

# Neuroprotective effect of ranolazine in mice's model of Alzheimer's disease

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## ABSTRACT

**Background.** Alzheimer's disease is a neurodegenerative disorder that worsens over time as more brain regions are affected and symptoms increase. It usually starts slowly and advances permanently. Ranolazine is a piperazine derivative used as a second-line treatment for people with chronic aortic stenosis who are unresponsive to other medications and have steady or ineffectively-managed angina pectoris. This study is intended to look into the possible neuroprotective effects of ranolazine scopolamine-induced Alzheimer's illness-like features in a mouse model.

**Methods.** This is a randomized controlled animal study that has been carried out in Department of Pharmacology from College of Medicine of Al-Nahrain University. Mice were separated into five groups equally (each group with 10); a control group, and an induction group (mice were administered scopolamine 1 mg per kg intraperitoneally every 24 hr for seven days to induce features similar to Alzheimer's disease). The mice in the remaining three treatment groups were given tested medications prophylactically for 14 days, then the induction was carried out with scopolamine 1 mg/kg i.p. once daily while the tested medication dosages were continued for an additional 7 days; these treatment groups included: donepezil group (5 mg/Kg/d), ranolazine group (40 mg/Kg/d), and combination groups - donepezil (5 mg/Kg/d) with ranolazine (40 mg/Kg/d); all were administered i.p. once daily. Behavioral parameters including the Y maze test and novel object recognition test were assessed for inflammatory cytokines and oxidative stress parameters.

**Result.** Ranolazine exhibits significant improvement in behavior and memory, oxidative stress parameter level, as well as inflammatory cytokines. When the scopolamine induction group was compared to the control group, the spontaneous alteration considerably decreased ( $p \leq 0.001$ ). However, compared to the induction group, all three donepezil, ranolazine, and combination (donepezil + ranolazine) groups had a highly significant increase in the spontaneous alteration and when compared with the control group, there were no statistically significant changes ( $p > 0.05$ ). In comparison with a control group, the scopolamine (induction group) revealed a highly significant reduction ( $p \leq 0.001$ ) in the recognition index. In contrast to the induction group, all three donepezil, ranolazine, and combination (donepezil + ranolazine) groups demonstrated a highly statistically significant improvement in the recognition index. When compared with the control group, there were no statistically significant changes ( $p > 0.05$ ).

**Conclusion.** The current investigation demonstrated ranolazine's neuroprotective action against scopolamine-induced AD-like characteristics in mouse models. The present work has demonstrated the considerable antioxidant and anti-inflammatory benefits of ranolazine, which may account for these positive results.

**Keywords:** Alzheimer's disease, neurodegenerative disorder, ranolazine, beta-amyloid protein, donepezil

## INTRODUCTION

Alzheimer's disease (AD) is a neurological illness that progresses irreversibly and usually begins slowly before becoming worse over time as more brain regions are impacted and symptoms multiply. The build-up of beta-amyloid protein fragments outside of neu-

rons and the twisting of tau protein fibers inside neurons are AD pathogenesis' main characteristics [1].

Numerous hypotheses about AD have been suggested, including those involving amyloid- $\beta$  ( $A\beta$ ), Tau, cholinergic neuron destruction, oxidative stress, inflammation, etc. Based on these hypotheses, nu-

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merous efforts have been made to create anti-AD medications [2].

Six medications have received FDA approval to be used in the treatment of Alzheimer's disease. Five of these medications—donepezil, galantamine, memantine, rivastigmine, and memantine in combination with donepezil—treat Alzheimer's symptoms only temporarily; they do not affect the underlying brain abnormalities associated with the illness [3].

One derivative of piperazine is ranolazine, which is [N (2,6-dimethyl-phenyl)-4[2-hydroxy-3(2-methoxy-phenoxy)propyl] 1 Piperazine acetamide, that is used as a second-line treatment for people with chronic aortic stenosis who are unresponsive to other medications and have steady or ineffectively-managed angina pectoris [4].

Ranolazine improves cardiovascular health by preventing late-phase inward sodium channel activity in ischemic cardiac myocytes. By lowering the intracellular sodium concentration, which in turn reduces intracellular calcium influx through the Na-Ca channel. Oxygen consumption is decreased as a result of decreased intracellular calcium. Blood pressure or heart rate are not impacted [5].

Ranolazine has been suggested as a potential treatment for neuropathic pain because of its ability to act as an anticonvulsant. It has been suggested that these benefits might be mediated by late INa or inwardly rectifying K<sup>+</sup> current, allowing the creation of new treatment plans for epileptic diseases or chronic pain [6].

