

Protective effects of Levosimendan in Scopolamine-induced mice model of Alzheimer's disease

By Nawres Lateef Alwaeli

Protective effects of Levosimendan in Scopolamine-induced mice model of Alzheimer's disease

Nawres Lateef Alwaeli ^a, Adeb Ahmed Al-Zubaidy ^b, Mariam Hadi Sadiq ^c

- ⁹ (a) Department of Pharmacology/College of Medicine/Al-Nahrain University, Baghdad, Iraq.
Email: nawresphd@gmail.com
- ⁴ (b) University of Warith Al-Anbiyaa/Collage of Medicine / Department of Pharmacology, Karbala, Iraq.
Email: adeebalzubaidy@uowa.edu.iq
- ⁹ (c) Department of Pharmacology/College of Medicine/Al-Nahrain University, Baghdad, Iraq.
Email: ph.marium@gmail.com

*Corresponding author:

Nawres Lateef Alwaeli
nawresphd@gmail.com

Abstract

Objectives Alzheimer's disease is the most prevalent neurodegenerative disease and accounts for approximately 70% of all dementia cases worldwide. Levosimendan is a positive inotropic agent with well-documented pleiotropic properties, including anti-inflammatory and antioxidant effects. The current research evaluated the possible effect of levosimendan alone or in combination with donepezil against a scopolamine-induced mice model of Alzheimer's disease.

Material and Methods: Mice were divided into 5 groups: the control group, the induction group (scopolamine 1 mg/kg /i.p once daily for 7 days), other groups that received the tested drugs prophylactically for 2 weeks and then induced with scopolamine together with the same doses of the tested drug for one week. These treatment groups included the levosimendan group (200 µg/kg i.p. once weekly for 3 weeks), the donepezil group (5 mg/kg i.p. once daily for 3 weeks), and the fifth group received a combination of levosimendan (200 µg/kg i.p. once weekly) and donepezil (5 mg/kg i.p. once daily). Cognitive performance was assessed by conducting behavioral tests (a novel objective recognition test and Y Maze test), and the levels of biological markers of oxidative stress (SOD and MDA), inflammatory cytokines (TNFα, IL1β and IL-6) and AChE in the brain tissue homogenate were measured utilizing available ELISA kits.

Results: levosimendan preserved spatial memory and recognition function in mice, significantly reduced AChE level, and significantly improved oxidative stress markers and inflammatory markers.

Conclusions: the current study showed that levosimendan produced a neuroprotective effect against the scopolamine-induced mice model of AD. This effect may be in part attributed to the antioxidant and anti-inflammatory properties of levosimendan.

Keywords: Levosimendan, Neuroinflammation, oxidative stress, Alzheimer's disease.

Introduction

Alzheimer's disease (AD) currently ranks as the most prevalent neurodegenerative disease, accounting for approximately 70% of all dementia cases worldwide [1]. AD is characterized by a gradual irreversible decline in cognitive functions, functional activities, and psychiatric symptoms that are severe enough to interfere with the everyday life of those affected both medically and socially [2].

The primary histopathological characteristics associated with AD are the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles. As the amyloid hypothesis suggests the accumulation of amyloid beta initiates a series of events including mitochondrial damage, inflammatory reactions, oxidative stress, and apoptosis. Collectively, these processes are responsible for the gradual disruption of finely balanced intercellular processes and impairments neuronal functioning [2, 3]. Several drugs have been approved for disease treatment including acetylcholine esterase inhibitors (donepezil, rivastigmine, and galantamine) and memantine; however, none of these drugs can cure the disease or halt its progression [4].

Levosimendan is an inotropic agent approved for the management of acute decompensated heart failure without interfering with the sympathetic nervous system or increasing myocardial oxygen consumption [5]. Significant data have been gathered over the years revealing a wide range of pleiotropic effects of levosimendan, beginning with the potent dilatory effect that ensures perfusion to central organs including the brain [6], kidneys [7], and liver [8].

