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Phloridzin Neuroprotective Potential in Alleviating Scopolamine-Induced Alzheimer's Disease in Male Wistar Rats

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KEYWORDS

Phloridzin, Cholinergic pathways, Scopolamine, Alzheimer's, Neuroprotective effect

ABSTRACT:

Introduction: The increasing prevalence of Alzheimer's disease (AD) has necessitated the search for effective therapeutic agents capable of mitigating its progression. Phloridzin, has been the subject of numerous studies highlighting its protective effects in various neurodegenerative disorders

Objectives: The main objective of this study was to evaluate the neuroprotective effect of Phloridzin against scopolamine-induced Alzheimer's disease in Wistar rats

Methods: Alzheimer's was induced by scopolamine in male Wistar rats. After 7 days of acclimatization, the rats were administered daily intraperitoneal treatment with scopolamine (0.7 mg/kg), and Phloridzin (10 mg/kg) was given orally for 13 days. The neuro-cognitive function of treated rats was evaluated by the Morris Water Maze test, along with assessments of locomotor activity, AChE activity, protein levels, antioxidant parameters, and brain histopathology (hippocampus).

Results: Scopolamine treated rats have shown a significant (*P<0.05) increase in AChE levels as compared to the normal group. However, AChE levels significantly (*P<0.05) decreased after the treatment with Phloridzin and a similar result was found in the total protein. Scopolamine administered rats were observed to have and to have an enhanced MDA level compared to NC, which was significantly (*P<0.05) reversed in Phloridzin-treated rats, indicating reduced lipid peroxidation. Reduced glutathione levels and glutathione peroxidase were significantly (*P<0.05) decreased, and SOD and catalase were significantly (*P<0.05) reduced in scopolamine-treated rats. These levels significantly increased after Phloridzin treatment.

Conclusions: Phloridzin improved the neuroprotective effect against Alzheimer's disease and enhanced neuronal signaling pathways, specifically cholinergic pathways. This drug has shown potent efficacy and therapeutic potential against scopolamine-treated rats in Alzheimer's disease

1. Introduction

Phloridzin is a natural compound and a dietary constituent that is found in fruit trees [apple]. It has been in use for over 150 years as a tool for physiology research, as a pharmaceutical, and as the as the latest data on the on the prevalence of Alzheimer’s disease. Over 45 million people worldwide are affected by dementia, and the main cause of Alzheimer’s disease (AD), is responsible for sixty-eight percent of cases. It is estimated that between 5 and 6 million Americans ages sixty-five and older have AD, and this count may reach 14 million by 2050 [1]. A rise in the count will raise the economic burden. The pathophysiology of AD, which contains extravascular beta-amyloid (β-amyloid) plaque aggregation and intra-cellular gliofibrillary and neurofibrillary tangles, forms hyperphosphorylated tau protein. According to recent research studies, AD remains a clinical assessment, although brain and spine fluids (cerebrospinal fluid) and positron emission tomography may enhance diagnostic perfection, which creates the abnormal neurosynaptic signal and neuron