The objective of the current study is to examine and assess ranolazine's possible neuroprotective properties in preventing Alzheimer's disease brought on by scopolamine in a mouse model

## METHODS

This is a randomized controlled animal study which has been carried out in the Department of Pharmacology from College of Medicine of Al-Nahrain University, between January 2022 and April 2024, and it was approved by the Institutional Review Board (IRB) under approval number: ( 2/3/1970 on 12/12/2021).

Fifty male mice weighing 25 and 35 grams and aged between two and three months, were kept in regular laboratory settings at a temperature between 20 and 22° C. After that, drugs were administered as follows: scopolamine (HyperChem, China), ranolazine (HyperChem, China), and donepezil (Hyper Chem, China) were dissolved in normal saline. Y maze and open field box were made in Baghdad locally. Five groups of mice were formed (ten mice in each group). Group one (control group): mice did not receive any medication. Group two (induction group): mice were injected with scopolamine intraperitone-

ally of dose of 1 mg per kg once daily for seven days to elicit characteristics similar to AD [7]. In the remaining three groups receiving treatment, mice were given the tested medications prophylactically for fourteen days, followed by an induction procedure using scopolamine intraperitoneally with a dose of 1 mg/kg once daily, then continued receiving the same dosage of the investigated drugs for an additional seven days. Among these treatment groups were, group three (donepezil group): donepezil 5 mg/Kg intraperitoneally once per day [8]; group four (ranolazine group): ranolazine 40 mg/Kg intraperitoneally once per day [9]; and group five (combination group) (Donepezil+ ranolazine): donepezil 5 mg/Kg and ranolazine 40 mg/Kg; both were injected intraperitoneally once per day. Behavioral evaluation containing cognitive assessment utilizing, novel object recognition (NOR) and a Y maze were carried out for three days in a row on day 25. After the end of the behavioral tests at day 25 the animals were anesthetized with diethyl ether and sacrificed.

## Behavioral tests

### A. Y - maze test

This device was designed like a Y shape, with three equal arms conveniently denoted by the letters A, B, and C. The arms had the following measurements: 20 cm long, 6 cm wide, and 15 cm high, and with 120° angle connecting them [10].

Each animal was subjected to this test for a total of 10 minutes. Each animal was inserted into one arm, as well as the order and number of arms it entered after that was noted. Complete arm entrance was defined as the hind paws fully enclosing any given arm, while the definition of alternation was when a mouse entered three distinct arms in a row. To prevent olfactory cues between tests, the Y - maze arena was cleaned with an ethanol 70% v/v solution [11].

The spontaneous alternation (%) was computed by multiplying the number of arm entries -2 divided by the sum of all the alternations, which resulted in the following equation: % Alternation is calculated through application of the subsequent formula: [(Number of alternations) / (Total number of arm entries -2)] × 100 [12].

### Novel object recognition (NOR)

The experimental tool was a white plastic open field box measured 40×40×20 cm). Three phases make up this evaluation:

(1) habituation: during the first day, all mice were permitted to recognize the open field box for around 15 minutes without being presented an object.

(2) training: every mouse was left in an open field for ten minutes on the next day, free to examine the two similar items.

(3) test: 90 minutes following the instruction session, one of the recognizable items was swapped out for a novel one, and mice ran for 5 minutes while the duration of time consumed with both objects was noted [13].

The recognition index is determined by using the following formula:  $[TB / (TA + TB) * 100]$ . Object exploration was described as active involvement with the object, such as smelling or putting the nose and/or forepaws in contact with the object. TA and TB are the times dedicated to examining known object A and unknown object B, correspondingly [14].

Following the behavioral assessments, diethyl ether was inhaled to induce anesthesia in mice. Mice were sacrificed, and mice brains were extracted promptly, and then cleansed with saline solution buffered with phosphate. One hemisphere of the brain was washed in ice-cold phosphate-buffered saline (pH 7.20-7.40, 0.02 mol/L) [15]. The mice's brain homogenate was utilized for evaluation of inflammatory cytokines (levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) and indicators of oxidative stress (MDA and SOD1) in mice brain homogenate using ELISA (BT LAB) in compliance with the guidelines provided by the manufacturer (Mouse Superoxide Dismutase, Cu-Zn, SOD1 ELISA Kit, BT LAB, Zhejiang, China).

### Sample size calculation

Fifty male mice weighing 25 and 35 grams and aged between two and three months.

