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Original article

Ranuncoside's attenuation of scopolamine-induced memory impairment in mice via Nrf2 and NF-κB signaling

Hafiza Sara Salahuddin [a], Sobia Attaullah [a,*], Shahid Ali Shah [b,c], SanaUllah Khan [d], Muhammad Zahid [a], Mujeeb Ullah [a], Khayyam [a], Sidra Salahuddin [e], Seema Gul [a], Mahdi H Alsugoor [f,*]

[a] Department of Zoology, Islamia College, Peshawar, Khyber Pakhtunkhwa, Pakistan
[b] Neuro Molecular Medicine Research Centre (NMMRC), Ring Road, Peshawar, KPK, Pakistan
[c] The University of Haripur, KPK, Pakistan
[d] Department of Zoology, University of Peshawar, Khyber Pakhtunkhwa, Pakistan
[e] Hayatabad Medical Complex, Peshawar, Khyber Pakhtunkhwa, Pakistan
[f] Department of Emergency Medical Services, College of Health Sciences-AlQunfudah, Umm Al- Qura University, Makkah 21912, Saudi Arabia

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ABSTRACT

Scopolamine is a well-known pharmacological agent responsible for causing memory impairment in animals, as well as oxidative stress and neuroinflammation inducer which lead to the development of Alzheimer disease. Although a cure for Alzheimer's disease is unavailable. Ranuncoside, a metabolite obtained from a medicinal plant has demonstrated antioxidant and anti-inflammatory properties in vitro, making it a promising treatment with potential anti-Alzheimer disease properties. However, as ranuncoside has not been evaluated for its antioxidant and anti-neuroinflammatory properties in any *in vivo* model, our study aimed to evaluate its neurotherapeutic efficacy against scopolamine-induced memory impairment in adult male albino mice.

Mice were randomly divided into four experimental groups. Mice of group I was injected with saline, group II was injected with scopolamine (1 mg/kg/day) for 3 weeks. After receiving a daily injection of scopolamine for 1 week, the mice of group III were injected with ranuncoside (10 mg/kg) every other day for 2 weeks along with scopolamine daily and group IV were injected with ranuncoside on 5th alternate days. Behavioral tests (i.e., Morris water maze and Y-maze) were performed to determine the memory-enhancing effect of ranuncoside against scopolamine's memory deleterious effect. Western blot analysis was also performed to further elucidate the anti-neuroinflammatory and antioxidant effects of ranuncoside against scopolamine-induced neuroinflammation and oxidative stress.

Our results showed memory-enhancing, anti-neuroinflammatory effect, and antioxidant effects of ranuncoside against scopolamine by increasing the expression of the endogenous antioxidant system (i.e., Nrf2 and HO-1), followed by blocking neuroinflammatory markers such as NF-κB, COX-2, and TNF-α. The results also revealed that ranuncoside possesses hypoglycemic and hypolipidemic effects against scopolamine-induced hyperglycemia and hyperlipidemia in mice as well as scopolamine's hyperglycemic effect. In conclusion, our findings suggest that ranuncoside could be a potential agent for the management of Alzheimer's disease, hyperglycemia, and hyperlipidemia.

Abbreviations: HO-1, Heme Oxygenase-1; Nrf2, Nuclear Factor-Erythroid 2 Related Factor 2; NF-κB, Nuclear Factor Kappa Light-Chain-Enhancer of Activated B-Cells; COX-2, Cyclooxygenase-2; TNF-α, Tumor Necrosis Factor-α; ROS, reactive oxygen species.

* Corresponding authors.
E-mail addresses: sarasalahuddin9@gmail.com (H. Sara Salahuddin), sobia@icp.edu.pk (S. Attaullah), alishahshahid@yahoo.com (S. Ali Shah), sanaullahkhan@uop.edu.pk (S. Khan), drmzahid@icp.edu.pk (M. Zahid), mujibkhanicp@gmail.com (M. Ullah), khayyam@icp.edu.pk (Khayyam), sidrasalahuddin321@gmail.com (S. Salahuddin), seemagull862@gmail.com (S. Gul), mhsugoor@uqu.edu.sa (M.H Alsugoor).

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1. Introduction

Alzheimer's disease, a complex neurodegenerative disorder, poses significant challenges, it typically progresses in old age and affects a substantial portion of the global population (Lucey, 2020; Serafín et al., 2020). The primary features of Alzheimer's disease include extracellular accumulation of amyloid beta (Aβ) peptides (i.e., Aβ1–40 and Aβ1–42) (Gul et al., 2023) and intracellular accumulation of neurofibrillary tangles (i.e., insoluble hyperphosphorylated tau protein) (Majdi et al., 2020; Saeed et al., 2020; Shah et al., 2015; Yun et al., 2021). Multiple factors have been attributed to Alzheimer's disease's pathogenesis, therefore exact mechanism underlying this disease remains elusive (Gadhave et al., 2021; Uddin et al., 2020; Wang et al., 2020; Zhu et al., 2020), although, Aβ accumulation and oxidative stress are widely accepted major factors responsible for Alzheimer's disease (Ullah et al., 2019). Further, research has revealed that neuroinflammation, characterized by the aggregation of Aβ, plays a significant role in the development of Alzheimer's disease. This neuroinflammation can trigger the activation of a mitochondrial apoptotic pathway and lead to synaptic dysfunction (Parodi-Rullán et al., 2019; Shin et al., 2019).

Using animal models, various studies shown that the antimuscarinic, scopolamine cause memory deficits particularly in short-term memory and cognitive impairment, by inducing oxidative stress and neuroinflammation, mimicking those observed in Alzheimer disease (Kim et al., 2021; Tang, 2019). Other recent findings have shown novel compounds in some medicinal plants that have antioxidant effects which counteract Alzheimer's disease (Pellegrini et al., 2019). During the phytochemical analysis, *Ranunculus muricatus*, a medicinal plant, exhibited the presence of a novel crystalline metabolite in its ethyl acetate fraction. This compound, named ranuncoside due to its origin in the Ranunculaceae species, holds potential significance for its unique characteristics and potential therapeutic applications. Ranuncoside's antioxidant, antibacterial, antifungal, and minor cytotoxic properties, as well as lipoxygenase and xanthine oxidase-inhibiting properties, have been documented (Raziq et al., 2017). Although ranuncoside has been shown to have potent anti-inflammatory and antioxidant activities in vitro, to the best of our knowledge, no study has been conducted to evaluate its effects in its isolated pure form on Alzheimer disease induced by oxidative stress in any animal model *in vivo*. Therefore, our study aimed to examine ranuncoside's potential anti-Alzheimer effect against scopolamine-induced reactive oxygen species (ROS), neuroinflammation, and memory impairment in the brains of adult male albino mice. Moreover, for the reason that no study has clarified ranuncoside's potential role in hyperlipidemia and hyperglycemia caused by scopolamine, we also aimed to examine ranuncoside's potential effect in an Alzheimer's disease mouse model with coexisting hyperglycemia and hyperlipidemia.

2. Materials and methods

2.1. Chemicals

All chemicals used in our experiments were sterile and stored according to their respective protocols. Purchased chemicals were dextrose, chloroform, tween, ammonium persulfate, and tetramethylethylenediamine (Daejung Chemicals and Metals Co. Ltd, Gyeonggi-do, Shiheung, South Korea); RNA wait (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China); phosphate-buffered saline, sodium dodecyl sulfate, scopolamine (Santa Cruz, CA, USA); enhanced chemiluminescence solution A and B (Bio-Rad Laboratories, Inc., Biotechnology Company Philadelphia, PA, USA); tissue protein extraction reagent and methanol (Thermo Scientific, Meridian Rd., Rockford, IL 61101 USA); Trizma base (Sigma Aldrich, Burlington, MA, USA); skim milk powder (Oxoid Ltd, Wade Road, Basingstoke, Hants, UK), Ranuncoside (provided by Umar Farooq). The primary antibodies were purchased from Santa Cruz, CA, USA, while the secondary antibody, i.e., anti-mouse IgG, was purchased from Promega, Madison, WI, USA (Table 1).

2.2. Mice and their groups

Male albino mice of strain BALB/C approximately 7–8 weeks old and weighing 25–30 g were obtained from the Veterinary Research Institute in Peshawar, Khyber Pakhtunkhwa, Pakistan. Mice were housed individually in separate cages (Biobase, China) in the breeding room of animal section of the Neuro-Molecular Medicine Research Centre (NMMRC), situated on Ring Road, Peshawar, Khyber Pakhtunkhwa, Pakistan, under a 12–12-hour dark-light cycle with controlled room temperature (25 ± °C) and humidity (60–65%) and were provided free access to water and food. All animal care as well as treatment procedure was carried out in accordance with the guidelines of the UK Animals (Scientific Procedures) Act 1986.