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signal transmission [2]. A huge number of studies have investigated the function of neuroinflammation in AD. In the beginning, neuroinflammation is found only in the end stage of the disease as a response to the sequelae of neurological differences found in the AD brain. However, recently, it has been revealed through studies of preclinical as well as clinical that immune system stimulation occurs at initiation in AD and that it plays a main role in its pathogenesis. Additionally, abnormal function of microglial clearance of β- amyloid plaques, massive release and production of chemokines and cellular kinase at the position of Aβ plaques, and the switch on the complimentary pathways and microglial activation play a vital role in intensifying the progression of AD [3]. Administration of Scopolamine is considered a psychopharmacological model of Alzheimer's disease (AD). In the context of AD, it is causing cholinergic abnormal function and enhancing Aβ deposition; these two are biomarkers of the disease. Aggregation of senile plaques and neurofibrillary tangles are the pathological features of AD. Amyloid-β (Aβ) protein is mainly responsible for senile plaques, and neurofibrillary tangles are neuronal intracellular structures composed of a hyperphosphorylated form of tau. Amyloid-β, which can be degraded through neprilysin, is derived from the protein APP precursor by the sequence of proteolytic fragmentation by β- and γ-secretases. Scopolamine may induce Amyloid-β accumulation in the brain and also cause the degeneration and atrophy of the brain in experimental rats. For example, if administration of scopolamine (2 mg/kg/day) intraperitoneally for 45 days in Wistar rats (male) enhanced APP mRNA expression (2.7-fold), Amyloid-β protein counts (2.4-fold), it would degrade the result of mRNA (0.35-fold). Scopolamine, which elevated a greater than 2-fold phosphorylated tau protein level, reduced the gene functions of tau protein. The finding strengthens the significance of posttranslational phosphorylation changes in intensifying tau pathology [4]. Tau protein kinase, which is improved by Scopolamine, also called microtubule affinity-regulating kinase 2, promotes tau hyperphosphorylation. Scopolamine enhances protein expression and glycogen synthase kinase 3β mRNA, which is also involved in AD pathogenesis via reduction of acetylcholine synthesis and up-regulation of Aβ production. It reduced acetylcholine levels to greater than 74% in the brains of Wistar rats (male). Further, scopolamine suppresses protein expression and choline acetyltransferase activity [5]. Scopolamine is a muscarinic receptor antagonist, resulting in the concomitant appearance of transient and cognitive amnesia electrophysiological changes that resemble those observed in AD and produce a blocking of the activity of the muscarinic acetylcholine receptors [6, 7]. Scopolamine induces the accumulation of tau plaque and amyloid beta; oxidative stress and apoptosis also cause brain neuronal death [8]. Scopolamine enhances the biomarkers that indicate mitochondrial dysfunction, neuroinflammation, and apoptosis in the brain [9]. Phloridzin is the main constituent of the unripe Malus root bark of apples, also obtained from strawberries. It is plentiful in vegetative parts (bark and leaves) and seeds. Very close to Pyrus communis [10]. Phloridizin is derived from Phloretin, which belongs to bicyclic flavonoids and is a phenolic phytoconstituent and a glucoside [1]. This compound has been investigated widely for pharmacological properties, including antioxidant, antidiabetic, and anti-inflammatory activities. Some researchers have analyzed phloridzin as a neuroprotective drug. It has also been reported that antidepressant activities. In earlier studies, Phloridzin has shown nootropic, neurotrophic, and neuroprotective activities against scopolamine-induced amnesia [3]. Phloridzin binds to the H2S signaling pathway, after which it reduces ROS production and oxidative damage, increases antioxidant enzyme activities, and inhibits the fungal infection, so according to [11] its mode of action and antioxidant properties, it is hypothesized that it shows a good effect in AD and provides good efficacy. Phloridzin's neuroprotective effects against scopolamine-induced Alzheimer's in male Wistar rats include elevating cholinergic function and reducing oxidative stress. These results suggest that phenol is a potential therapeutic agent for Alzheimer's disease, although further research is needed to fully clarify its therapeutic effects and mechanisms of action. Some previous studies suggest that Phloridzin has neuroprotective effects, antioxidant, anti-inflammatory, and antidiabetic properties [3]. The current study aims to investigate its neuroprotective effect against scopolamine-induced Alzheimer's in male Wistar rats by using the Morris Water Maze test and various biochemical parameters. The hypotheses of this study are that Phloridzin treated rats will elevate antioxidant enzyme activities, such as catalase and superoxide dismutase, against scopolamine-treated rats. Phloridzin will exert neuroprotective effects against scopolamine induced hippocampal damage. We selected male Wistar rats for their genetic background and well-characterized

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physiology, which makes them suitable for studying Alzheimer's. They were also used to induce Alzheimer's due to their ability to block acetylcholine receptors, decrease the disease's pathology. This model is a suitable and relevant platform to evaluate Phloridzin neuroprotective effects and its potential as an Alzheimer's treatment.

2. Objectives

The main objective of the current study was to investigate the potential neuroprotective effect of Phloridzin against scopolamine-induce Alzheimer's disease. Multiple studies have highlighted the protective effects of Phloridzin in numerous neurodegenerative disorders.

3. Methods

Animals

Wistar rats (male) weighing 250 ± 10 g were taken from the CDRI, India, and were maintained under standard animal husbandry protocol with access to water and food. The temperature was maintained at 21 ± 3 °C. Ethical approval for this study protocol was obtained from the Institutional Animal Ethics Committee (IAEC No.: IU/IAEC/21/04) under CPCSEA guidelines.