### Statistics

All data were displayed as mean  $\pm$  standard deviation. The statistical comparisons were performed using an independent t-test and a one-way ANOVA (analysis of variance) test; p-values of 0.05 or less were considered statistically significant. Excel 2010 and the Statistical Package for Social Sciences (SPSS) version 23 were used to analyze the data [16].

### Ethics

Ethical approval for conducting this study was issued and approved by the Department of Pharmacology, College of Medicine, Al-Nahrain University, Iraq (<https://www.colmed-alnahrain.edu.iq/?&lang=en>) according to the letter NO. 2/3/1970 dated in December 12, 2021.

## RESULTS

**Y-maze test:** When scopolamine (the induction group) was compared to the control group, the spontaneous alteration considerably decreased ( $p \leq 0.001$ ). However, compared to the induction group, all three donepezil, ranolazine, and combination (donepezil + ranolazine) groups had a highly significant increase

in the spontaneous alteration and there were no statistically significant differences between the experimental group and the control group ( $p > 0.05$ ) (Table 1).

**NOR test:** In comparison with the control group, the scopolamine (induction group) revealed a highly significant reduction ( $p \leq 0.001$ ) in the recognition index. In contrast to the induction group, all three donepezil, ranolazine, and combination (donepezil+ranolazine) groups demonstrated a highly statistically significant improvement in the recognition index. There were no statistically significant differences between the experimental group and the control group ( $p > 0.05$ ).

**TABLE 1.** Effects of ranolazine and donepezil on behavioral tests

Groups	Y - maze	NOR
Control	66.58 $\pm$ 2.82	63.58 $\pm$ 2.81
Induction (scopolamine)	53.72 $\pm$ 4.32 <sup>###</sup>	50.16 $\pm$ 4.0 <sup>###</sup>
Donepezil	67.58 $\pm$ 4.0 <sup>**</sup>	64.77 $\pm$ 5.35 <sup>**</sup>
Ranolazine	63.62 $\pm$ 5.36 <sup>**</sup>	60.98 $\pm$ 2.69 <sup>**</sup>
Donepezil+Ranolazine	64.6 $\pm$ 4.38 <sup>**</sup>	63.12 $\pm$ 5.18 <sup>**</sup>

*n = 10 mice/group, the information is given as mean  $\pm$  standard deviation, with the following classifications: \*: statistically significant ( $p < 0.05$ ), \*\*: highly statistically significant ( $p \leq 0.001$ ) compared with the induction (scopolamine) group, # statistically significant ( $p \leq 0.05$ ), ### highly statistically significant ( $p \leq 0.001$ ) compared with the control group*

### Evaluation of oxidative stress

The induction group demonstrated in comparison to the control group a highly significant increase in MDA level and a highly significant drop in SOD1. All of the donepezil, ranolazine, and combination (donepezil+ranolazine) groups displayed MDA levels significantly decreased whereas, SOD1 levels significantly increased in comparison to the induction group. The MDA and SOD1 level of the control group was shown to be statistically non-significantly different ( $p > 0.05$ ) in the donepezil, ranolazine, and combination (donepezil + ranolazine) groups (Table 2).

**TABLE 2.** Effects of ranolazine and donepezil on oxidative stress parameters

Groups	MDA (ng/ml)	SOD1 (ng/ml)
Control	1.74 $\pm$ 0.21	16.93 $\pm$ 2.55
Induction (scopolamine)	2.57 $\pm$ 0.29 <sup>###</sup>	10.87 $\pm$ 2.32 <sup>###</sup>
Donepezil	1.65 $\pm$ 0.21 <sup>**</sup>	14.51 $\pm$ 3.59 <sup>*</sup>
Ranolazine	1.81 $\pm$ 0.21 <sup>**</sup>	16.17 $\pm$ 3.91 <sup>**</sup>
Donepezil+Ranolazine	1.72 $\pm$ 0.27 <sup>**</sup>	15.65 $\pm$ 1.432 <sup>**</sup>

*n = 10 mice/group, the information is given as mean  $\pm$  standard deviation, with the following classifications: \*: statistically significant ( $p < 0.05$ ), \*\*: highly statistically significant ( $p \leq 0.001$ ) compared with the induction (scopolamine) group, # statistically significant ( $p \leq 0.05$ ), ### highly statistically significant ( $p \leq 0.001$ ) compared with the control group*

