Study Effects of *Actaea racemosa* on Neurotoxicity in White Male Rats Exposed to Hyperhomocysteinemia

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Abstract

Introduction: Homocysteine (Hcy) plays a critical function in the metabolism of methionine. Individuals who are affected by genetic variants of hyperhomocysteinemia face early vascular damage and cognitive impairment. Hyperhomocysteinemia is a complex disorder, emerging from a mix of dietary, behavioral, and pathogenic aspects. **Aim**: evaluted the possible role of Actaea Racemosa as neuroprotective against varied degrative marketers. **Method**: Thirty male albino rats were separated into 5 groups, 6 rats each, Sham animal which not got anything. Vehicle which got 1% starch gel solution, Control which administration of D-L-thioacetaldehyde homocysteine hydrochloride, Treatment animals which administered Actaea racemosa , and finaly Combination group: The animals were exposed to the same regimen as the control group, in addition to being injected 100 mg/kg of Actaea racemosa extract intragastrical once daily for eight weeks. **Results**: Compared to the control group, our data revealed that Actaea racemosa significantly ($p<0.05$) elevated the levels of acetylcholine esterase (AchE, U/ml), dopamine (pg/ml), glutamate (mM/ml), SOD (ng/ml), and CAT (U/mg) in both the AR and combination groups. Simultaneously, Actaea racemosa therapy led to a marked reduction in neurofilament light (NfL, pg/ml), TNF-α (ng/ml), IL-1β (ng/ml), MDA (ng/ml), Hz (μMOL/L), β-amyloid (pg/ml), and t-tau (pg/ml). **Conclusion**: Neuroprotective effects of Actaea Racemosa in mitigating cerebral injury through the upregulation of neuro-hormones and antioxidant proteins, alongside the downregulation of pro-inflammatory markers and neurodegenerative proteins.

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Keywords: Homocysteine, Actaea Racemosa, dopamine, anti-oxidant, inflammation.

1. INTRODUCTION

Homocysteine (Hcy) plays a vital role in the metabolism of methionine. Individuals who are affected by hereditary forms of hyperhomocysteinemia experience early vascular damage and cognitive impairment. Hyperhomocysteinemia is a multifactorial condition, arising from a combination of nutritional, behavioral, and pathogenic variables (1). In recent years, there has been a growing body of evidence linking hyperhomocysteinemia to a range of diseases, particularly neurodegenerative disorders. The potential mechanisms by which Hcy contributes to neurodegeneration include modifications to protein structure and function, oxidative stress, disruptions in cellular metabolism, epigenetic changes, deposition of pathological aggregates, endothelial damage, and atherothrombosis (2).

There are various reasons why cerebral tissues with hyperhomocysteinemia are important. In addition to being connected to Alzheimer's disease and vascular dementia, hyperhomocysteinemia has been identified as a risk factor for stroke and carotid artery disease (atherosclerosis). The effects of hyperhomocysteinemia on cerebral vascular biology and the underlying molecular pathways have only recently been elucidated by experimental studies (3).

Inflammation affects the brain's neurotransmitter systems. These systems include serotonin, dopamine and glutamate pathways, as well as the kynurenine pathway. The kynurenine pathway produces a chemical called quinolinic acid, which can damage the brain. Studies using imaging techniques have shown that changes to neurotransmitter pathways are linked to inflammation. Inflammation can cause changes in the brain that affect motivation, movement, anxiety, arousal and alarm (4). Acetylcholinesterase is a well-known protein because it plays a key role in breaking down acetylcholine, which is important for nerve transmission. As well as this, it has other functions that are not related to catalysis (5). One of these is linked to nerve injury, and it could have a big impact on how diseases progress and how aggressive they are. It is also involved in tumors, where a lower amount of AChE would mean more cells survive because the apoptosome doesn't form as well (6).