Treatments

All drugs used in this study were sourced as follows: Phloridzin from Cayman Chemical Company and scopolamine from INSCHEM. The rats were grouped into four groups, each consisting of five animals: normal control (NC), scopolamine (0.7 mg/kg; i.p.), scopolamine + Donepezil (0.5 mg/kg), and scopolamine + phloridzin (10 mg/kg), over 13 days [3, 12, 13]. Doses were calculated based on standard dose calculation methods. Locomotor activities were assessed before and after the Morris water maze (MWM) on days 6 and 13. The MWM test took place from the 8th to the 12th day, followed by euthanasia on the 14th day, with brain samples collected for biochemical analysis [13].

Assessment of behavioral parameters

Locomotor Activity

The locomotor test was measured by a digital actophotometer (LABGO) in a controlled environment to minimize external disturbances. The apparatus recorded the number of times the light beam was penetrated by the rats, and the result was counted every 5 minutes [14].

Morris Water Maze (MWM)

The MWM test is responsible for spatial learning and memory [15]. Rats were practiced in a circular pool of 180 cm diameter and 60 cm depth with a portable platform. The circular pool was separated into four quadrants (Q1–Q4) and recorded their escape latency time (ELT) during practice [16]. The platform was removed on the 12th day, and rats were left to explore for 90 seconds in the pool, and time spent in the target quadrant (TSTQ) was recorded as a measure of retrieval.

Preparation of the Brain Homogenate

Following euthanasia, brains were collected, rinsed with normal saline (NS), and homogenized with 0.1 M phosphate buffer (pH 7.4) at a 10:1 ratio (w/v). Centrifuged at 10,000 rpm for 10 minutes using a REMI CM-12 PLUS cooling microcentrifuge, and the collected supernatant was utilized for biochemical parameters [13].

Estimation of Biochemical Parameters

Protein Estimation

The Bradford method was employed to quantify protein content in brain tissue. A mixture of brain homogenate sample (20 µL) and Bradford reagent (200 µL) was left for incubation for up to 15 minutes at 37 °C. Measured the absorbance at 596 nm by a UV spectrophotometer (UV-1800 Shimadzu spectrophotometer, Japan). Bovine serum albumin (0.1–1 mg) was used as a standard. The protein compound in the brain sample was in mg/µ [13].

Estimation of Acetylcholine Esterase

The assessment of acetylcholinesterase activity was performed by the Ellman protocol. We combined 50 microliters of brain homogenate, 3 mL of 0.1 M phosphate buffer (pH 8), 0.1 mL of 14 mM Ach iodide, and 0.1 mL of 10 mM 5,5-dithiobis (2-nitrobenzoate) and properly blended the solution. Afterward, the solution was left for 5 minutes for incubation. We then recorded the increase in absorbance at 412 nm for 2 minutes at 30-second intervals using a UV-1800 Shimadzu spectrophotometer from Japan. Acetylcholinesterase activity was quantified in enzyme units per milligram (U/mg) of protein [13, 17, 18].

Estimation of Reduced Glutathione (GSH)

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The GSH concentration was determined by UV spectrophotometry. For this, a reaction mixture was prepared with 50 µL of the tissue supernatant, 1.1 mL of 0.25 M SPB (pH 7.4), and 130 µL of a 0.04% solution of 5,5-dithiobis (2-nitrobenzoate).

Then make up the volume with distilled water (1.5 mL), and then record the absorbance at 412 nm. The expression was reported in micromoles per milligram of protein [19, 20].

Estimation of Glutathione Peroxidase (GPx)

The analysis of GPx followed the procedure outlined in [21]. Two hundred microliters of the supernatant were combined with a solution comprising 1 mL of 0.4 M phosphate buffer (pH 7.0), 1 mL of 5 mM NaN3, and 1 mL of 4 mM glutathione. After a pre-incubation of 5 minutes at 37 °C, 1 mL of 4 mM hydrogen peroxide was added, and after this, the mixture was left for 5 minutes for incubation. The outcomes were presented as micromoles per milligram of protein.

Estimation of Catalase

Catalase activity was performed by a previous study [22]. A volume of 0.1 mL of the supernatant was mixed with 2.9 mL of a 10 mM hydrogen peroxide solution in a 50 mM potassium phosphate buffer (pH 7). The decreased absorbance (240 nm) was recorded for 3 minutes by UV spectrophotometer. The findings were reported as µm/mg per protein.