Furthermore, the opening of ATP-dependent potassium channels in the mitochondria may broaden the spectrum of cellular responses implying a protective mechanism against stressful conditions [9]. Moreover, in recent studies, levosimendan has been shown to exert a neuroprotective effect on cisplatin-induced toxicity [10], produce an anticonvulsant effect [11], and reduce tau pathology [12].

The present study aimed to investigate the possible neuroprotective effect of levosimendan on a mouse model of Alzheimer's disease induced by scopolamine.

Methods

The present study was carried out at the Department of Pharmacology, College of Medicine / Al-Nahrain University and Alrazy Center for Research and Diagnostic Kits, Baghdad –Iraq from January 2022 to November 2022. The authorizing committee of the College of Medicine / Al-Nahrain University assessed the research protocol before granting study permission (committee approval number 219 - 18/1/2022).

Animals

Male albino mice were obtained from the Alrazy Center for Research and Diagnostic Kits. The animals weighed between 25 and 30 grams and were between 2.5 and 3 months old. The mice were placed in sterilized cages, with 10 mice per cage, kept in a standardized environment with controlled temperature and lighting, and then left without intervention for one week for acclimatization.

Experimental design

Mice were randomly divided into 5 groups, of 10 mice each:

Group 1 (negative Control): Mice received intraperitoneal saline solution for 21 days.

Group 2 (Induction): Mice received only 1 mg/kg scopolamine (IP) [13] for seven days to establish the AD model.

The treatment groups received the test drugs prophylactically for two weeks, and then induction with scopolamine started in the third week, together with the administration of the test drugs.

Group 3 (donepezil): Mice received donepezil (5mg/kg, IP) once daily [13].

Group 4 (levosimendan): Mice received 200 μ g/kg levosimendan IP once weekly [14].

Group 5 (levosimendan and donepezil): Mice received donepezil (5 mg/kg, i.p .) once daily and levosimendan (200 μ g/kg, i.p.) once weekly.

Behavioral tests

The tests were conducted on day 22 and over three successive days:

Spontaneous Y maze test

A Y-shaped maze that was custom-designed to have three arms that were oriented at 120 degrees from each other was used for testing. The test concluded by permitting the mice to move spontaneously through the maze and by estimating the spontaneous alterations, which reflect spatial working memory. Mice with unaltered working memory can recall the recently visited arms of the maze and explore new arms. Mice were placed in a specific location in the maze and left to freely move in the maze for 10 minutes. Sequential inputs into the three arms constitute an alternation. The tests were video recorded; arm entries and total arm alternations were counted to calculate the spatial alternation, for which a high percentage implied that the mouse retained memory for the arms it had previously visited, whereas a low percentage indicated defective spatial memory, which imply hippocampal dysfunction [15]. Spontaneous alternation (%) was calculated using the following equation [16]:

$$\text{Alternation \%} = \frac{\text{Alternation No.}}{(\text{Total arms Entries} - 2)} \times 100$$

Novel object recognition test

The basic experimental design required three sessions. In the first (14) (habituation), The mice were placed in a sterilized empty arena for 10 minutes. The second session

(training) was 24 hours later, the mice were returned to the arena and allowed to explore two identical objects (10 minutes). The third (testing) session was carried out 4 hours after the end of the second session; The mice were placed back in the arena but one of the previous objects was substituted with a novel one, after which the mice were allowed to explore (10 minutes). Rodents have an innate attraction for novelty; therefore, a mouse that recognizes a familiar item will spend more time examining the novel object. After each test, the arena and objects were sterilized and cleaned with 70% alcohol [17, 18]. The tests were video recorded and the time spent investigating each object was recorded. The recognition index is calculated using the following formula [16]:

$$\text{Recognition Index} = \frac{T_2}{T_1 + T_2} \times 100$$

T₁ is the time spent exploring the familiar object and T₂ is the time spent exploring the novel object.