The mice were equally divided into four experimental groups (n = 5). Group I (Control group or C), Group II (Scopolamine treated group or SCOP), Group III (Scopolamine plus Ranuncoside treated group or SCOP + R) and Group IV (Ranuncosid treated group or R). Mice of group 1 received normal saline (0.9%) on daily basis as described in previous literature (Naseer et al., 2014; Jahan et al., 2022), group II received injection of scopolamine (1 mg/kg/-day) for a period of three weeks, according to the protocol described in previous literature (Yadang et al., 2020; Noroozi et al., 2022; Sandhu et al., 2022; Syed et al., 2022), group III was subjected injections of scopolamine (1 mg/kg/day) for three weeks, along with ranuncoside (10 mg/kg) administered every other day for a duration of two weeks while group IV was treated with ranuncoside (10 mg/kg) after a gap of every fifth day. The injections were delivered intraperitoneally with great care on the specified days. Fig. 1 described the experimental layout of the drug administration schedule, behavioral evaluation, blood glucose tests, and isolation of brain samples after euthanasia.

2.3. Behavioral tests

After treatment, a behavioral study involving a Morris water maze (MWM) test and Y-maze test was conducted to determine the learning abilities and memory of the mice, respectively (Imran et al., 2021). The researcher who led the behavioral tests was completely blinded to the groups.

2.3.1. Morris water maze (MWM) test

The apparatus used during the test consisted a water filled circular metal tank (diameter of approximately 100 cm and a height

Table 1
List of primary and secondary antibodies with catalog number.

S.#	Antibodies Name	Catalog #
1	Anti-HO-1	sc-136960
2	Anti-Nrf-2	sc-365949
3	Anti- NF–κb	sc- 8008
4	Anti-COX-2	sc-376661
5	Anti-TNF-α	sc- 52,746
6	Anti-β actin	sc-47778
7	Goat anti-mouse (IgG-HRPs) secondary antibodies	W4028

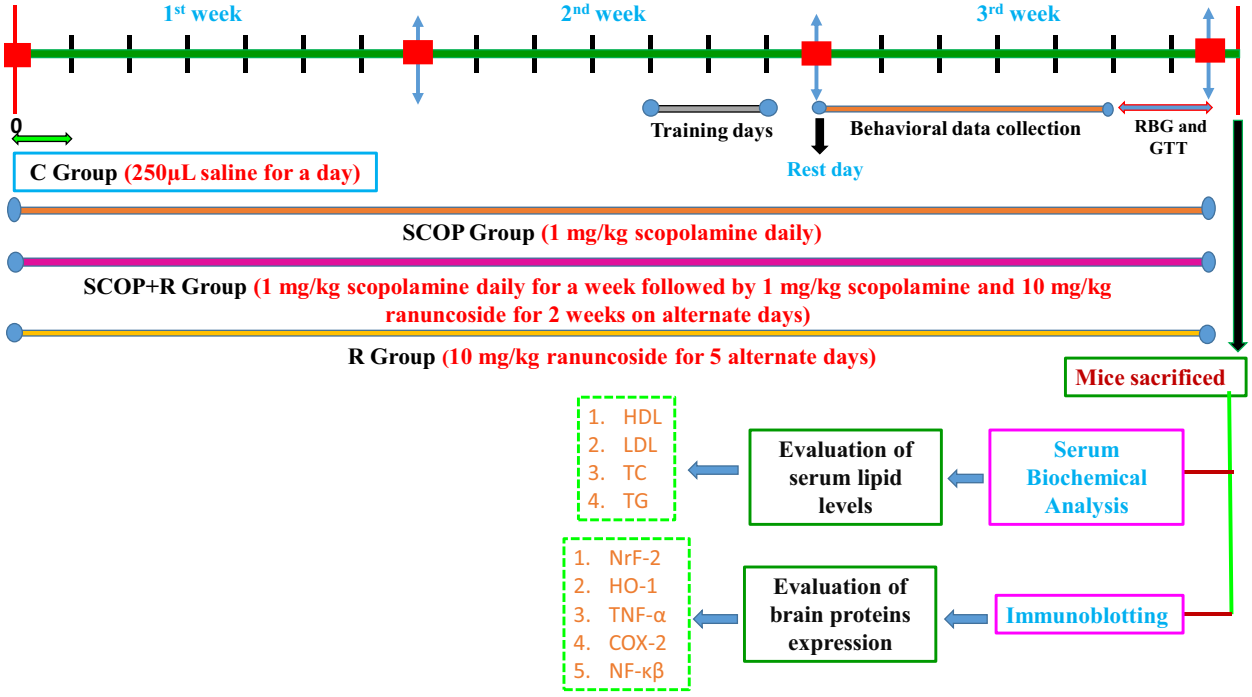

Fig. 1. Experimental layout of animal grouping, drug administration schedule, behavioral evaluation, blood glucose tests (Random Blood Glucose and Glucose Tolerance Test), lipid level, and isolation of brain samples after euthanasia.

of 40 cm) having a platform (diameter of 10 cm and a height of 20 cm), at a depth of up to 26 cm in one of the four quadrants of the tank. All experimental mice were given daily twice swimming session of 60 sec for five consecutive days in the water pool with platform where starting pole was changed on each day to get acclimated to the platform. If a mouse failed to locate the platform within the given time, it was gently placed on the platform for 10 sec to familiarize itself with its location. After 24 h rest, a probe test was conducted. During the probe test, all mice were allowed to search missing platform for 60 sec. The mean escape latency, which indicated the time taken to find the platform, was recorded for all the groups.

2.3.2. Y-maze test

Y-maze is an apparatus having 3 arms of 50 cm in length, 20 cm in height and had a width of 10 cm at the bottom, tapering to 10 cm at the top, lying at angle of 120○. All mice received two trials training a day for two consecutive days. Trial session was started by placing the mice at the center of the apparatus and was allowed to move freely for 8 min to discover the arms. Next, an experimental session was followed for four days. Throughout the sessions, mice' total arm entries and successive number of triplets were noted. Spontaneous alternation behavior of mice (consecutive entry of the mouse into the three arms in overlapping sets of triplets) was calculated by the formula

$$Spontaneousalternation\% = \frac{Succeesivetripletset}{totalnumberofarmentries - 2} \times 100$$

2.4. Random blood glucose (RBG) detection

The blood glucose concentrations (mg/dL) of all groups were randomly measured on days 4, 8, and 12 with a glucometer (On Call Plus, San Diego, CA, USA).

2.5. Glucose tolerance test (GTT)

After the dextrose injection, blood glucose levels were checked after 15 min, 30 min, 60 min, 120 min, and 180 min (Huang et al., 2018).

2.6. Protein extraction from mice brains

The brains were carefully removed after all mice were euthanized, for protein extraction followed the protocol described in an earlier study (Farooq et al., 2021; Gul et al., 2023).

2.7. Lipid profile test

Blood samples were collected from all mice to conduct a lipid profile test included high-density lipoprotein (HDL), low-density lipoprotein (LDL), Triglycerides and Total cholesterol using the Easy Tech kit (Clinical Chemistry Analyzer) as reported in earlier studies (Barthold et al., 2020; Feng et al., 2019; Khan et al., 2018).

2.8. Western blot analysis

The western blot procedure was carried out as previously described by Farooq et al., (2021). All brain tissues were homogenized, centrifuged and then loaded to perform 12–15% SDS PAGE. To record the desired protein's molecular weight; a pre-stained protein marker (Gang Nam-STAIN™, iNtRon Biotechnology, Inc., Seongnam, Korea) that covers a broad range of molecular weights, i.e., 10–245 kDa range was used throughout the study. It was followed by the transfer of proteins to PVDF membrane (Santa Cruz Biotechnology, Santa Cruz, CA, USA) using Semi Dry Trans-Blot (Bio-Rad). A mouse derived primary monoclonal antibodies were applied at 4 °C overnight (1:1000 in TBST) to detect different targeted proteins (HO-1, COX-2, Nrf-2, TNF-α, NF-κB, and β-actin). The binding of non-specific proteins was reduced by using 5% (w/v) skim milk which blocked the membranes. The blots were

rinsed, and then incubated for two hours at room temperature with horseradish-peroxidase-conjugated goat anti-mouse (IgG-HRPs) secondary antibody (1:2000 in TBST). The bands of the western blot were visualized using enchanced ochemiluminescence solution, a detection reagent according to the manufacturer's instructions (Amersham Pharmacia Biotech, Uppsala, Sweden). Results obtained were developed on the X-ray films, which were later scanned.

2.9. Statistical analysis

To perform the statistical analysis of the scanned X-rays, Prism 6 (Graph Pad Software, San Diego, CA, USA) was used for the Student's *t* test followed by one-way analysis of variance and Tukey's Multiple Comparision Post Hoc Test, whereas Image J was used to analyze the optical densities of the scanned X-ray films of the western blot and immunofluorescence images by densitometry. Protein densities were recorded as the $M \pm SEM$ in arbitrary units. # represented significant difference among C group and SCOP group while * showed that SCOP group was significantly different from SCOP + R group; *, # $P < 0.05$, **, ## $P < 0.01$, ***, ### $P < 0.001$. A $P < 0.05$ was considered a statistically significant value.