**TABLE 3.** Effects of ranolazine and donepezil on inflammatory cytokines

Groups	TNF- $\alpha$ (pg/ml)	IL-1 $\beta$ (pg/ml)	IL-6 (pg/ml)
Control	130.77 $\pm$ 18.32	693.56 $\pm$ 150.18	156.45 $\pm$ 39.04
scopolamine	189.89 $\pm$ 33.93 <sup>###</sup>	983.69 $\pm$ 109.73 <sup>###</sup>	227.51 $\pm$ 35.19 <sup>###</sup>
Donepezil	125.91 $\pm$ 21.21 <sup>**</sup>	744.48 $\pm$ 34.39 <sup>**</sup>	158.68 $\pm$ 15.92 <sup>**</sup>
Ranolazine	126.16 $\pm$ 13.72 <sup>**</sup>	680.27 $\pm$ 74.31 <sup>**</sup>	162.45 $\pm$ 17.11 <sup>**</sup>
Donepezil+Ranolazine	126.44 $\pm$ 15.43 <sup>**</sup>	728.28 $\pm$ 120.59 <sup>**</sup>	144.38 $\pm$ 27.09 <sup>**</sup>

*n* = 10 mice/group, the information is given as mean  $\pm$  standard deviation, with the following classifications: \*: statistically significant ( $p < 0.05$ ), \*\*: highly statistically significant ( $p \leq 0.001$ ) compared with the induction (scopolamine) group, # statistically significant ( $p \leq 0.05$ ), ## highly statistically significant ( $p \leq 0.001$ ) compared with the control group.

### Evaluation of inflammatory cytokines

The levels of IL-1 $\beta$ , IL-6, and TNF $\alpha$  in the induction group were significantly higher than those in the control group. However, compared to the induction group, all of the donepezil, ranolazine, and combination (donepezil + ranolazine) groups demonstrated a highly significant decrease in the levels of these three cytokines. There were non-significant alterations in the levels of TNF $\alpha$ , IL-6, and IL-1 $\beta$  in donepezil, ranolazine, and combination (donepezil + ranolazine) groups in contrast to the control group (Table 3).

### DISCUSSION

When combination was used, the score was lower than donepezil alone. When scopolamine (the induction group) was compared to the control group, the spontaneous alteration considerably decreased ( $p \leq 0.001$ ). However, compared to induction group, all three donepezil, ranolazine, and combination (donepezil + ranolazine) groups had a highly significant increase in spontaneous alteration. In comparison with a control group, the scopolamine induction group revealed a highly significant reduction ( $p \leq 0.001$ ) in the recognition index. Compared to induction group, all three donepezil, ranolazine, and combination (donepezil + ranolazine) groups demonstrated a highly statistically significant improvement in the recognition index.

Alzheimer's disease is among the most common neurodegenerative illnesses that worsen over time which results in serious suffering for patients and their relatives [17].

Although there is mounting evidence that AD is a complicated illness resulting from several causes with different molecular targets, the precise pathophysiology of AD is still unknown. Thus, while developing a novel medication, synaptic malfunction, oxidative stress, or the early stages of neuroinflammation must be considered [18].

Scopolamine hydrochloride has been used in the current study to induce memory impairment in a mouse model. In experimental models, it's frequently employed to induce dementia-like amnesia caused

by AD scopolamine has been employed to evaluate the therapeutic efficacy of medications in the experimental model of neurodegenerative disease to identify anti-dementia medications since it is linked to oxidative stress and synapse loss throughout the brain [19].

We employed the novel object recognition (NOR) test and the Y-maze to evaluate cognitive performance

(memory and learning). In earlier research, the scopolamine induction group demonstrated the lowest level of motion in the Y-maze behavioral test when compared to the control group, in terms of the percentage of spontaneous alternations and total arm entries [20].

In the novel object recognition (NOR) test, the scopolamine group's recognition index substantially decreased compared to the group under control, indicating a potential impairment by process of learning and recognition. The results revealed that scopolamine, an anticholinergic medication that blocks muscarinic receptors, interferes with learning and both short- and long-term memory performance, as previously reported by Rajashri K. [21].

The current study's findings confirmed previous suggestions that there was a collapse in the brain's antioxidant defense mechanism, as seen by a higher level of MDA in the group receiving scopolamine medication than in the control group [7].

In the present study, superoxide dismutase (SOD1) was remarkably decreased in the brain homogenate when compared to the control group. Among all the antioxidant enzymes, superoxide dismutase (SOD1) is the primary enzyme, SOD1 is responsible for detoxifying superoxide anions, which has harmful effects on cell membranes [22].