Tissue Sample

Collection and Preparation

Diethyl ether was used to induce anesthesia. The animals were sacrificed and their brains were extracted and rinsed thoroughly with phosphate-buffered saline (PBS) to eliminate any residual blood and then weighed. Brain tissue was then homogenized using buffer phosphate saline (tissue weight (gm.) / PBS volume (mL) = 1:9), and a homogenizer. The resulting homogenate was subjected to centrifugation in a cold centrifuge device at 3000 rpm for 20 minutes. The desired supernatant layers were carefully collected for biological analysis [16, 19, 20].

Biological analysis:

Inflammatory markers (IL-1 β , IL-6, and TNF- α), oxidative stress markers (SOD and MDA), and AChE levels were measured utilizing readily available ELISA kits (obtained from a bioassay technology laboratory, China and Sunlong Biotech, China). The analysis was performed following the instructions provided by the manufacturer.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) (version 23) was used to analyze the collected data to determine the differences between groups. Independent t-test and analysis of variance test (ANOVA) were used, a significance level of P \leq 0.05 was considered to indicate a significant difference, and P \leq 0.001 was considered to indicate a highly significant difference.

Results

Y maze: effect on spatial alterations

One week of 1 mg/kg i.p. scopolamine administration resulted in significant impairment in spatial working memory compared to a control group ($p < 0.05$). Donepezil, levosimendan, and a combination of (donepezil + levosimendan) had comparable effects that were significantly higher than those of the induction group ($p < 0.05$), with no significant difference from the control group. (Table 1)

NORT: effect on the recognition index

The recognition index was significantly lower in the scopolamine-treated group than in the control group ($p \leq 0.05$). However, treatment with either donepezil or levosimendan reduced the effect of scopolamine on the recognition index ($p \leq 0.05$), with no significant difference from the control group. On the other hand, the effect of the combination of (levosimendan + donepezil) was not statistically significant compared to that of either the induction or control group. (Table 1).

Table 1. Effect of treatment protocol on behavioral tests

Group	Ymaze	NORT
Control	64.97 ± 11.53*	67.90 ± 9.16*
Induction (scopolamine)	51.75 ± 7.78#	50.86 ± 11.48#
Donepezil	66.29 ± 8.86*	66.23 ± 9.37*
Levosimendan	63.15 ± 6.04*	66.90 ± 9.59*
Levosimendan and donepezil	62.75 ± 15.91*	54.52 ± 16.27

n = 10 mice/group, Data are expressed as the mean ± SD, # Statistically significant ($p \leq 0.05$) compared with a control group, * Statistically significant ($p \leq 0.05$) with induction (scopolamine) group

Choline esterase enzyme assessment

The current research showed that induction with scopolamine caused a highly significant increase in the level of the enzyme choline esterase compared with that in healthy controls ($p \leq 0.001$). Compared with those in the induction group, AChE levels in the groups treated with donepezil, levosimendan, or their combination (levosimendan + donepezil) were highly significantly lower (Table 2).

Table 2. Effect of treatment protocol on AChE levels

Group	AChE level (ng/ml)
Control	1.420 ± 0.10**
Induction (scopolamine)	2.31 ± 0.55##
Donepezil	1.608 ± 0.08**
Levosimendan	1.72 ± 0.14**
Levosimendan and donepezil	1.430 ± 0.11**

n = 10 mice/group, Data are expressed as the mean ± SD, ## Highly statistically significant ($p \leq 0.001$) compared with a control group, **: Highly statistically significant ($p \leq 0.001$) compared with induction (scopolamine) group

Assessment of oxidative stress markers

Compared with the control group, the induction group presented a significant increase in MDA levels and a significant decrease in SOD. Levosimendan, donepezil, and the combination (levosimendan + donepezil) had effects on MDA levels that were significantly lower than those of the induction group, with no significant difference from the control group. Donepezil resulted in a non-statistical significant difference in SOD level in comparison to either the control or induction group. Levosimendan resulted in a comparable level with a control group and the combination (levosimendan + donepezil) resulted in a highly significant increase in SOD level compared to the induction group ($p \leq 0.001$) and significantly higher than the donepezil group ($p \leq 0.05$). (Table 3).