2.10. Institutional review board Statement

The Ethics Committee of the NMMRC in Peshawar granted approval for all experimental procedures involving animals on June 25, 2021 (Ref. NMMRC/03/2019).

3. Results

3.1. Effect of scopolamine and ranuncoside on the memory of mice

The findings of MWM test revealed contrasting effects of treatment among the different groups of mice. The decreased mean escape latencies reported in C group mice indicated improved performance in searching of platform. SCOP group took significantly longer time to reach the platform and their performance over consecutive days also decreased (F = 10.88). SCOP + R group exhibited shorter escape latencies compared to SCOP group (F = 12.32), and their performance improved day after day. The R group also displayed reduced escape latencies, demonstrating a positive effect of the drug on mice reaching the platform (Fig. 2A). Reduced escape latencies in SCOP + R and R group indicated a beneficial impact of the drug on reaching the platform.

During the probe test, the C group spent a significant amount of time in navigating the target quadrant. On the other hand, SCOP group spent the least amount of time (F = 75). However, the SCOP + R group exhibited a significant increase in time spent in the target quadrant (F = 56.75) and R group spent a considerably higher amount of time, indicated effectiveness of the drug administration on both group's spatial memory (Fig. 2B).

Similarly the findings of Y-maze test (Fig. 2C) demonstrated distinct patterns of spontaneous alternation among mice's groups. The spontaneous alternation of C group exhibited highest percentage of spontaneous alternation due to greater tendency of reaching the arms of maze, while SCOP group had the lowest percentage of spontaneous alternation (F = 262.1), indicating impaired working memory. However, SCOP + R group demonstrated a significant increase in the percentage of spontaneous alternation (F = 198.1) due to better exploration of different arms of the maze, suggesting an improved working memory compared to SCOP group and lastly R group showed a relatively high percentage of spontaneous alternation. This increase indicated the positive influence of ranuncoside by effectively reversal of memory impairment in mice of SCOP + R and R group.

Considering the results of both behavioral tests, our study demonstrated that the best memory performance of C group showed while SCOP group showed impaired performance. Conversely, SCOP + R group showed relatively positive improvements in performance while R group displayed much better performance.

3.2. Effect of scopolamine and ranuncoside on mice blood glucose level

Scopolamine induced hyperglycemia after being injected for three weeks in mice. The SCOP group (F = 370.4) exhibited higher levels of blood glucose compared to the control group. On the other hand, the introduction of ranuncoside, either in combination with scopolamine (F = 312.8) or alone, resulted in lower glucose levels in the blood.

For further validity of these findings, GTT was performed at different time points after administering a glucose load. In this study, at all time points tested (including 0 and between 15 min to 3 h), the SCOP group (F = 26.31) consistently displayed higher concentration of glucose in the blood compared to the SCOP + R (F = 19.97) and R group (Fig. 3A and 3B).

3.3. Scopolamine and ranuncoside effect on serum lipid levels in mice

The results of the lipid profile test (Fig. 4A–4D) revealed that the SCOP group (F = 40.75) exhibited a decrease in HDL content compared to the C group. However, the administration of ranuncoside reversed this effect, leading to an increase in HDL levels, as observed in the SCOP + R group (F = 31.25). Conversely, the SCOP group (F = 3062) had the highest content of LDL, but ranuncoside administration counteracted this effect by reducing LDL levels in the SCOP + R group (F = 2698). Total cholesterol levels were also highest in the SCOP group (F = 2003), but the hypolipidemic effects of scopolamine were neutralized by ranuncoside, as evidenced in the SCOP + R group (F = 387.0). Furthermore, the SCOP group (F = 2108) exhibited elevated triglyceride content, which was effectively reduced by ranuncoside, as indicated in the SCOP + R group (F = 601.7).

3.4. Effects of scopolamine and ranuncoside on oxidative stress markers, i.e., Nrf2 or HO-1 in mice brains

Scopolamine caused the inhibition of Nrf2 (F = 195.9) and HO-1 (F = 204.5) in the brains of mice, whereas ranuncoside upregulated the expression of Nrf2 (F = 101.6) as well as HO-1 (F = 232.0), in turn reduced scopolamine-induced oxidative stress, as shown in Fig. 5A–5C.

3.5. Scopolamine and ranuncoside effect on NF-κB and its downstream signaling, i.e., COX-2 and TNF-α in mice brains

The administration of scopolamine caused neuroinflammation in mice by upregulating NF-κB (F = 175.0) and its downstream signaling protein molecules i.e., COX-2 (F = 565.1) and TNF-α (F = 386.8), whereas ranuncoside inhibited the neuroinflammatory markers i.e., NF-κB (F = 237.0), COX-2 (F = 484.4), and TNF-α (F = 421.7) in mice and, in turn, reduced scopolamine-induced neuroinflammation (Fig. 6A–6D).

4. Discussion

This study aimed to identify a novel therapeutic agent for treating Alzheimer's disease, hyperglycemia, and hyperlipidemia. It was observed that ranuncoside eliminated scopolamine-generated

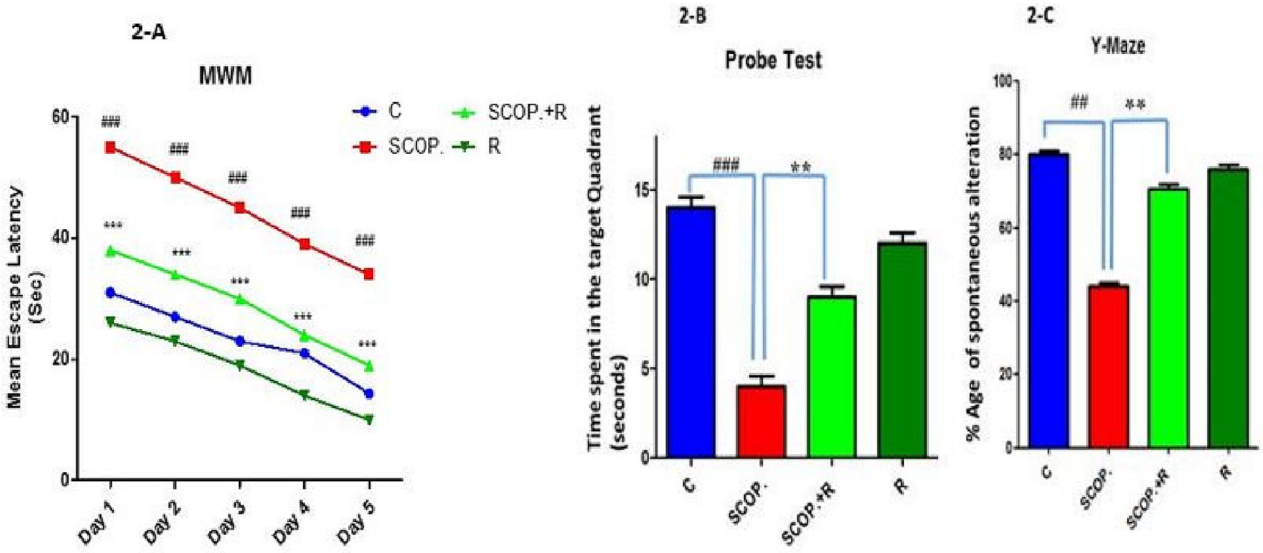

Fig. 2. Scopolamine-induced memory deficits improved following the administration of ranuncoside in mice. Behavioral tasks, including the Y-maze and MWM tests, as well as probe tests for all four experimental groups was presented in respective bar graphs along with their *p* values ($^{**, \#\#}$ $p < 0.01$, $^{***, \#\#\#}$ $p < 0.001$), for identifying the statistical difference between the C, SCOP, SCOP + R and R group. **(A)** Graph of the MWM test results showing the mean escape latency time for each group. **(B)** Histogram showing the probe test results for the four groups and the time spent in the target quadrant on the day of probing. **(C)** Histogram depicting the Y-maze test results in the form of the percentage of spontaneous alternation in the groups.