Estimation of Superoxide Dismutase

Superoxide dismutase (SOD) activity was assessed using the specified procedure. A mixture consisting of 0.1 mL of 2.6 mM pyrogallol solution in 10 mM HCl, 2.8 mL of 0.1 M PPB (pH 7.4), and 0.1 mL of brain homogenate was prepared. The absorbance rate increase was monitored at 325 nm for around 3 minutes. One unit of SOD was represented as the quantity required to inhibit fifty percent of pyrogallol in a 3 mL solution. The results were reported as µm/mg per protein [13, 20].

Estimation of Malondialdehyde (MDA)

We quantitatively assessed malondialdehyde (MDA), the final result of lipid peroxidation, in brain homogenate following the reported method [23]. To prepare the sample, 0.2 mL of tissue homogenate was mixed with 0.2 mL of 8.1% (w/v) sodium dodecyl sulfate, 1.5 mL of 0.8% (w/v) TBA, acetic acid (pH 3.50); 1.5 mL of 20% (v/v). The mixture underwent incubation (95 °C) in a water bath for 60 minutes. Then the solution was added to 1 mL of distilled water, followed by a mixture of n-butanol (5 mL) and pyridine (15:1 v/v ratio). Subsequently, after centrifugation at 4000 rpm for 10 minutes, the supernatant absorbance was measured at 532 nm. The concentration of MDA results in micromoles per milligram of protein.

Histopathological examination

After isolated hippocampus section of brain from Wistar rat. The brain samples fixed in formalin (10%) were further processed and fixed in paraffin blocks to obtain 5 µm sections. These sections were stained with haematoxylin (H) and eosin (E). Finally, the stained sections were observed under a light microscope.

Statistical analysis

The data were presented as the standard error of their means [mean ± SEM]. The behavioral parameters of escape latency and TSTQ were tested by a one-way ANOVA followed by the Dunnett's test. The biochemical parameter data were analyzed by a one-way ANOVA followed by the Dunnett's test. Statistical analysis was carried out by GraphPad Prism 5 software (8.0.1). A value of significant (*P < 0.05), very significant (**P < 0.01), and highly significant (***P < 0.001) was considered significant

4. Results

Effects of Phloridzin on Behavioral Parameters

Locomotor Activity: During the studies on the sixth and thirteenth days of assessment, the scopolamine-treated rat group exhibited a significant (*P < 0.05) higher locomotor activity compared to the NC group. The Phloridzin group displayed a significant (*P < 0.05) reduction in locomotor activity in comparison to the toxic control group. No inference of sedative effects was observed in the Morris Water Maze results. Consequently, ELT and TSTQ in MWM indicated enhanced memory (Figure 1A). The observation of ELT was done between the 8th and 11th days of the experimental schedule. On the 8^{th}, 9^{th}, and 10^{th} days, significant (*P < 0.05) changes were observed in the toxic control group rats and Phloridzin group rats. On the eleventh day, ELT was lower in all treated groups, with significant (*P < 0.05) differences between the normal groups and the Phloridzin groups. On the twelfth day, the TSTQ was measured, serving as the recovery.

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Scopolamine group rats spent less time in the target quadrant (TSTQ) as compared to normal, Phloridzin and donepezil-treated rats are spent time significantly (*P < 0.05) more time than scopolamine treated rats (Figure 1B, 1C). Behavioral activities during acquisition and retrieval trials: ELT and TSTQ were evaluated during learning and memory. It is significant that the MWM test, which investigates memory and spatial learning, was employed to assess differences in the central cholinergic system. In the MWM test, Wistar rats exhibited a significant reduced in ELT on the 4th day compared to day 1, which refers to standard learning capacity. Additionally, on the 5th day of assessment, there was a significant enhancement in TSTQ compared with other quadrants' time spent, i.e., indicating normal retrieval capacity. These findings indicate that scopolamine has the ability to disrupt the typical learning and memory functions of male Wistar rats. However, when Phloridzin was given for 13 consecutive days, it significantly reversed the learning and memory dysfunction. The analysis of locomotor activity did not reveal any evidence of sedative effects in the MWM test. Instead, it was evident that time spent target quadrant and escape latency time in the Morris water maze were the key factors contributing to improved memory. As a result, Phloridzin appears to have the potential to enhance long-term memory against scopolamine-administered rats.