Table 3. Effect of treatment protocol on oxidative stress markers

Group	MDA (ng/ml)	SOD (ng/ml)
Control	1.853± 0.54*	16.25 ± 2.83*
Induction (scopolamine)	2.628±0.249#	11.63 ± 3.14#
Donepezil	1.937± 0.37*	14.45 ± 4.44
Levosimendan	2.0842±0.22*	17.34 ± 3.15*
Levosimendan and donepezil	1.970±0.189*	20.88± 5.27**.#

n = 10mice/group, Data are expressed as the mean ± SD, # Statistically significant ($p \leq 0.05$) compared with control group *: Statistically significant ($p \leq 0.05$), **: Highly statistically significant ($p \leq 0.001$) compared with induction (scopolamine) group, .# statistically significant compared with donepezil group ($p \leq 0.05$).

Assessment of the Inflammatory markers

In comparison to the control group, the induction group resulted in a significant increase in inflammatory markers TNF- α , IL-1 β , and IL-6 ($p \leq 0.05$). Both levosimendan and donepezil significantly attenuated the increase in inflammatory markers (TNF- α , IL-1 β , and IL-6) compared to those in the induction group. However the combination (levosimendan + donepezil) significantly decreased the levels of TNF- α and IL-1 β compared to the induction group, but the level of IL-6 remained high, with no significant difference from that in the induction group (Table 4).

Table 4. Effect of treatment protocol on inflammatory markers levels.

Group	TNF- α (ng/ml)	IL-1 β (ng/ml)	IL-6(ng/ml)
Control	135.509 ± 18.81*	693.562 ± 150.19*	142.95 ± 46.27*
Induction (scopolamine)	202.523 ± 75.50#	1118.541 ± 171.60#	229.81 ± 32.93#
Donepezil	134.291 ± 18.71*	744.480 ± 34.94*	176.28 ± 24.82*
Levosimendan	111.761 ± 41.24*	734.149 ± 152.38*	165.96 ± 29.37*
Levosimendan and donepezil	132.215 ± 46.27*	825.695 ± 223.55*	205.63 ± 69.73#

n = 10mice/group, Data are expressed as the mean ± SD, # Statistically significant ($p \leq 0.05$) compared with control group *: Statistically significant ($p \leq 0.05$) compared with induction (scopolamine) group.

Discussion

Alzheimer's disease is a progressive neurodegenerative disease that affects approximately 50 million individuals worldwide [21]. Despite extensive studies attempting to introduce drugs that target the primary pathogenesis of this disease, the

approved treatment for clinical use provides symptomatic relief only [3]. The underlying pathogenesis of AD is very complex; however, it is well-documented that inflammation and oxidative stress are critical components of disease pathology, and chronic activation of any of these processes can initiate a vicious cycle of disease pathology that leads to neuronal damage [22, 23].

Scopolamine acts as a muscarinic receptor blocker that induces amnesia and impairs learning in both humans and animals [24]. In addition, scopolamine seems to induce amnesia by increasing AChE activity & mimics several other biochemical characteristics of AD, for instance, by inducing amyloid beta deposition, neuroinflammation, and oxidative stress in mouse brains [25, 26]. These properties have led to its use as an animal model for AD [27, 28]. The Y maze and NORT are hippocampus cortex-dependent learning and memory tests that have been widely employed in mouse models to test cognitive function [29]. The present study revealed that the administration of scopolamine significantly reduced spatial alterations and recognition indices compared to those in the control group. This finding confirms previous studies by de Bruin & Pouzet [30] and Ishola et al. [31].

Levosimendan was introduced to medical practice over two decades ago as an inotropic agent for the management of acute decompensated heart failure [32]. In addition to that, levosimendan has been shown to exert several other beneficial effects, including vasodilator effects [33], mainly by opening K_{ATP} channels and, to a lesser extent, by inhibiting the phosphodiesterase III isoenzyme [32]. The vasodilation effect increases blood perfusion to vital organs, including the brain [34]. AD is strongly associated with a gradual decrease in blood flow to the brain, resulting in a significant decrease in cerebral blood flow. In humans, a fall of 20% in blood flow leads to the inability to maintain attention, while a reduction of more than 30% in rats impairs spatial memory [35]. Levosimendan contains a hydrazone functional group in its molecular structure [36]. According to a previous molecular docking study, a synthesized hydrazone-containing compound had a direct significant inhibitory effect on AChE [37].