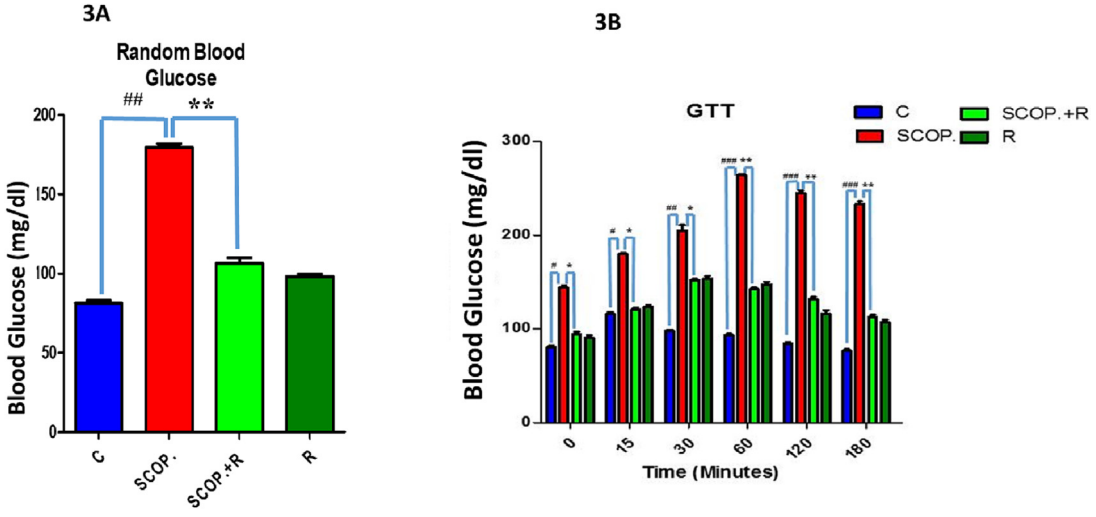

Fig. 3. Ranuncoside administration lowered the scopolamine induced high blood glucose level in mice. (**A**) Changes in the RBG level of all experimental groups are represented in a bar graph along with the statistics applied ($^{**, \#\#}$ $p < 0.01$) for the comparison. **(B)** GTT results (histogram) at 0 time interval or at any other time between 15 min and 3 h, showing that, in all cases, the SCOP group mice had a higher concentration of glucose, whereas the SCOP + R group had a lower concentration. *, # p < 0.05, **, ## p < 0.01, ***, ### p < 0.001 was the statistics applied to compare the C, SCOP, SCOP + R and R groups.

oxidative stress mediated by neuroinflammation and associated memory impairment *in vivo* by following the Nrf2 or NF-κB pathway in the brains of adult male albino mice. Moreover, ranuncoside could reduce oxidative stress by increasing the expression of endogenous antioxidant enzymes such as Nrf2 and HO-1, thereby improving memory. Likewise, ranuncoside suppressed NF-κB and its subsequent signaling molecules (i.e., TNF-α and COX-2) to minimize scopolamine-induced neuroinflammation in mice brains. On top of that, it has been revealed that scopolamine possesses a hyperglycemic effect that could be reduced by the hypoglycemic potency of ranuncoside. Moreover, ranuncoside has a hypolipidemic effect against scopolamine-induced hyperlipidemia. Those discoveries suggest that ranuncoside is a promising therapeutic medicine for treating Alzheimer's disease in the future.

For this purpose researchers have used different extracts as therapeutic agents that can effectively reduce the prevalence of Alzheimer's disease. An *in vivo* model of Aβ (25–35), or isoflurane-induced memory dysfunction, apigenin was used as treatment, revealed in memory assessment tasks (Kim et al., 2021). Lee et al., (2017) reported the neuroprotective effect of α-pinene (a volatile component) via spontaneous alternation behavior in a scopolamine-induced amnesia model. *Amanita caesarea* polysaccharides is also used to recover the memory impairment in a mouse model (Li et al., 2019). Similarly, scopolamine-induced memory deficits in humans were reverted to normal by physostigmine (Prohovnik et al., 1997). Likewise, scopolamine-induced memory deficits have been recovered by danshensu and donepezil (Bae et al., 2019) and a cyclopentanone derivative has

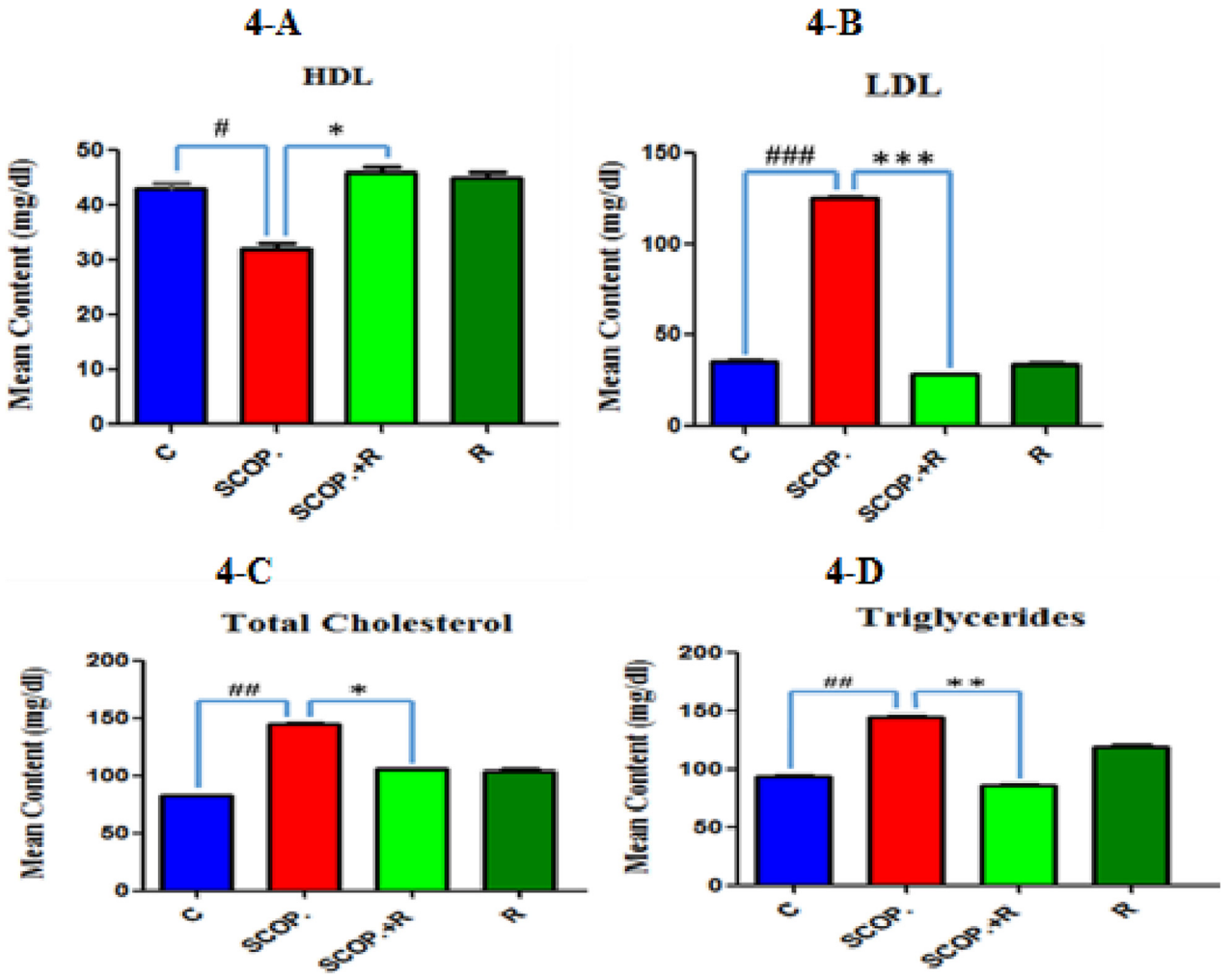

Fig. 4. Lipid profile test (HDL, LDL, Total cholesterol and Triglycerides) of mice were statistically compared between the C, SCOP, SCOP + R and R groups. The data was presented in the form of bar graphs with different *p* values ($^{*,\#}$ $p < 0.05$, $^{**,\#\#}$ $p < 0.01$, $^{***,\#\#\#}$ $p < 0.001$). The X-axis denotes the various experimental groups, whereas the Y-axis showed the amount of lipids (mg/dl).

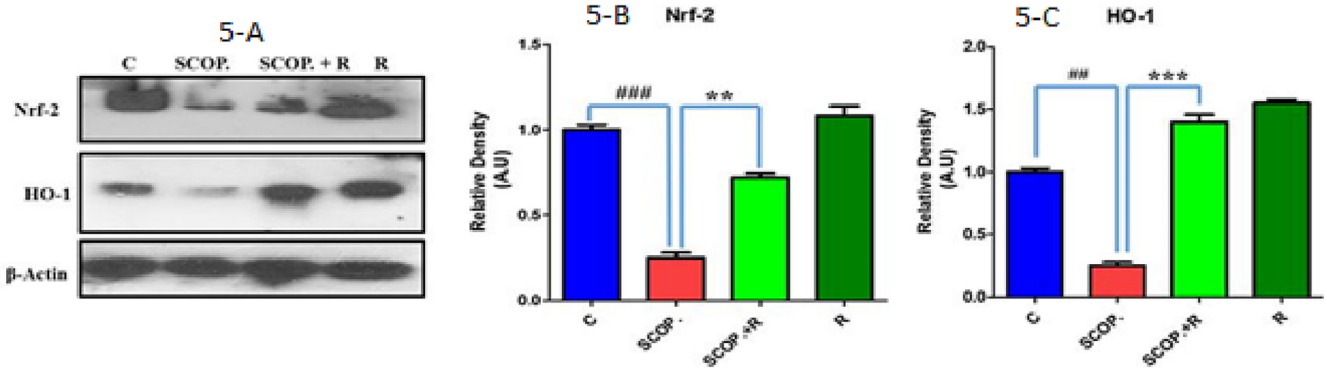

Fig. 5. Ranuncoside reduced oxidative stress amid the long-term administration of scopolamine in mice brains. **(A)** Western blot results of both the antioxidant enzymes (i.e., Nrf2 and HO-1) and β-actin were used to determine protein expression and interpret western blot results accurately on the scanned X-rays for all experimental groups. **(B-C)** Respective histograms of all experimental groups for Nrf2 and HO-1 protein expression along with the statistics applied ($^{**,\#\#}$ $p < 0.01$, $^{***,\#\#\#}$ $p < 0.001$) for comparison between the C, SCOP, SCOP + R and R groups.

also been identified as a memory enhancer in 5XF Alzheimer's disease mice (Ullah et al., 2019). Woo et al., (2020) have confirmed that administering *Euonymus alatus* extract had a memory-enhancing effect in scopolamine-treated mice. Another study has shown that Aβ (1–42)-treated mice had less interest in entering different arms of the Y-maze, which was later neutralized with the administration of quinovic acid and, in turn, enhanced the memory of mice (Saeed et al., 2020). The neurotherapeutic efficacy of lutein on scopolamine-induced memory loss in mice and zebrafish has also been documented (Patel et al., 2021).