1. MATERIAL AND METHODS

1.1. ANIMALS

Thirty male albino rats (Rattus norvegicus), with a weight range of 220–280 g, were procured from the animal house at the Faculty of Science, Karbala University. The rats were housed in well-ventilated conditions under a 12-hour light/dark cycle at a controlled temperature of 25 ± 2 °C. The animals were provided with a standard diet for laboratory rodents and had unlimited access to water (7). The experiment was approved by the Al-Kufa University Animal Care and Research Committee and was conducted in accordance with the Laboratory Animal Care Guide.

1.2. ANIMAL GROUPS

The animals were randomly distributed into the following groups:

1. **Sham Group**: The animals were without getting anything.
2. **Vehicle Group**: The animals received an intragastric administration of a 1% starch gel solution (1 ml/100 g rat weight) once daily for a period of eight weeks.
3. **Control group**: The induction of hyperhomocysteinemia was achieved through the administration of D-L-thioacetaldehyde homocysteine hydrochloride (Acros Organics, Italy) at a dose of 200 mg/kg body weight to the experimental group.
4. **Treatment group**: The animals were administered 100 mg/kg of Actaea racemosa extract intragastrical once daily for eight weeks.
5. **Combination group**: The animals were subjected to the same regimen as the control group, in addition to being administered 100 mg/kg of Actaea racemosa extract intragastrical once daily for eight weeks.

At the completion of the experiment, the animals were euthanized by decapitation under general anesthesia, after which their brains were isolated for analyses (8, 9).

1.3. DETERMINATION OF STUDY MARKERS

The rats were anaesthetized and blood samples were collected directly from the left ventricle prior to sacrifice. ELISA kits (Westang Biotech Co., Ltd, Shanghai, China) were utilized to measure the serum levels, with the procedure conducted in accordance with the manufacturer's instructions. Triple tests were performed for each sample.

1.4. Ethical approval

Ethical approval was obtained from the Animal Care and Use Committee of Kufa University (Kufa, Iraq, approval number: KUF24355) for all experiments performed.

1.5. STATISTICAL ANALYSIS

Statistical analysis was conducted utilizing the GraphPad Prism 9.0 software. A two-way ANOVA with Bonferroni correction was employed to assess the statistical significance of differences between groups.

For the evaluation of serum levels, a one-way ANOVA with Bonferroni correction was utilized. An unpaired t-test was applied for the purpose of comparisons between two groups. A p-value of less than 0.05 was deemed to be statistically significant.

2. RESULTS

2.1. EFFECT OF ACTAEA RACEMOSA TREATMENT ON NEUROHORMONES

In this study, we employed an ELISA analysis of midbrain tissues to evaluate the impact of Actaea racemosa treatment on the progression of hyperhomocysteinemia. Actaea racemosa treatment resulted in elevated neurotransmissions hormone levels (table 1 and Figure 1). Consequently, control group showed significant difference ($p<0.05$) as compared with sham and vehicle groups while there was insignificant difference ($p>0.05$) between AR when compared with both sham and vehicle groups respectively.