In the present study, levosimendan induced a highly significant reduction in AChE compared with that in the induction group. In addition, the combination of levosimendan with donepezil further reduced AChE compared with donepezil or levosimendan alone. This effect of levosimendan can be from action on improving other pathways involved in disease pathology, including antioxidant and anti-inflammatory effects [14, 38].

In terms of the impact on oxidative stress markers, the antioxidant effect of levosimendan has been supported by previous studies [39, 40]. Additionally, combining levosimendan with donepezil resulted in a significantly higher level of SOD compared to the induction group, indicating a positive effect of combination therapy. Moreover, levosimendan treatment alone significantly attenuated the lipid peroxidation level (MDA) compared with that in the induction group, which was in accordance with previous studies [41, 42]. Levosimendan can activate K_{ATP} channels located in the mitochondrial membrane [33]; this effect in particular may provide defense against oxidative stress and ischemic

conditions [41, 43]. The opening of mitochondrial K_{ATP} channels allows K^+ influx and membrane depolarization which reduce the effect of calcium overload, prevent mitochondrial dysfunction, and maintain cellular energy metabolism [43, 44].

The effects of in levosimendan the present study on proinflammatory cytokines were consistent with previous studies [45, 46] and confirmed its anti-inflammatory role. These effects could explain, at least in part, the beneficial effects of levosimendan in counteracting the effect of scopolamine induction. Furthermore, the combination of levosimendan with donepezil unexpectedly did not have the synergistic anti-inflammatory effect that was seen in oxidative stress markers and AChE activity, although both drugs have been shown to cause NF- κ B downregulation [38, 47] and suppress cytokines release [45, 48]. NF- κ B is an important factor in the transcription of several proinflammatory cytokines by glial cells [49].

Sareila et al. [38] demonstrated that levosimendan can reduce the activity of the NF- κ B transcription pathway. Additionally, microglia possess mitochondrial K_{ATP} channels that can regulate their activity and the production of proinflammatory cytokines. The opening of these channels by levosimendan may help to decrease microglial excitatory activity and maintain it below a certain threshold [50].

Conclusions

The present investigation showed that levosimendan pretreatment improved the cholinergic system function in the hippocampus possibly by reducing neuroinflammation, decreasing AChE activity, and improving oxidative stress status. Additionally, the combined use of levosimendan and donepezil had a synergistic effect, primarily reducing oxidative stress and AChE activity with a lesser impact on inflammatory markers.

Acknowledgment

The author is grateful to Alnahrain University /College of Medicine for all the facilities used for this study.

Funding

None

4

Conflicts of interest

None

Ethical approval

The institution's approving council at the College of Medicine at Al-Nahrain University evaluated the study and certified the most recent installment in January 2022. The research was performed according to the Declaration of Helsinki guidelines for ethics.