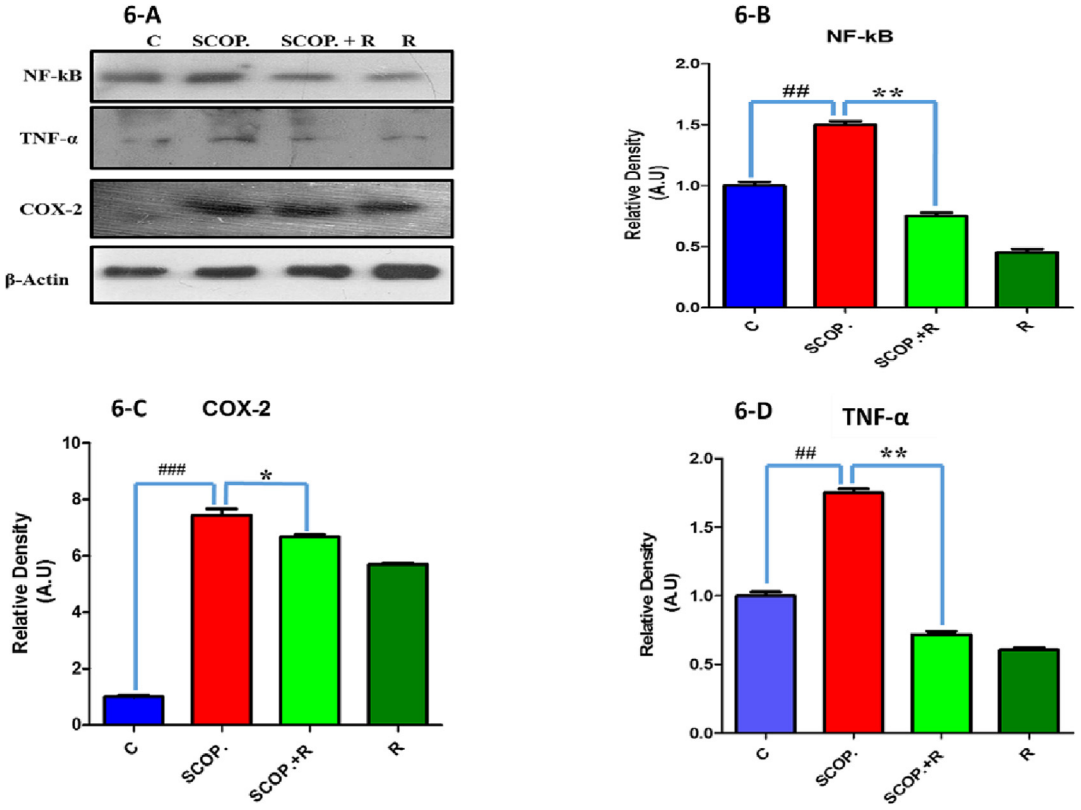

Fig. 6. Ranuncoside inhibited the upregulation of scopolamine-induced NF-κB and its downstream signaling (i.e., COX-2 and TNF-α) in mice brain. **(A)** Immunoblot results of NF-κB, TNF-α, COX-2, and β-actin proteins. **(B–D)** bar graphs of all four groups for the expression of NF-κB, COX-2 and TNF-α, proteins, in which *p* values ($^{*,\#}$ $p < 0.05$, $^{**,\#\#}$ $p < 0.01$, $^{***,\#\#\#}$ $p < 0.001$) denoted significant differences between the C, SCOP, SCOP + R and R groups.

During the MWM test, the mice of control group exhibited high activity comparatively other groups, and even on the day of probing their performance was relatively good. In contrast, the scopolamine treated group reported difficulty in locating the platform, leading poor performance on the day of probing. On the other hand, scopolamine + ranuncoside treated group demonstrated notable improvement, as well as positive results on the day of probing, indicating that ranuncoside successfully reversed their memory impairment induced by scopolamine. These findings highlight the beneficial impact of ranuncoside in ameliorating memory deficits in mice caused by scopolamine administration.

Our study evidenced that SCOP group had increased blood glucose concentration compared with all other groups, and exhibited the hyperglycemic properties of scopolamine. Consistent with our findings, others have reported that treating male mice with olanzapine raised their blood glucose levels (Medak et al., 2020), while a preliminary study by Uto et al., (2021) revealed the hyperglycemic activity of dexamethasone in male mice as well. Previous reports have documented the role of streptozotocin in inducing hyperglycemia in rats (Gupta et al., 2011), while its property of causing hyperglycemia in mice has also been reported (Wu et al., 2021).

As various multiple abnormalities are associated with hyperglycemic conditions, there is an imperative need for its effective treatments. Consequently, researchers have dedicated their efforts to explore novel therapeutic interventions aimed at treating hyperglycemia. Our study explored ranuncoside's anti-hyperglycemic effect against the effects of scopolamine in the mice leading to the establishment of new approaches for treating hyperglycemia. A study confirmed the ability of catalpol to lower blood glucose in mice (Wu et al., 2021), other revealed the ameliorating effect of aqueous Ajwa seeds extract on hyperglycemia induced by streptozotocin and nicotinamide in a rat model (Mani et al., 2022). The *Pluchea indica* tea's antihyperglycemic capability was reported by Sirichaiwetchakoon et al., (2021), hypoglycemic effect of doxepin on obese diabetic mice by Chen et al., (2020) and caffeoylquinic acid derivatives from *P. indica* mitigating effect on hyperglycemia was reported in previous studies (Araujo et al., 2015; Cui, 2009; Sirichaiwetchakoon et al., 2021; Wu et al., 2018).

The current biochemical analysis revealed that the administration of scopolamine induced hyperlipidemia in mice, leading to valuable information regarding the hyperlipidemic lowering properties of scopolamine. On other words, ranuncoside was able to reverse the effects of scopolamine on HDL, LDL, TC, and TG levels, thereby promoting a normal lipid profile. This finding enhances our understanding of the potential therapeutic options for managing hyperlipidemia and its associated complications. Different studies have also identified various compounds with potent hypolipidemic activity. Among their results, *Enterococcus faecium* WEFA23 reduced cholesterol levels in rats fed a high-fat diet (Barthold et al., 2020; Zhang et al., 2017), while the combination of probiotics reduced serum TC and LDL levels (Barthold et al., 2020; Bordoni et al., 2013). The use of atorvastatin to reverse hypercholesterolemia induced by a high-fat diet in rats has also been documented. Moreover, in a study on hyperlipidemia induced by a high-fat diet in rats, reduced serum TC, TG, and LDL levels were attributed to the ameliorative effects of 3,5,6,7,8,3′,4′-hepta methoxyflavone on HFD induced hyperlipidemia in rats (Feng et al., 2019). Other evidence suggests using nobiletin to improve hyperlipidemia in obese mice (Lee et al., 2013), and elevated serum lipid profile was shown to be inhibited by obtusifolin while counteracting hyperglycemia and oxidative stress in albino rats (Tang & Zhong, 2014).

The western blot analysis indicated that scopolamine blocked the expression of endogenous antioxidant enzymes in the brains

of the mice, including Nrf2 and HO-1. On the one hand, Nrf2 is a transcription factor that upregulates antioxidant enzymes in cellular defense amid oxidative stress. It also degrades ROS, while its upregulation increases the amount of HO-1 output, which reduces oxidative stress (Krajka-Kuźniak and Baer-Dubowska, 2021). On the other, HO-1 is an antioxidant enzyme that eliminates tau protein and possesses an anti-inflammatory effect (Fujita et al., 2012; Schipper et al., 2009). To extend studies involving the use of antioxidant drugs as therapeutic agents for scopolamine-induced oxidative stress in patients with Alzheimer's disease (Abu Almaaty et al., 2021), we evaluated ranuncoside's role as a potent antioxidant. We found that it upregulated the expression of Nrf2 and HO-1 proteins, thereby lessening scopolamine-induced oxidative stress. Recently, anthocyanin has been reported to reduce oxidative stress in D-galactose-treated rats (Rehman et al., 2017) and *Amanita caesarea* polysaccharides to reduce Nrf2-mediated oxidative stress (Li et al., 2019). In another study, vanillic acid attenuated oxidative stress in a free radical scavenging assay and, in turn, reduced the production of ROS (Ul Amin et al., 2017). Fig. 7 revealed the pathway that ranuncoside induces its potential neuroprotective effect by preventing neuroinflammation, generating reactive oxygen species, and recovering the memory deficits induced by scopolamine via the Nrf2 and/or NF-κB pathway.