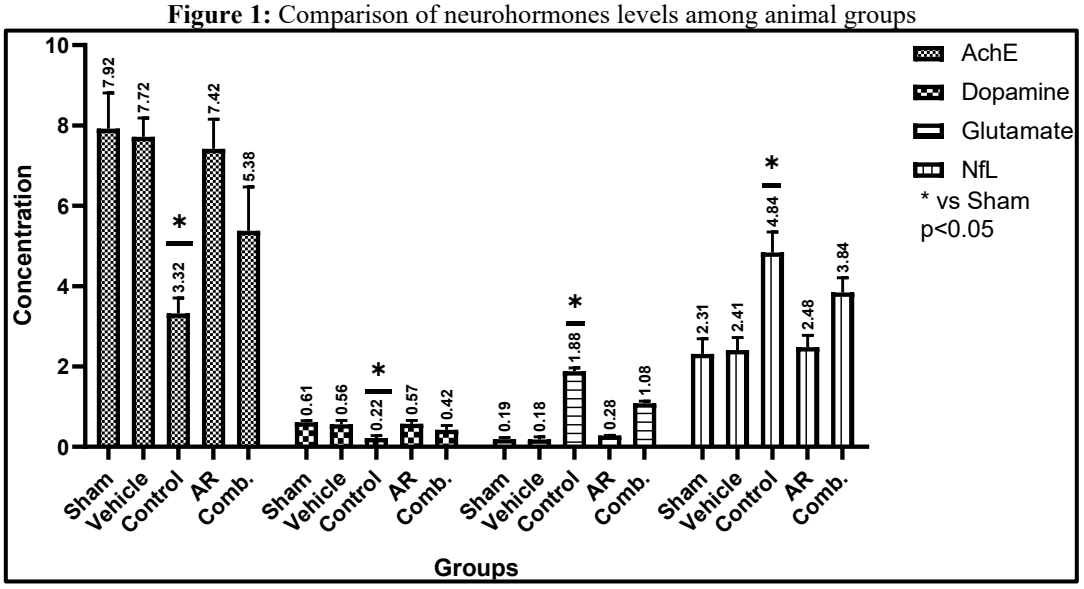
However, our results showed a substantial increase significantly ($p< 0.05$) in acetylcholine esterase (AchE U/ml), dopamine (pg/ml), and glutamate (mM/ml) was observed in both AR and combination groups as compared with control. However, Actaea racemosa treatment concomitantly reduced neurofilament light (NfL pg/ml).

Table 1: Comparison of neurohormones levels among animal groups

Groups / Parameters	AchE U/ml	Dopamine (pg/ml)	Glutamate (mM/ml)	NfL pg/mL
Sham	7.92±0.63	0.61±0.03	0.19±0.03	2.31±0.27
Vehicle	7.72±0.33	0.56±0.07	0.18±0.05	2.41±0.22
Control	3.32±0.27*	0.22±0.04*	1.88±0.06*	4.84±0.36*
AR	7.42± 0.52	0.57±0.06	0.28±0.002	2.48±0.21
Comb.	5.38±0.77*	0.42±0.08*	1.08±0.04*	3.84±0.26*

AchE: acetylcholine esterase, NfL: Neurofilament light, *AR: Actaea Racemosa* * significant

Figure 1: Comparison of neurohormones levels among animal groups



2.2. EFFECT OF ACTAEA RACEMOSA TREATMENT ON NEUROINFLAMMATOR AND OXIDATIVE STRESS

ELISA analysis of midbrain tissues was conducted to evaluate the impact of Actaea racemosa treatment on the progression of hyperhomocysteinemia. The results demonstrated that Actaea racemosa treatment resulted in reduced neuro-inflammatory and oxidative stress marker levels (see Table 2 and Figure 2). Consequently, a significant difference ($p<0.05$) was observed between the control group and the sham and vehicle groups, while no significant difference ($p>0.05$) was observed between AR and the sham and vehicle groups, respectively.

However, the present study demonstrated a significantly elevated level of TNF-α (ng/ml), IL-1β (ng/ml), and MDA (ng/ml) in control groups as compared with sham groups. While our results showed that there was significant lowered ($p < 0.05$) in the levels of SOD (ng/ml) and CAT (U/mg) in the sham, vehicle, and AR groups compared with the control group. The combination groups demonstrated significant differences compared with the control group.