References

1. Hansson O. Biomarkers for neurodegenerative diseases. *Nature medicine*. 2021;27(6):954-63.
2. Long JM, Holtzman DM. Alzheimer disease: an update on pathobiology and treatment strategies. *Cell*. 2019;179(2):312-39.
3. Jevtic S, Sengar AS, Salter MW, McLaurin J. The role of the immune system in Alzheimer disease: Etiology and treatment. *Ageing research reviews*. 2017;40:84-94.
4. Sharma P, Srivastava P, Seth A, Tripathi PN, Banerjee AG, Shrivastava SK. Comprehensive review of mechanisms of pathogenesis involved in Alzheimer's disease and potential therapeutic strategies. *Progress in neurobiology*. 2019;174:53-89.
5. Innes CA, Wagstaff AJ. Levosimendan: a review of its use in the management of acute decompensated heart failure. *Drugs*. 2003;63:2651-71.
6. Kelm RF, Wagenführer J, Bauer H, Schmidtmann I, Engelhard K, Noppens RR. Effects of levosimendan on hemodynamics, local cerebral blood flow, neuronal injury, and neuroinflammation after asphyctic cardiac arrest in rats. *Critical Care Medicine*. 2014;42(6):e410-e9.
7. Lannemyr L, Ricksten SE, Rundqvist B, Andersson B, Bartfay SE, Ljungman C, et al. Differential effects of levosimendan and dobutamine on glomerular filtration rate in patients with heart failure and renal impairment: a randomized double-blind controlled trial. *Journal of the American Heart Association*. 2018;7(16):e008455.
8. Farmakis D, Alvarez J, Gal TB, Brito D, Fedele F, Fonseca C, et al. Levosimendan beyond inotropy and acute heart failure: evidence of pleiotropic effects on the heart and other organs: an expert panel position paper. *International Journal of Cardiology*. 2016;222:303-12.
9. Facundo HT, Fornazari M, Kowaltowski AJ. Tissue protection mediated by mitochondrial K⁺ channels. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. 2006;1762(2):202-12.
10. Kavaz E, Ekinci N, Tekin H, Sayyed M, Aygün B, Perişanoğlu U. Estimation of gamma radiation shielding qualification of newly developed glasses by using WinXCOM and MCNPX code. *Progress in nuclear energy*. 2019;115:12-20.
11. Gooshe M, Tabaeizadeh M, Aleyasin AR, Mojahedi P, Ghasemi K, Yousefi F, et al. Levosimendan exerts anticonvulsant properties against PTZ-induced seizures in mice through activation of nNOS/NO pathway: role for KATP channel. *Life Sciences*. 2017;168:38-46.
12. Lim S, Shin S, Sung Y, Lee HE, Kim KH, Song JY, et al. Levosimendan inhibits disulfide tau oligomerization and ameliorates tau pathology in TauP301L-BiFC mice. *Experimental & Molecular Medicine*. 2023;55(3):612-27.
13. Yadang FSA, Nguezeye Y, Kom CW, Betote PHD, Mamat A, Tchokouaha LRY, et al. Scopolamine-induced memory impairment in mice: neuroprotective effects of *Carissa edulis* (Forssk.) Valh (Apocynaceae) aqueous extract. *International Journal of Alzheimer's Disease*. 2020;2020.
14. Rababa'h AM, Alzoubi KH, Baydoun S, Khabour OF. Levosimendan prevents memory impairment induced by diabetes in rats: role of oxidative stress. *Current Alzheimer Research*. 2019;16(14):1300-8.
15. Prieur EA, Jadavji NM. Assessing spatial working memory using the spontaneous alternation Y-maze test in aged male mice. *Bio-protocol*. 2019;9(3):e3162-e.
16. Hamood HM, Al-Zubaidy AA. Neuroprotective effects of *Vitex agnus castus* extract in rats' model of Alzheimer's disease. *EurAsian Journal of Biosciences*. 2020;14(2).
17. Leger M, Quiedeville A, Bouet V, Haelewyn B, Boulouard M, Schumann-Bard P, et al. Object recognition test in mice. *Nature protocols*. 2013;8(12):2531-7.
18. Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. *JoVE (Journal of Visualized Experiments)*. 2017(126):e55718.
19. Foudah AI, Devi S, Alam A, Salkini MA, Ross SA. Anticholinergic effect of resveratrol with vitamin E on scopolamine-induced Alzheimer's disease in rats: Mechanistic approach to prevent inflammation. *Frontiers in Pharmacology*. 2023;14:1115721.
20. Ghasemi S, Moradzadeh M, Hosseini M, Beheshti F, Sadeghnia HR. Beneficial effects of *Urtica dioica* on scopolamine-induced memory impairment in rats: protection against acetylcholinesterase activity and neuronal oxidative damage. *Drug and chemical toxicology*. 2019;42(2):167-75.