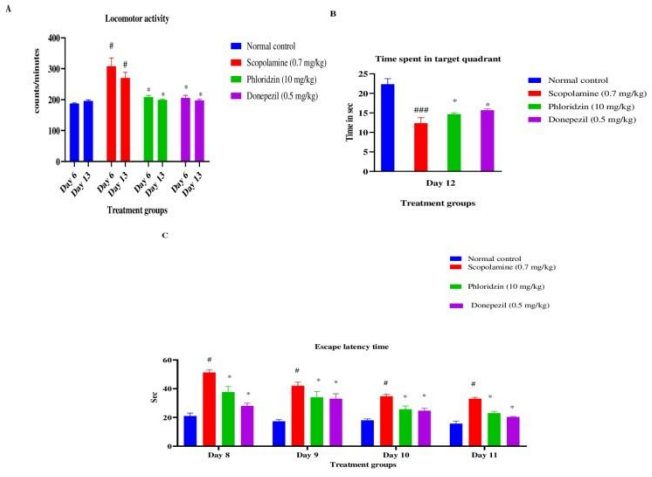

Figure 1**:** Effect of Phloridzin at the 6th and 13th days of the evaluation of locomotor activity in a rat (A). Effect of Phloridzin on behavioral parameters (B and C): ELT and TSTQ (learning ability and memory); all values are expressed as the mean ± SE of 5 animals per group. $^{\#}$P < 0.05(significant),$^{\#\#}$ P < 0.01 (very significant),$^{\#\#\#}$P < 0.001 (highly significant): when toxic controlvsnormal controland *P< 0.05 (significant),**P< 0.01(very significant), ***P < 0.001(highly significant),: toxic control (scopolamine 0.7mg/kg) vs treatment control (Phloridzin 10mg/kg) and toxic control vs standard drug (donepezil 0.5mg/ kg).

Effects of Phloridzin on Biochemical Parameters

The AChE activity was observed to be extended in rats given scopolamine in contrast to the control group. In Phloridzin-treated rats, there was a decrease in AChE activity (Figure 2A). AChE levels were significantly (*P < 0.05) lower in rats administered phloridzin compared to those receiving scopolamine. The scopolamine-treated rats group showed a high AChE level in comparison to NC. However, a significant (*P < 0.05) decrease in AChE levels was found after dosing with Phloridzin. Similar results were found in total protein estimation in the brain (Figure 2C). Rats subjected to Scopolamine exhibited a significant (*P < 0.05) elevation in MDA levels when compared to NC. In contrast, Phloridzin-treated rats demonstrated a significant (*P < 0.05) reduction in MDA levels, suggesting mitigation of lipid peroxidation, as illustrated in Figure 2B. Reduced glutathione was significantly (*P < 0.05) decreased, and glutathione peroxidase was significantly (*P < 0.05) reduced in scopolamine-treated rats. It was found to be significantly increased after treatment with Phloridzin (Figures 2D and E). Catalase and SOD activities were significantly (*P < 0.05) reduced in the scopolamine treated group compared to NC. This was significantly (*P < 0.05) increased after treatment with Phloridzin (Figures 2F and 2G). All values are expressed as the mean ± SEM.

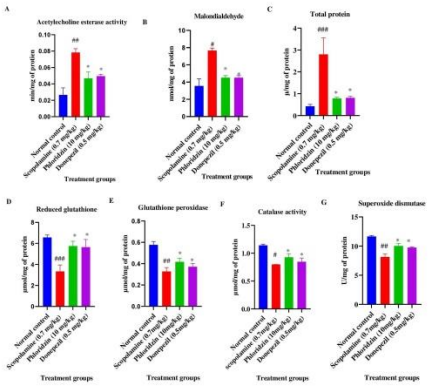

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Figure 2: Effect of Phloridzin on biochemical parameters such as (A) acetylcholine esterase, (B) Malondihyde, (C) total protein, (D) reduced glutathione, (E) glutathione peroxidase, (F) catalase activity and (G) superoxide's dismutase: all values are expressed as the mean ± SE of 5 animals per group. $^{\#}P < 0.05$(significant), $^{\#\#} P < 0.01$(very significant), $^{\#\#\#}P < 0.001$(highly significant): when toxic control vs normal control and *P< 0.05 (significant), **P< 0.01(very significant), ***P < 0.001 (highly significant): toxic control (scopolamine 0.7mg/kg) vs treatment control (Phloridzin 10mg/kg) and toxic control vs standard drug (donepezil 0.5mg/ kg).