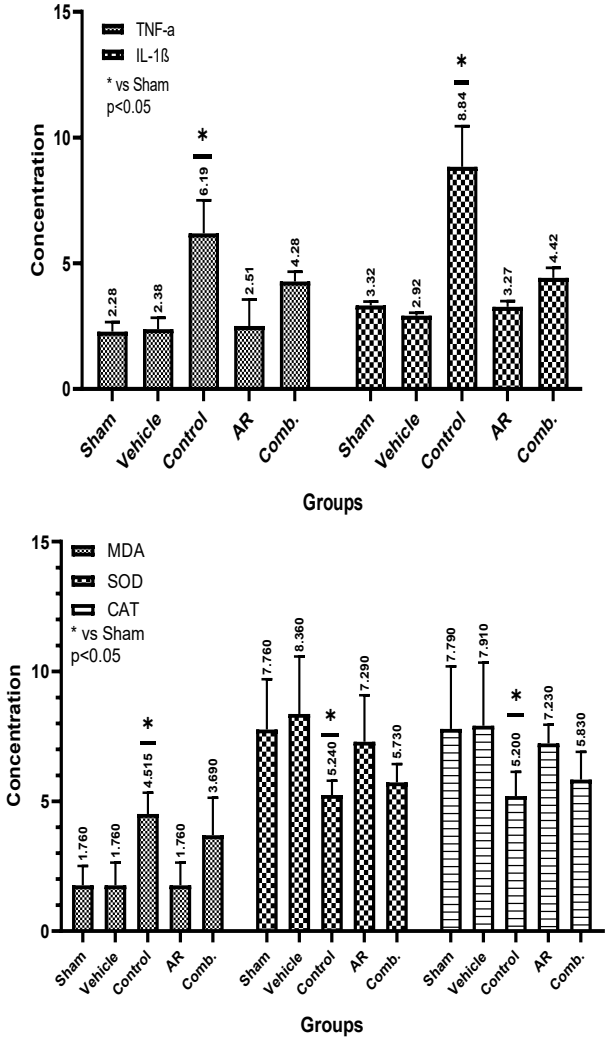
Table 2: Comparison of Neuroinflammatory and oxidative stress levels among animal groups

Groups / Parameters	TNF-α (ng/ml)	IL-1β (ng/ml)	MDA (ng/ml)	SOD (ng/ml)	CAT (U/mg)
Sham	2.28±0.27	3.32±0.11	1.76±0.03	7.76±0.87	7.79±0.20
Vehicle	2.38±0.32	2.92±0.08	1.76±0.08	8.36±1.07	7.91±0.22

Control	6.19±0.93*	5.68±0.14*	4.52±0.05	5.24±1.39	5.20±1.66*
AR	2.51±0.74	8.84±0.16	1.76±0.05	7.29±0.27	7.23±0.51
Comb.	4.28±0.27*	4.42±0.28*	3.69 ±0.02	5.73±0.88	5.83±1.26*

TNF-α: tumor necrosis factor; IL-1β: interleukin 1-beta; MDA: Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase. AR: Actaea Racemosa. * Significant

Figure 2: Comparison of Neuroinflammatory and oxidative stress levels among animal groups



2.3. EFFECT OF ACTAEA RACEMOSA TREATMENT ON NEURODEGERATIVE MARKERS

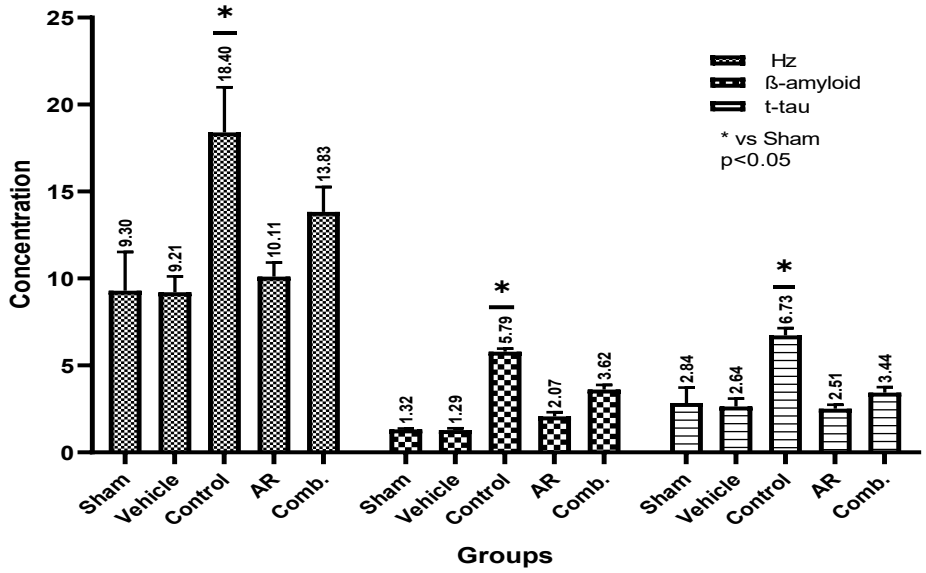
The results showed that Actaea racemosa treatment resulted in reduced levels of neurodegenerative markers (see Table 2 and Figure 2). ELISA analysis of brain tissue was performed to evaluate the effect of Actaea racemosa treatment on the progression of hyperhomocysteinemia . Our data showed that a significant difference ($p<0.05$) was observed between the control group and the sham and vehicle groups, while no significant difference ($p>0.05$) was observed between AR and the sham and vehicle groups. The present study showed a significantly increased level of Hz (µMOL/L), β-amyloid (pg/ml), and t-tau (pg/ml) in the control groups compared to the sham groups. The combination groups showed significant differences compared to the control group.