21. Andrade-Guerrero J, Santiago-Balmaseda A, Jeronimo-Aguilar P, Vargas-Rodríguez I, Cadena-Suárez AR, Sánchez-Garibay C, et al. Alzheimer's disease: an updated overview of its genetics. *International journal of molecular sciences*. 2023;24(4):3754.
22. Onyango IG, Jauregui GV, Čarná M, Bennett Jr JP, Stokin GB. Neuroinflammation in Alzheimer's disease. *Biomedicines*. 2021;9(5):524.
23. Ju Y, Tam KY. Pathological mechanisms and therapeutic strategies for Alzheimer's disease. *Neural Regeneration Research*. 2022;17(3):543-9.
24. Rabbani M, Ghannadi A, Malekian N. Evaluation of the effect of *Cyperus rotundus* L. in scopolamine-induced learning deficit in mice. *Advanced biomedical research*. 2014;3(1):217.
25. San Tang K. The cellular and molecular processes associated with scopolamine-induced memory deficit: A model of Alzheimer's biomarkers. *Life sciences*. 2019;233:116695.
26. Kazmi I, Al-Abbasi FA, Afzal M, Nadeem MS, Altayb HN. Sterubin protects against chemically-induced Alzheimer's disease by reducing biomarkers of inflammation-IL-6/IL- β /TNF- α and oxidative stress-SOD/MDA in rats. *Saudi Journal of Biological Sciences*. 2023;30(2):103560.
27. Olayinka J, Eduviere A, Adeoluwa O, Fafure A, Adebajo A, Ozolua R. Quercetin mitigates memory deficits in scopolamine mice model via protection against neuroinflammation and neurodegeneration. *Life Sciences*. 2022;292:120326.
28. Muhammad T, Ali T, Ikram M, Khan A, Alam SI, Kim MO. Melatonin rescue oxidative stress-mediated neuroinflammation/neurodegeneration and memory impairment in scopolamine-induced amnesia mice model. *Journal of Neuroimmune Pharmacology*. 2019;14:278-94.
29. Okada K, Hashimoto K, Kobayashi K. Cholinergic regulation of object recognition memory. *Frontiers in Behavioral Neuroscience*. 2022;16:996089.
30. de Bruin N, Pouzet B. Beneficial effects of galantamine on performance in the object recognition task in Swiss mice: deficits induced by scopolamine and by prolonging the retention interval. *Pharmacology Biochemistry and Behavior*. 2006;85(1):253-60.
31. Ishola IO, Olubodun-Obadun TG, Ojulari MA, Adeyemi OO. Rutin ameliorates scopolamine-induced learning and memory impairments through enhancement of antioxidant defense system and cholinergic signaling. *Drug metabolism and personalized therapy*. 2021;36(1):53-61.
32. Markou T, Makridou Z, Galatou E, Lazou A. Multiple signalling pathways underlie the protective effect of levosimendan in cardiac myocytes. *European journal of pharmacology*. 2011;667(1-3):298-305.
33. Al-Chalabi A, Heunks LM, Papp Z, Pollesello P. Potential of the cardiovascular drug levosimendan in the management of amyotrophic lateral sclerosis: an overview of a working hypothesis. *Journal of Cardiovascular Pharmacology*. 2019;74(5):389-99.
34. Roehl AB, Zoremba N, Kipp M, Schiefer J, Goetzenich A, Bleilevens C, et al. The effects of levosimendan on brain metabolism during initial recovery from global transient ischaemia/hypoxia. *BMC neurology*. 2012;12:1-10.
35. Korte N, Nortley R, Attwell D. Cerebral blood flow decrease as an early pathological mechanism in Alzheimer's disease. *Acta neuropathologica*. 2020;140(6):793-810.
36. Reddy GMK, Singamsetti JM, Kaliyaperumal M, Doddipalla R, Ivaturi R, Rumalla CS, et al. Degradation studies of levosimendan isolation, identification, and structure confirmation of stress degradation products using LCMS, mass mediated Prep-HPLC, NMR, HRMS, SFC and FTIR. *Journal of Liquid Chromatography & Related Technologies*. 2020;43(1-2):17-28.
37. Khodja IA, Boulebd H, Bensouici C, Belfaitah A. Design, synthesis, biological evaluation, molecular docking, DFT calculations and in silico ADME analysis of (benz) imidazole-hydrazone derivatives as promising antioxidant, antifungal, and anti-acetylcholinesterase agents. *Journal of Molecular Structure*. 2020;1218:128527.
38. Sareila O, Korhonen R, Auvinen H, Hämäläinen M, Kankaanranta H, Nissinen E, et al. Effects of levo- and dextrosimendan on NF- κ B-mediated transcription, iNOS expression and NO production in response to inflammatory stimuli. *British journal of pharmacology*. 2008;155(6):884-95.
39. Gecit I, Kavak S, Yüksel MB, Basel H, Bektaş H, Gümrukçuoğlu HA, et al. Effect of short-term treatment with levosimendan on oxidative stress in renal tissues of rats. *Toxicology and industrial health*. 2014;30(1):47-51.