In this study, we collected brain tissue (hippocampus) and utilized hematoxylin and eosin (H&E) staining to visualize the potential neuroprotective impact of Phloridzin. Following scopolamine administration, we observed neuronal loss, reduced blood flow, and hippocampal atrophy within the hippocampal region. Phloridzin treatment showed a neuroprotection role against Alzheimer's disease and reduced the hippocampal damage area. Further, the results of the present research have shown that Phloridzin and standard drug-treated Wistar rats help to improve neurocognitive function when compared to the toxic control group (scopolamine-administered rats) (Figure 3). The current research reveals that Phloridzin may have a protective function in neurological disorders due to its antioxidant properties triggered by scopolamine. Enhanced antioxidant and cholinergic signaling activities imply that Phloridzin could potentially be beneficial in addressing Parkinson's and dementia.

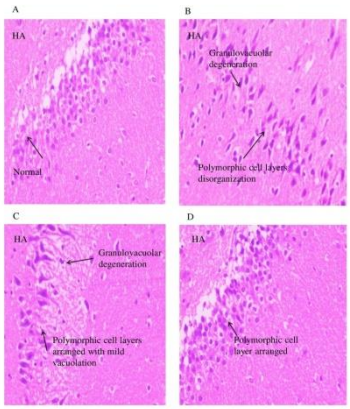

Figure 3 **:**(A) Normal Control group: Normal arrangement of pyramidal cell and polymorphic cell. (B) Toxic Control group (scopolamine 0.7mg/kg): H&E stained hippocampal area (HA) section shows mild polymorphic cell degeneation along with disorganisation. Some area shows granulovacuolar degeneration of displaying degenerative pyramidal cell and changes in pyramidal layer. (C)Treatment group (Phloridzin 10mg/kg): showing significant repair of the rearrangement of pyramidal layer with mild vacuolation. (D)Standard group (Donepezil 0.5mg/ kg): showing significant repair of the rearrangement of pyramidal layer with granulovacular degeneration.

5. Discussion

On the sixth and thirteenth days of assessing locomotor activity, rats administered scopolamine displayed heightened locomotor activity compare to the control group. Precisely, rats given Phloridzin exhibited diminished locomotor activity compared to those treated with scopolamine. Phloridzin demonstrated statistically significant (*P < 0.05) alterations in locomotor activity. Following the experimental protocol, we monitored ELT from the eighth to the eleventh day. Statistically significant (*P < 0.05) differences were found between the scopolamine-treated and Phloridzin-treated rat groups on the 8th day. By the 11th day, we observed a reduction in escape latency time across all treated groups, with a significant (*P < 0.05) difference between the normal and Phloridzin groups. The experimental protocol evaluated the time spent in the target quadrant (TSTQ) on the 12th day, by giving an index of regaining. Scopolamine-dosed rats have shown comparatively lower TSTQ compared to normal, Phloridzin, and Donepezil-treated rats. Throughout the experimental protocol, behavioral parameters, including ELT and TSTQ, were assessed to assess learning ability and memory retrieval. The Morris water maze (MWM) test is important for observing memory enhancement and spatial learning. It was used to detect changes in the central cholinergic system, as per the studies by Ahmed et al. [18, 24]. In the MWM test, rats displayed reduced escape latency time from day 1 to the 4th day. All the experimental groups demonstrated typical learning abilities. Furthermore, on the 5th day, there was a significant increase in the TSTQ compared to other quadrants of time spent. These findings from my research study suggest that rats possess the ability to significantly (*P < 0.05) recover, indicating that scopolamine induces abnormal memory processes and hampers learning abilities in them. Administering Phloridzin for 13 consecutive days mitigated the detrimental effects of scopolamine on learning and memory functions. Our results from the assessment of