Table 3: Comparison of Neurovegetative makers levels among animal groups

Groups / Parameters	Hz (µMOL/L)	β-amyloid (pg/ml)	t-tau (pg/mL)
Sham	9.30± 1.57	1.32±0.04	2.84±0.63
Vehicle	8.56± 1.29	1.29±0.07	2.64±0.32
Control	18.4±1.83*	5.792±0.13*	6.73±0.29
AR	10.11± 0.57	2.02±0.12	2.51±0.16
Comb.	13.83 ±1.01*	3.62±0.18*	3.44±0.22

Hz: Homocysteine; AR: Actaea Racemosa. * Significant

Figure 3: Comparison of Neurovegetative makers levels among animal groups



DISCUSSION

Hyperhomocysteinemia, which is defined as an elevated level of homocysteine, has been shown to cause significant changes to blood vessel walls. These changes include oxidative stress, proinflammatory responses (e.g., increased tumour necrosis factor-α and iNOS) and endothelial dysfunction. The oxidative stress in question stems from reduced antioxidant enzyme activity and increased superoxide anion production, a precursor to reactive oxygen and nitrogen species (10, 11). Results of this study suggest that Actaea racemosa can control not just oxidative stress but also the inflammatory response and brain damage. This results in the achievement of prevention of a-synuclein accumulation, and loss of dopaminergic neuron.

The findings indicate that the administration of AR not only mitigates the detrimental effects of hyperhomocysteinemia but also plays a crucial role in maintaining neuronal health. This protective mechanism may involve the stabilization of neurotransmitter levels, thereby preserving normal synaptic function. Further research is warranted to explore the underlying pathways through which AR exerts its neuroprotective effects and to evaluate its potential therapeutic applications in neurodegenerative disorders linked to hyperhomocysteinemia.

AchE is a protein of significant scientific study due to its role in the hydrolysis of acetylcholine during nerve transmission (12). A decrease in the activity or level of AchE results in an increase in the acetylcholine level in the brain, which in turn causes changes in neuronal excitability, influences synaptic transmission, induces synaptic plasticity, and coordinates the firing of groups of neurons. Consequently, it elicits alterations in neuronal networks throughout the brain, modifies their response to both internal and external stimuli, and potentiates behaviors that are adaptive to environmental stimuli while concomitantly diminishing responses to ongoing stimuli (13).

Research has demonstrated that inflammation lowers dopamine levels in the brain by means of synaptic availability and monoamine release reduction. This may result in a reduced supply of monoaminergic neurotransmitters to cortical areas, a fundamental process that contributes to the pathophysiology of neural injury (4). Glutamate plays a pivotal role in the maintenance of optimal energy levels, which are essential for the functioning of the central nervous system (CNS). Furthermore, glutamate is crucial for neuroplasticity, a process that facilitates adaptation to environmental changes. This discussion will explore the significance of glutamate in everyday functioning and the methods by which to maintain healthy levels to enhance resilience during periods of stress (14).