40. Akhtar MS, Pillai KK, Hassan Q, Ansari SH, Ali J, Akhtar M, et al. Levosimendan suppresses oxidative injury, apoptotic signaling and mitochondrial degeneration in streptozotocin-induced diabetic cardiomyopathy. *Clinical and Experimental Hypertension*. 2016;38(1):10-22.
41. Abdelrahman AM, Al Suleimani Y, Shalaby A, Ashique M, Manoj P, Al-Saadi H, et al. Effect of levosimendan, a calcium sensitizer, on cisplatin-induced nephrotoxicity in rats. *Toxicology reports*. 2019;6:232-8.
42. Aycan A, Oksuz E, Gonullu E, Kume T, Ergur B, Akyol ME, et al. A new approach in the treatment of traumatic brain injury: The effects of levosimendan on necrosis, apoptosis, and oxidative stress. *World Neurosurgery*. 2022;168:e432-e41.
43. Torraco A, Carozzo R, Piemonte F, Pastore A, Tozzi G, Verrigni D, et al. Effects of levosimendan on mitochondrial function in patients with septic shock: a randomized trial. *Biochimie*. 2014;102:166-73.
44. Roehl AB, Hein M, Loetscher PD, Rossaint J, Weis J, Rossaint R, et al. Neuroprotective properties of levosimendan in an in vitro model of traumatic brain injury. *BMC neurology*. 2010;10:1-4.
45. Shi J, Chen Y, Zhi H, An H, Hu Z. Levosimendan protects from sepsis-inducing cardiac dysfunction by suppressing inflammation, oxidative stress and regulating cardiac mitophagy via the PINK-1-Parkin pathway in mice. *Annals of Translational Medicine*. 2022;10(4).
46. Karas G, Scheltens P, Rombouts S, Van Schijndel R, Klein M, Jones B, et al. Precuneus atrophy in early-onset Alzheimer's disease: a morphometric structural MRI study. *Neuroradiology*. 2007;49:967-76.
47. Sochocka M, Zaczyńska E, Taboń A, Czarny A, Leszek J, Sobczyński M. The influence of donepezil and EGb 761 on the innate immunity of human leukocytes: effect on the NF-κB system. *International immunopharmacology*. 2010;10(12):1505-13.
48. Kim J, Lee H-j, Park SK, Park J-H, Jeong H-R, Lee S, et al. Donepezil regulates LPS and Aβ-stimulated neuroinflammation through MAPK/NLRP3 inflammasome/STAT3 signaling. *International journal of molecular sciences*. 2021;22(19):10637.
49. Brás JP, Bravo J, Freitas J, Barbosa MA, Santos SG, Summavielle T, et al. TNF-alpha-induced microglia activation requires miR-342: impact on NF-kB signaling and neurotoxicity. *Cell death & disease*. 2020;11(6):415.
50. Rodríguez MJ, Martínez-Moreno M, Ortega FJ, Mahy N. Targeting Microglial K ATP Channels to Treat Neurodegenerative Diseases: A Mitochondrial Issue. *Oxidative Medicine and Cellular Longevity*. 2013;2013.