Among our other findings, scopolamine-induced oxidative stress caused neuroinflammation in Alzheimer's disease mice by upregulating NF-κB and its subsequent signaling protein molecules (i.e., COX-2 and TNF-α), which are neuroinflammatory markers secreted by microglia. Increased ROS production stimulates NF-κB (Rehman et al., 2017), which has essential inflammatory properties, by producing proinflammatory cytokines and enzymes (Krajka-Kuźniak and Baer-Dubowska, 2021). In the brain, COX-2 mediates inflammation and is considered to be the best target for discovering a cure for Alzheimer's disease (Prabhakaran et al., 2021), while TNF-α also causes inflammation by over-activating microglial cells and producing inflammatory cytokines, thereby impairing memory (Chen et al., 2021; McCoy and Tansey, 2008; Morris et al., 2013).

The ranuncoside inactivated the expression of astrocytes and microglia in the Alzheimer's disease mice model by decreasing the Nrf2 and NF-κB inflammatory pathways and inflammatory cytokines, including TNF-α and COX-2. Those findings suggest that ranuncoside can inhibit the inflammatory signaling pathways in mice with Alzheimer's disease. Other researchers have shown that using forsythoside B inactivated astrocytes and microglia as well as inhibited neuroinflammation by suppressing NF-κB (Kong et al., 2020). NF-κB and its downstream signaling molecules have also been shown to be inhibited by another vital therapeutic agent, vitamin D (Ali et al., 2021), and vanillic acid's role in reducing Aβ-induced neuroinflammation has also been documented (Ul Amin et al., 2017).

5. Conclusion

Our study confirmed that ranuncoside could reduce scopolamine-induced oxidative stress and neuroinflammation by upregulating the Nrf2 or NF-κB pathway and improved memory in mice. It is a potent natural neurotherapeutic agent that can be used as a supplement in poly-drug treatment for Alzheimer's disease. Additionally, we discovered that scopolamine is a hyperglycemic agent; and introduced the promising effects of ranuncoside as an antihyperglycemic and antihyperlipidemic agent.

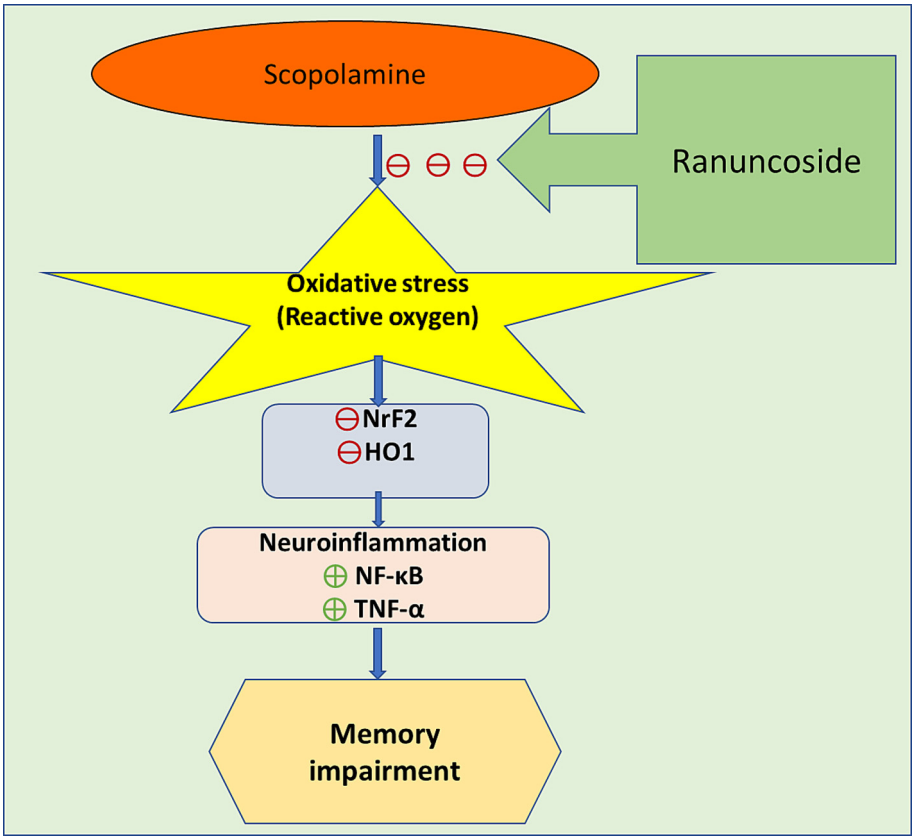

Fig. 7. Schematic diagram reveal that ranuncoside induces its potential anti-Alzheimer effect by preventing neuroinflammation, generating reactive oxygen species, and recovering the memory deficits induced by scopolamine via the Nrf2 and/or NF-κB pathway.

6. Recommendation

Although we analyzed ranuncoside's antioxidant and neuroprotective effects against scopolamine-induced oxidative stress and neuroinflammation leading to memory loss in mice, more detailed studies are recommended to determine the molecules that played pharmacological roles in our research.

7. Data availability statement

The authors declare that the data supporting the findings of this study are available upon request.

CRediT authorship contribution statement

Hafiza Sara Salahuddin: Conceptualization, Methodology, Validation, Resources, Supervision, Project administration. **Sobia Attaullah:** Conceptualization, Validation, Resources, Writing – review & editing. **Shahid Ali Shah:** Conceptualization, Resources, Validation, Writing – original draft. **SanaUllah Khan:** Investigation, Writing – review & editing, Visualization, Project administration. **Muhammad Zahid:** Formal analysis, Data curation. **Mujeeb Ullah:** Writing – review & editing, Data curation. **Khayyam:** Validation. **Sidra Salahuddin:** Investigation, Resources, Writing – original draft. **Seema Gul:** Investigation, Visualization, Resources. **Mahdi H Alsugoor:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abu Almaaty, A.H., Mosaad, R.M., Hassan, M.K., Ali, E.H.A., Mahmoud, G.A., Ahmed, H., Anber, N., Alkahtani, S., Abdel-Daim, M.M., Aleya, L., Hammad, S., 2021. Urtica dioica extracts abolish scopolamine-induced neuropathies in rats. Environmental Science and Pollution Research 28. https://doi.org/10.1007/s11356-020-12025-y.

Ali, A., Shah, S.A., Zaman, N., Uddin, M.N., Khan, W., Ali, A., Riaz, M., Kamil, A., 2021. Vitamin D exerts neuroprotection via SIRT1/nrf-2/ NF-kB signaling pathways against D-galactose-induced memory impairment in adult mice. Neurochem Int 142. https://doi.org/10.1016/j.neuint.2020.104893.

Araujo, C.M., Lúcio, K.D.P., Silva, M.E., Isoldi, M.C., de Souza, G.H.B., Brandão, G.C., Schulz, R., Costa, D.C., 2015. Morus nigra leaf extract improves glycemic response and redox profile in the liver of diabetic rats. Food Funct 6. https://doi.org/10.1039/c5fo00474h.

Bae, H.J., Sowndhararajan, K., Park, H.B., Kim, S.Y., Kim, S., Kim, D.H., Choi, J.W., Jang, D.S., Ryu, J.H., Park, S.J., 2019. Danshensu attenuates scopolamine and amyloid-β-induced cognitive impairments through the activation of PKA-CREB signaling in mice. Neurochem Int 131. https://doi.org/10.1016/j.neuint.2019.104537.

Barthold, D., Joyce, G., Brinton, R.D., Wharton, W., Kehoe, P.G., Zissimopoulos, J., 2020. Association of combination statin and antihypertensive therapy with reduced Alzheimer's disease and related dementia risk. PLoS One 15. https://doi.org/10.1371/journal.pone.0229541.

Bordoni, A., Amaretti, A., Leonardi, A., Boschetti, E., Danesi, F., Matteuzzi, D., Roncaglia, L., Raimondi, S., Rossi, M., 2013. Cholesterol-lowering probiotics: In vitro selection and in vivo testing of bifidobacteria. Appl Microbiol Biotechnol 97. https://doi.org/10.1007/s00253-013-5088-2.

Chen, Z., Liu, X., Luo, Y., Wang, J., Meng, Y., Sun, L., Chang, Y., Cui, Q., Yang, J., 2020. Repurposing doxepin to ameliorate steatosis and hyperglycemia by activating FAM3A signaling pathway. Diabetes 69. https://doi.org/10.2337/db19-1038.