The administration of AR resulted in decreased MDA levels and increased concentrations of SOD CAT, TNF-α, and IL-1β indicating its potential antioxidative and anti-inflammatory effects. These findings suggest that AR may play a protective role against oxidative stress and inflammation induced by hyperhomocysteinemia that contribute to oxidative stress and increased inflammatory mediators by disrupting the balance of antioxidant enzymes and promoting lipid peroxidation in addition to inflammatory markers. Further investigation is warranted to explore the underlying mechanisms and potential therapeutic interventions to mitigate these effects.

In an in vivo examination, it has been previously proven that the Actaea racemosa promotes the Nrf2/HO-1 pathway (15). Nrf2, a transcription factor that activates genes with cytoprotective properties, is implicated in antioxidant and anti-inflammatory reactions (11, 16). Several phase II detoxification enzymes, including NQO1, HO-1, and many others, are encoded by these cytoprotective genes, as well as Excessive oxidative stress increases activation of NF-kB, demonstrating a connection between these two pathways (17). NF-kB is a transcription factor that modulates the inflammatory response by

activating numerous genes that code for pro-inflammatory cytokines and immunoregulatory mediators (18, 19)

The administration of AR resulted in significantly lower concentrations of Hz, β-amyloid, and t-tau, demonstrating its potential to ameliorate brain damage. These data imply that AR may give a protective effect against degeneration caused by hyperhomocysteinemia, which contributes to oxidative stress and increased inflammatory mediators.

Homocysteine induces apoptosis in rat hippocampus neurons. Following exposure to homocysteine, DNA strand breaks and the associated activation of poly-ADP-ribose polymerase (PARP) together with NAD depletion happen fast, occurring before mitochondrial failure, oxidative stress, and caspase activation (20). Amyloid-beta is a complex biological molecule that interacts with various receptors and forms insoluble assemblies. Over time, its abnormal deposits disrupt normal neuronal conditions. Amyloid-beta impairs synaptic activity, promotes neuritis, initiates neurodegeneration, and alters synaptic proteins in many neuronal diseases (21). Data suggests that neuronal death mediated by tau can happen even without the creation of tangles. Consequently, an increasing number of research are focusing on how anomalies in tau, such as atypical phosphorylation, glycosylation, or truncation, contribute to toxicity. While studies from experimental models of tauopathies strongly imply that pathologically altered tau and tau aggregates play a role in neurodegeneration, the particular neurotoxic species remain unclear, as do the methods via which they promote neuronal death (22).

CONCLUSION

The neuroprotective action of Actaea Racemosa against cerebral injury is achieved by the up-regulation of neurohormones and antioxidant proteins, with the down-regulation of pro-inflammatory markers and neurodegenerative proteins. This dual process not only boosts the brain's resilience to oxidative stress but also mitigates inflammation-driven neuronal damage, giving a promising treatment approach for neurodegenerative illnesses and brain traumas.