The current study's findings confirm the oxidative stress state following scopolamine administration where the elevated MDA level and decreased SOD1 level in the induction group's brain homogenate.

Behavioral anomalies and memory impairments produced by scopolamine are demonstrated by spatial memory and learning. Substantial impairment of cognitive function is caused by scopolamine, which has been associated with elevated levels of neuroinflammatory indicators, oxidative stress, AChE, IL-1 $\beta$ , TNF- $\alpha$ , IL-6, and IFN in the brain [23].

The abnormal productions of inflammatory cytokines induce neuronal damage, preceding the progression of AD. According to earlier research, administering scopolamine for experimental animal models significantly raised the neuroinflammatory markers which lead to neuronal damage [20].

The current investigation showed that the injec-

tion of scopolamine elevated neurotoxic cytokines and inflammatory mediators, such as IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 as previously reported [24].

This study showed that ranolazine significantly improved the recognition index and the average of spontaneous alteration, suggesting that the drug may have a protective effect against the cognitive impairment brought on by scopolamine; the current study's findings agree with those of Cassano et al. (2022), who demonstrated that rats given metformin or ranolazine over an extended period, these drugs have protective benefits against the onset of cognitive decline by reducing memory impairment [25].

Cassano et al. (2020) demonstrated that in a diabetic rat's model, ranolazine keeps memory loss (measured by the latency time needed to enter a dark compartment during an exam) at a distance, protecting against the onset of cognitive decline, as well as memory and learning (as shown by a novel object recognition test). Ranolazine-treated diabetic animals exhibited indications of improved inflammatory characteristics, suggesting that the drug may have positive effects against depression and cognitive impairment primarily through its anti-inflammatory action by reducing TNF- $\alpha$  and IL-6 [26].

The present study revealed that ranolazine exhibited a significant reduction in MDA level and a significant elevation in SOD1 level, indicating its powerful antioxidant effects. These findings appear to be consistent with previous studies [27].

Dogan et al. (2023) revealed that ranolazine reduces the oxidative damage caused by MTX in cardiomyocytes to some extent by reducing oxidative stress via reducing the MDA activity, maintaining T-SH, CAT, and TAC activity levels, and suppressing the development of the HIF-1 $\alpha$  inflammatory pathway. Consequently, ranolazine provides a defense against hypoxia and oxidative damage [27].

In the present study, ranolazine exhibited a significant decrease in proinflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$ , and IL-6) confirmed by these findings which demonstrate that, in DM rats, ranolazine substantially decreased the amounts of pro-inflammatory cytokines like NF- $\kappa$ B, p-IKK $\alpha$ , IL-6, TNF- $\alpha$ , and IL-1 $\beta$  in the hippocampal regions. The evidence for ranolazine comes from its capacity for reducing hippocam-

pus neurodegeneration following T2DM induction [28].

Aldasoro et al. (2016) [29], observed that in the primary culture of astrocytes, ranolazine improved the antioxidant enzymes' expression Cu/Zn-SOD and Mn-SOD and reduced TNF- $\alpha$  and IL-1 $\beta$  as pro-inflammatory cytokines. It additionally raised the expression of the anti-inflammatory (PPAR- $\gamma$ ). Furthermore, ranolazine decreased the production of LDH and enhanced astrocyte survival besides proliferation. In the central nervous system, ranolazine could serve as neuroprotective medication via enhancing antioxidant and anti-inflammatory chemicals, preventing necrosis and apoptosis, decreasing inflammatory processes, and enhancing astrocyte viability. Astrocytes have a crucial function in defending neurons from inflammation and oxidation. One important mechanism they use to do this is the biogenesis of mitochondrial cells [30].

### Limitation

Small sample size and short time of work are the main limitation, in addition, the death of animals and their replacement, and the difficulties of behavioral tests.

### CONCLUSIONS

Ranolazine and its combinations at prescribed doses in the current study enhanced memory deficits and learning in an Alzheimer's disease mouse model induced by scopolamine probably via their antioxidant and anti-inflammatory properties, confirmed by a substantial increase in antioxidant mediator (SOD1) and a substantial reduction in inflammatory cytokines (TNF-, IL-1 $\beta$ , and IL-6) and the oxidative stress marker (MDA).

### Recommendations

Further advanced studies are recommended to determine the optimal dosages and clarify the precise mechanisms underlying the preventative effects of ranolazine and famotidine against scopolamine-induced AD-like features in animal models.

*Conflicts of interest:* none declared

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