Chen, R., Zhang, Q., Yan, Y., Zhang, Y., Zhang, T., 2021. Legumain Knockout Protects Against Aβ1–42-Induced AD-like Cognitive Deficits and Synaptic Plasticity Dysfunction Via Inhibiting Neuroinflammation Without Cleaving APP. Mol Neurobiol 58. https://doi.org/10.1007/s12035-020-02219-3.

Cui, C.-B., 2009. Inhibitory Activity of Caffeoylquinic Acids from the Aerial Parts of Artemisia princeps on Rat Lens Aldose Reductase and on the Formation of Advanced Glycation End Products. J Korean Soc Appl Biol Chem 52. https://doi.org/10.3839/jksabc.2009.109.

Farooq, U., Khan, T., Shah, S.A., Hossain, M.S., Ali, Y., Ullah, R., Raziq, N., Shahid, M., Capasso, R., 2021. Isolation, characterization and neuroprotective activity of folecitin: An in vivo study. Life 11. https://doi.org/10.3390/life11080825.

Feng, K., Zhu, X., Chen, T., Peng, B., Lu, M., Zheng, H., Huang, Q., Ho, C.T., Chen, Y., Cao, Y., 2019. Prevention of Obesity and Hyperlipidemia by Heptamethoxyflavone in High-fat Diet-induced Rats. J Agric Food Chem 67. https://doi.org/10.1021/acs.jafc.8b05632.

Fujita, K., Yamafuji, M., Nakabeppu, Y., Noda, M., 2012. Therapeutic approach to neurodegenerative diseases by medical gases: Focusing on redox signaling and related antioxidant enzymes. Oxid Med Cell Longev. https://doi.org/10.1155/2012/324256.

Gadhave, K., Kumar, D., Uversky, V.N., Giri, R., 2021. A multitude of signaling pathways associated with Alzheimer's disease and their roles in AD pathogenesis and therapy. Med Res Rev. https://doi.org/10.1002/med.21719.

Gul, S., Attaullah, S., Alsugoor, M.H., Bawazeer, S., Shah, S.A., Khan, S.. ..Ullah, M., 2023. Folicitin abrogates scopolamine induced oxidative stress, hyperlipidemia mediated neuronal synapse and memory dysfunction in mice. Heliyon. 15 9 (6). https://doi.org/10.1016/j.heliyon.2023.e16930.

Gupta, R., Sharma, A.K., Dobhal, M.P., Sharma, M.C., Gupta, R.S., 2011. Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia. J Diabetes 3. https://doi.org/10.1111/j.1753-0407.2010.00107.x.

Huang, F., Zhang, F., Xu, D., Zhang, Z., Xu, F., Tao, X., Qiu, L., Wei, H., 2018. Enterococcus faecium WEFA23 from infants lessens high-fat-diet-induced hyperlipidemia via cholesterol 7-alpha-hydroxylase gene by altering the composition of gut microbiota in rats. J Dairy Sci 101. https://doi.org/10.3168/jds.2017-13713.

Imran, I., Javaid, S., Waheed, A., Rasool, M.F., Majeed, A., Samad, N., Saeed, H., Alqahtani, F., Ahmed, M.M., Alaqil, F.A., 2021. Grewia asiatica Berry Juice Diminishes Anxiety, Depression, and Scopolamine-Induced Learning and Memory Impairment in Behavioral Experimental Animal Models. Front Nutr 7. https://doi.org/10.3389/fnut.2020.587367.

Jahan, R., Yousaf, M., Khan, H., Bibi, N., Ijaz, M., Rehan, T., Shah, S.A., 2022. ZOMEC via the p-Akt/Nrf2 Pathway Restored PTZ-Induced Oxidative Stress-Mediated Memory Dysfunction in Mouse Model. BioMed Research International. https://doi.org/10.1155/2022/8902262.

Khan, T.J., Ahmed, Y.M., Zamzami, M.A., Mohamed, S.A., Khan, I., Baothman, O.A.S., Mehanna, M.G., Yasir, M., 2018. Effect of atorvastatin on the gut microbiota of high fat diet-induced hypercholesterolemic rats. Sci Rep 8. https://doi.org/10.1038/s41598-017-19013-2.

Kim, Y.J., Kim, J.H., He, M.T., Lee, A.Y., Cho, E.J., 2021. Apigenin ameliorates scopolamine-induced cognitive dysfunction and neuronal damage in mice. Molecules 26. https://doi.org/10.3390/molecules26175192.

Kong, F., Jiang, X., Wang, R., Zhai, S., Zhang, Y., Wang, D., 2020. Forsythoside B attenuates memory impairment and neuroinflammation via inhibition on NF-κB signaling in Alzheimer's disease. J Neuroinflammation 17. https://doi.org/10.1186/s12974-020-01967-2.

Krajka-Kuźniak, V., Baer-Dubowska, W., 2021. Modulation of Nrf2 and NF-κB signaling pathways by naturally occurring compounds in relation to cancer prevention and therapy. Are combinations better than single compounds? Int J Mol Sci. https://doi.org/10.3390/ijms22158223.

Lee, Y.S., Cha, B.Y., Choi, S.S., Choi, B.K., Yonezawa, T., Teruya, T., Nagai, K., Woo, J.T., 2013. Nobiletin improves obesity and insulin resistance in high-fat diet-induced obese mice. Journal of Nutritional Biochemistry 24. https://doi.org/10.1016/j.jnutbio.2012.03.014.

Lee, G.Y., Lee, C., Park, G.H., Jang, J.H., 2017. Amelioration of Scopolamine-Induced Learning and Memory Impairment by α -Pinene in C57BL/6 Mice. Evidence-based Complementary and Alternative Medicine 2017. https://doi.org/10.1155/2017/4926815.

Li, Z., Chen, X., Zhang, Y., Liu, X., Wang, C., Teng, L., Wang, D., 2019. Protective roles of Amanita caesarea polysaccharides against Alzheimer's disease via Nrf2 pathway. Int J Biol Macromol 121. https://doi.org/10.1016/j.ijbiomac.2018.09.216.

Lucey, B.P., 2020. It's complicated: The relationship between sleep and Alzheimer's disease in humans. Neurobiol Dis. https://doi.org/10.1016/j.nbd.2020.105031.

Majdi, A., Sadigh-Eteghad, S., Rahigh Aghsan, S., Farajdokht, F., Vatandoust, S.M., Namvaran, A., Mahmoudi, J., 2020. Amyloid-β, tau, and the cholinergic system in Alzheimer's disease: Seeking direction in a tangle of clues. Rev Neurosci 31. https://doi.org/10.1515/revneuro-2019-0089.

Mani, V., Arfeen, M., Sajid, S., Almogbel, Y., 2022. Aqueous Ajwa dates seeds extract improves memory impairment in type-2 diabetes mellitus rats by reducing blood glucose levels and enhancing brain cholinergic transmission. Saudi J Biol Sci 29. https://doi.org/10.1016/j.sjbs.2021.12.060.

McCoy, M.K., Tansey, M.G., 2008. TNF signaling inhibition in the CNS: Implications for normal brain function and neurodegenerative disease. J Neuroinflammation. https://doi.org/10.1186/1742-2094-5-45.

Medak, K.D., Shamshoum, H., Peppler, W.T., Wright, D.C., 2020. GLP1 receptor agonism protects against acute olanzapine-induced hyperglycemia. Am J Physiol Endocrinol Metab 319. https://doi.org/10.1152/AJPENDO.00309.2020.

Morris, G.P., Clark, I.A., Zinn, R., Vissel, B., 2013. Microglia: A new frontier for synaptic plasticity, learning and memory, and neurodegenerative disease research. Neurobiol Learn Mem 105. https://doi.org/10.1016/j.nlm.2013.07.002.

Naseer, M.I., Ullah, I., Narasimhan, M.L., Lee, H.Y., Bressan, R.A., Yoon, G.H., Yun, D.J., Kim, M.O., 2014. Neuroprotective effect of osmotin against ethanol-induced apoptotic neurodegeneration in the developing rat brain. Cell Death Dis. 5 (3), e1150–e.

Noroozi, N., Shayan, M., Maleki, A., Eslami, F., Rahimi, N., Zakeri, R., Dehpour, A.R., 2022. Protective effects of dapsone on scopolamine-induced memory impairment in mice: Involvement of nitric oxide pathway. Dementia and Geriatric Cognitive Disorders Extra 12 (1), 43–50.

Parodi-Rullán, R., Sone, J.Y., Fossati, S., Albensi, B., 2019. Endothelial Mitochondrial Dysfunction in Cerebral Amyloid Angiopathy and Alzheimer's Disease. Journal of Alzheimer's Disease. https://doi.org/10.3233/JAD-190357.

Patel, C., Patel, P., Sarkar, D., Bulsara, J., Soni, A., Isapure, K., Acharya, S., 2021. Neuroprotective Effect of Lutein in Scopolamine-Induced Alzheimer's Disease in Mice and Zebrafish. Revista Brasileira de Farmacognosia 31. https://doi.org/10.1007/s43450-021-00202-0.