REFERENCES

1. Price BR, Wilcock DM, Weekman EM. Hyperhomocysteinemia as a risk factor for vascular contributions to cognitive impairment and dementia. Frontiers in Aging Neuroscience. 2018;10:350.
2. Bonetti F, Brombo G, Zuliani G. The relationship between hyperhomocysteinemia and neurodegeneration. Neurodegenerative disease management. 2016;6(2):133-45.
3. Faraci FM, Lentz SR. Hyperhomocysteinemia, Oxidative Stress, and Cerebral Vascular Dysfunction. Stroke. 2004;35(2):345-7.
4. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nature Reviews Immunology. 2016;16(1):22-34.
5. Dasgupta P, Rastogi S, Pillai S, Ordonez-Ercan D, Morris M, Haura E, Chellappan S. Nicotine induces cell proliferation by β-arrestin–mediated activation of Src and Rb–Raf-1 pathways. The Journal of clinical investigation. 2006;116(8):2208-17.
6. Richbart SD, Merritt JC, Nolan NA, Dasgupta P. Acetylcholinesterase and human cancers. Advances in Cancer Research. 2021;152:1-66.
7. Lee YT, Laxton V, Lin HY, Chan YWF, Fitzgerald-Smith S, To TLO, et al. Animal models of atherosclerosis. Biomedical Reports. 2017;6(3):259-66.
8. Hu Y, Dietrich H, Metzler B, Wick G, Xu Q. Hyperexpression and Activation of Extracellular Signal–Regulated Kinases (ERK1/2) in Atherosclerotic Lesions of Cholesterol-Fed Rabbits. Arteriosclerosis, thrombosis, and vascular biology. 2000;20(1):18-26.
9. Adekunl A, Adelusi T, Fatoki J, Oyedokun B. A Diet-induced Atherosclerosis in Rabbit Model Provides an Insight into Essential Elements Concentrations in Cardiovascular Disease. 2013.
10. Dos Santos TM, Júnior OVR, Alves VS, Coutinho-Silva R, Savio LEB, Wyse AT. Hyperhomocysteinemia alters cytokine gene expression, cytochrome c oxidase activity and oxidative stress in striatum and cerebellum of rodents. Life Sciences. 2021;277:119386.
11. Hassan SM, Mohammed MH, Jawad MJ, Abbas AN. Use of Infliximab to Attenuate Cerebral Apoptosis Induced by Cerebral Ischemia/reperfusion in Male Rats. Indexed in Pubmed/medline, Scopus, Embase, Ebsco, Index Copernicus, Polish Ministry of Education and Science, Polish Medical Bibliography. 2023;76(2):326-31.
12. Picciotto MR, Higley MJ, Mineur YS. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron. 2012;76(1):116-29.
13. Pérez-Aguilar B, Marquardt JU, Muñoz-Delgado E, López-Durán RM, Gutiérrez-Ruiz MC, Gomez-Quiroz LE, Gómez-Olivares JL. Changes in the acetylcholinesterase enzymatic activity in tumor development and progression. Cancers. 2023;15(18):4629.
14. Zhou Y, Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. Journal of neural transmission. 2014;121:799-817.
15. Peritore AF, Crupi R, Scuto M, Gugliandolo E, Siracusa R, Impellizzeri D, et al. The role of annexin A1 and formyl peptide receptor 2/3 signaling in chronic corticosterone-induced depression-like behaviors and impairment in hippocampal-dependent memory. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders). 2020;19(1):27-43.

16. Lastres-Becker I, García-Yagüe AJ, Scannevin RH, Casarejos MJ, Kügler S, Rábano A, Cuadrado A. Repurposing the NRF2 activator dimethyl fumarate as therapy against synucleinopathy in Parkinson's disease. Antioxidants & redox signaling. 2016;25(2):61-77.
17. Lingappan K. NF-κB in oxidative stress. Current opinion in toxicology. 2018;7:81-6.
18. Barnabei L, Laplantine E, Mbongo W, Rieux-Laucat F, Weil R. NF-κB: at the borders of autoimmunity and inflammation. Frontiers in Immunology. 2021;12:716469.
19. Saheb HA, Abbas FG, Alhassani ZK, Ali SMH, Abbas SN, Hassan SM, Merzah ZS. Etanercept's Protective Effect Against Lung Inflammation Caused by Polymicrobial Sepsis Induced by CLP in Male Rats. Azerbaijan Pharmaceutical and Pharmacotherapy Journal. 2023;22:11-4.
20. Kruman, II, Culmsee C, Chan SL, Kruman Y, Guo Z, Penix L, Mattson MP. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. J Neurosci. 2000;20(18):6920-6.
21. Sadigh-Eteghad S, Sabermarouf B, Majdi A, Talebi M, Farhoudi M, Mahmoudi J. Amyloid-beta: a crucial factor in Alzheimer's disease. Med Princ Pract. 2015;24(1):1-10.
22. Gendron TF, Petrucelli L. The role of tau in neurodegeneration. Mol Neurodegener. 2009;4:13.