharmacol 2014; 88: 508-16.
3. Pchelina SN: Alpha-synuclein as a biomarker of Parkinson's disease. Annals of Clinical and Experimental Neurology 2011; 4: 46-51.
4. Alessenko AV: Potential role of sphingolipids in neuro-pathogenesis of Alzheimer's disease. Biomedical Chemistry 2013; 1: 25-50.
5. Amikishieva AV: Behavioral phenotyping: current methods and equipment. Vavilov Journal of Genetics and Breeding 2009; 3: 529-542.
6. Moyseenok AG, Omel'yanchik SN, Shevalier AA and Evkovich IN: Method for modeling of neurodegenerative disorder of central nervous system in rats. Patent BY 11160 C1 2008; 10: 30.
7. Malinovskaya NA: Role of NAD + - dependent mechanisms in regulation of neuro-glial interactions in the cerebral ischemia and neurodegeneration. Author's Abstract of the Doc Sci Med Dissertation Kemerovo 2014.
8. Tarasova AYU: Neophilia and anxiety in manifestation of cognitive abilities (as exemplified by the laboratory mice). Author's Abstract of the C and of Sci Biology Dissertation Moscow 2018; 79.
9. Dubrovina NI and Loskutova LV: Dopaminergic mechanisms of memory and attention. Novosibirsk, Nauka 2003.
10. Conti E, Gregori, M, Radice I, Da-Re F, Grana D, Re F, Salvati E, Masserini M, Ferrarese C, Zoia CP and Tremolizzo L: Multifunctional liposomes interact with abeta in human biological fluids: Therapeutic Implications for Alzheimer's Disease Neurochemistry International 2017; doi: 10.1016/j.neuint.2017.02.012.
11. Handloser D, Widmer V and Reich E: Separation of phospholipids by HPTLC an investigation of important parameters. Journal of Liquid Chromatography and Related Technologies 2008; 31: 1857-70.
12. Isakina MV: Role of lipids in the processes of conduction and regeneration of the compromised somatic nerves. Abstract of Doctor Sci Biology Dissertation Saransk 2016; 55.
13. Methodical regulations in study on processes of free-radical oxidation and antioxidant support system. Assay for malondialdehyde in blood. Voronezh 2010; 37-39.
14. Belikova MV, Pastukhova CA, Luk'antseva GV, Krasnova SP and Lutsenko AV: Effect of regular normobaric hypoxia on dopamine in the striatum upon ageing. Galitskyi Medical Bulletin 2015; 3(1): 28-30.
15. Tiwari V, Kuhad A, Bishnoi M and Chopra K: Chronic treatment with tocotrienol, an isoform of vitamin E, prevents intracerebroventricular streptozotocin-induced cognitive impairment and oxidative nitrosative stress in rats. Pharmacology Biochemistry and Behavior 2009; 93: 183-89.
16. Koudinov AP: Role of lipids in the neuroplasticity and neurodegeneration. Abstract of Doctor Sci Biology Dissertation Moscow 2007.
17. Alessenko AV: New research on Alzheimer's disease. Welsh EM Edition 2006.
18. Baralab S, Pariyarab R, Kimb J, Lee H and Seo J: Quercetin-3-O-glucuronide promotes the proliferation and migration of neural stem cells. Neurobiology of Aging 2017; 52: 39-52.

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