Pellegrini, C., Fornai, M., Antonioli, L., Blandizzi, C., Calderone, V., 2019. Phytochemicals as novel therapeutic strategies for nlrp3 inflammasome-related neurological, metabolic, and inflammatory diseases. Int J Mol Sci. https://doi.org/10.3390/ijms20122876.

Prabhakaran, J., Molotkov, A., Mintz, A., Mann, J.J., 2021. Progress in pet imaging of neuroinflammation targeting cox-2 enzyme. Molecules. https://doi.org/10.3390/molecules26113208.

Prohovnik, I., Arnold, S.E., Smith, G., Lucas, L.R., 1997. Physostigmine reversal of scopolamine-induced hypofrontality. Journal of Cerebral Blood Flow and Metabolism 17. https://doi.org/10.1097/00004647-199702000-00012.

Raziq, N., Saeed, M., Ali, M.S., Zafar, S., Shahid, M., Lateef, M., 2017. A new glycosidic antioxidant from Ranunculus muricatus L. (Ranunculaceae) exhibited lipoxygenasae and xanthine oxidase inhibition properties. Nat Prod Res 31. https://doi.org/10.1080/14786419.2016.1236098.

Rehman, S.U., Shah, S.A., Ali, T., Chung, J. il, Kim, M.O., 2017. Anthocyanins Reversed D-Galactose-Induced Oxidative Stress and Neuroinflammation Mediated Cognitive Impairment in Adult Rats. Mol Neurobiol 54. https://doi.org/10.1007/s12035-015-9604-5.

Saeed, K., Shah, S.A., Ullah, R., Alam, S.I., Park, J.S., Saleem, S., Jo, M.H., Kim, M.W., Hahm, J.R., Kim, M.O., 2020. Quinovic Acid Impedes Cholesterol Dyshomeostasis, Oxidative Stress, and Neurodegeneration in an Amyloid- β -Induced Mouse Model. Oxid Med Cell Longev 2020. https://doi.org/10.1155/2020/9523758.

Sandhu, M., Irfan, H.M., Shah, S.A., Ahmed, M., Naz, I., Akram, M., Farooq, A.S., 2022. Friedelin Attenuates Neuronal Dysfunction and Memory Impairment by Inhibition of the Activated JNK/NF-κB Signalling Pathway in Scopolamine-Induced Mice Model of Neurodegeneration. Molecules 27 (14), 4513.

Schipper, H.M., Song, W., Zukor, H., Hascalovici, J.R., Zeligman, D., 2009. Heme oxygenase-1 and neurodegeneration: Expanding frontiers of engagement. J Neurochem. https://doi.org/10.1111/j.1471-4159.2009.06160.x.

Serafín, V., Gamella, M., Pedrero, M., Montero-Calle, A., Razzino, C.A., Yáñez-Sedeño, P., Barderas, R., Campuzano, S., Pingarrón, J.M., 2020. Enlightening the advancements in electrochemical bioanalysis for the diagnosis of Alzheimer's disease and other neurodegenerative disorders. J Pharm Biomed Anal. https://doi.org/10.1016/j.jpba.2020.113437.

Shah, S.A., Yoon, G.H., Ahmad, A., Ullah, F., Amin, F.U., Kim, M.O., 2015. Nanoscale-alumina induces oxidative stress and accelerates amyloid beta (Aβ) production in ICR female mice. Nanoscale 7. https://doi.org/10.1039/c5nr03598h.

Shin, S.J., Jeon, S.G., Kim, J. il, Jeong, Y.O., Kim, S., Park, Y.H., Lee, S.K., Park, H.H., Hong, S.B., Oh, S., Hwang, J.Y., Kim, H.S., Park, H., Nam, Y., Lee, Y.Y., Kim, J.J., Park, S.H., Kim, J.S., Moon, M., 2019. Red ginseng attenuates aβ-induced mitochondrial dysfunction and aβ-mediated pathology in an animal model of alzheimer's disease. Int J Mol Sci 20. Doi: 10.3390/ijms20123030

Sirichaiwetchakoon, K., Churproong, S., Kupittayanant, S., Eumkeb, G., 2021. The Effect of Pluchea indica (L.) Less. Tea on Blood Glucose and Lipid Profile in People with Prediabetes: A Randomized Clinical Trial. Journal of Alternative and Complementary Medicine 27. https://doi.org/10.1089/acm.2020.0246.

Syed, Z., Shal, B., Azhar, A., Amanat, S., Khan, A., Ali, H., Khan, S., 2022. Pharmacological mechanism of xanthoangelol underlying Nrf-2/TRPV1 and anti-apoptotic pathway against scopolamine-induced amnesia in mice. Biomedicine & Pharmacotherapy 150, 113073.

Tang, K.S., 2019. The cellular and molecular processes associated with scopolamine-induced memory deficit: A model of Alzheimer's biomarkers. Life Sci. https://doi.org/10.1016/j.lfs.2019.116695.

Tang, Y., Zhong, Z., 2014. Obtusifolin Treatment Improves Hyperlipidemia and Hyperglycemia: Possible Mechanism Involving Oxidative Stress. Cell Biochem Biophys 70. https://doi.org/10.1007/s12013-014-0124-0.

Uddin, M.S., Rahman, M.A., Kabir, M.T., Behl, T., Mathew, B., Perveen, A., Barreto, G. E., Bin-Jumah, M.N., Abdel-Daim, M.M., Ashraf, G.M., 2020. Multifarious roles of mTOR signaling in cognitive aging and cerebrovascular dysfunction of Alzheimer's disease. IUBMB Life. https://doi.org/10.1002/iub.2324.

Ul Amin, F., Shah, S.A., Kim, M.O., 2017. Vanillic acid attenuates Aβ1-42-induced oxidative stress and cognitive impairment in mice. Sci Rep 7. https://doi.org/10.1038/srep40753.

Ullah, R., Khan, M., Shah, S.A., Saeed, K., Kim, M.O., 2019. Natural antioxidant anthocyanins—a hidden therapeutic candidate in metabolic disorders with major focus in neurodegeneration. Nutrients. https://doi.org/10.3390/nu11061195.

Uto, A., Miyashita, K., Endo, S., Sato, M., Ryuzaki, M., Kinouchi, K., Mitsuishi, M., Meguro, S., Itoh, H., 2021. Transient Dexamethasone Loading Induces Prolonged Hyperglycemia in Male Mice with Histone Acetylation in Dpp-4 Promoter. Endocrinology (United States) 162. https://doi.org/10.1210/endocr/bqab193.

Wang, W., Zhao, F., Ma, X., Perry, G., Zhu, X., 2020. Mitochondria dysfunction in the pathogenesis of Alzheimer's disease: Recent advances. Mol Neurodegener. https://doi.org/10.1186/s13024-020-00376-6.

Winny Yun, J., Washington, C., McCormick, J., Stevenson, E., Steven Alexander, J., 2021. Amyloid beta peptides and Th1 cytokines modulate human brain vascular smooth muscle tonic contractile capacity in vitro: Relevance to alzheimer's disease? Pathophysiology 28. https://doi.org/10.3390/pathophysiology28010006.

Woo, Y., Lim, J.S., Oh, J., Lee, J.S., Kim, J.S., 2020. Neuroprotective effects of euonymus alatus extract on scopolamine-induced memory deficits in mice. Antioxidants 9. https://doi.org/10.3390/antiox9050449.

Wu, X., Wang, J., Song, L., Guan, Y., Cao, C., Cui, Y., Zhang, Y., Liu, C., 2021. Catalpol Weakens Depressive-like Behavior in Mice with Streptozotocin-induced Hyperglycemia via PI3K/AKT/Nrf2/HO-1 Signaling Pathway. Neuroscience 473. https://doi.org/10.1016/j.neuroscience.2021.07.029.

Wu, T., Yang, L., Guo, X., Zhang, M., Liu, R., Sui, W., 2018. Raspberry anthocyanin consumption prevents diet-induced obesity by alleviating oxidative stress and modulating hepatic lipid metabolism. Food Funct 9. https://doi.org/10.1039/c7fo02061a.

Yadang, F.S.A., Nguezeye, Y., Kom, C.W., Betote, P.H.D., Mamat, A., Tchokouaha, L.R. Y., Bum, E.N., 2020. Scopolamine-induced memory impairment in mice: neuroprotective effects of Carissa edulis (Forssk.) Valh (Apocynaceae) aqueous extract. International Journal of Alzheimer's Disease.

Zhang, F., Qiu, L., Xu, X., Liu, Z., Zhan, H., Tao, X., Shah, N.P., Wei, H., 2017. Beneficial effects of probiotic cholesterol-lowering strain of Enterococcus faecium WEFA23 from infants on diet-induced metabolic syndrome in rats. J Dairy Sci 100. https://doi.org/10.3168/jds.2016-11870.

Zhu, F., Li, C., Chu, F., Tian, X., Zhu, J., 2020. Target Dysbiosis of Gut Microbes as a Future Therapeutic Manipulation in Alzheimer's Disease. Front Aging Neurosci. https://doi.org/10.3389/fnagi.2020.544235.