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locomotor activity indicate that there was no interference or sedative effect during the MWM test, according to the Cakmak et al. study. Both ELT and TSTQ in the MWM test solely led to improved memory. According to some research studies [25, 26], it can be concluded that Phloridzin has a little bit of potential to enhance long-term memory impaired by scopolamine. These biomarkers used to evaluate the neuroprotective and Alzheimer's mitigating effects of Phloridzin in the study included AChE activity, MDA levels, GSH and GPx levels, SOD, and catalase activities. Acetylcholinesterase is the main brain biochemical marker related to Alzheimer's disease. This specific biomarker plays a crucial role in the breakdown of acetylcholine (ACh) neurotransmitters. Acetylcholinesterase performs the hydrolysis of acetylcholine into choline and acetate, effectively terminating their impact at cholinergic synapses [27, 28]. The measurement of AChE activity was employed to investigate the influence of Phloridzin on cholinergic function, a critical factor governing various aspects of memory and cognitive processes. This study uncovered that scopolamine led to an elevation in acetylcholinesterase levels compared to rats treated with Phloridzin. Previous research on phloridzin's inhibitory effects demonstrated that it could inhibit 90–95% of AChE activity, particularly in lipopolysaccharide conditions [3, 29]. In addition to AChE activity, we evaluated the MDA level as an indicator of lipid peroxidation within the brain. Rats administered with scopolamine exhibited higher MDA levels in comparison to those in normal, phloridzin-treated, and donepezil-treated rats. Lipid peroxidation stands as a significant marker for various neurological and other disorders, representing one of the end products resulting from the peroxidation of polyunsaturated fatty acids within cells and serving as a gauge for free radical generation. Lipid peroxidation is a major indicator of neurological and other disorders. It is one of the end products of polyunsaturated fatty acid peroxidation in cells and a measure of free radical generation. Lipids are initially targeted components that undergo excessive lipid peroxidation, causing Alzheimer's disease and other neurodegenerative diseases [30, 31]. In this research, scopolamine significantly induced lipid peroxidation and cerebrospinal fluid proteins, while decreasing antioxidant activities. These biochemical changes enhance oxidative stress. In scopolamine-treated rats, elevated MDA levels pointed towards increased lipid peroxidation within the brain it reverse after treated with Phloridzin, MDA levels showed a significant reduction, indicating a decrease in lipid peroxidation. In this study, reduced glutathione levels exhibited a significant decrease in the toxic control groups when compared to the normal control group. The Phloridzin group demonstrated an increase in reduced glutathione levels compared to rats administered with scopolamine. Furthermore, glutathione peroxidase levels in scopolamine-treated rats were notably lower than those in the normal control group. GSH antioxidants help protect against oxidative stress. Glutathione peroxidase catalyzes the reduction of hydroperoxides (H2O2) by GSH, reducing cellular damage and protecting against oxidative stress [32, 33]. GSH and GPx levels exhibit a decrease in rats treated with scopolamine, attributed to the elevated MDA levels in the brain. The primary defense mechanism against hydroperoxide-induced reactive oxygen species, as per previous research [22], We found alignment with prior studies, where glutathione peroxidase and reduced glutathione levels decreased upon scopolamine administration but showed significantly ($*P < 0.05$) improved with Phloridzin treatment. In this study, we observed a significant ($*P < 0.05$) decrease in the activity of SOD and catalase. SOD levels significantly ($*P < 0.05$) increased after Phloridzin administration in brain homogenates, while catalase activity was significantly ($*P < 0.05$) higher in the Phloridzin group as compared to the scopolamine group. Moreover, a statistically significant ($*P < 0.05$) difference was evident between the Phloridzin, Donepezil, and Scopolamine groups. SOD and catalase, two pivotal antioxidant enzymes, play crucial roles. SOD functions by neutralizing superoxide anions, which can harm cell membranes [34]. Catalase, on the other hand, serves as a primary antioxidant enzyme, combating hydroperoxide radicals and mitigating reactive oxygen species in Alzheimer's disease. This study suggests that the observed significant increase in SOD and catalase activities in the Phloridzin group may be attributed to their potent antioxidant capabilities [35]. This investigation reaffirms that Phloridzin possesses antioxidant properties against scopolamine-induced effects. Phloridzin helps in memory retrieval and influences memory storage. The study explores Phloridzin has potential to alleviate Alzheimer's disease in rats induced by scopolamine. It contributes to understanding Alzheimer's pathology and potential therapies by showing Phloridzin has the ability to enhance cognitive function, reduce oxidative stress, and improve cholinergic signaling. Behavioral and

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biochemical analyses provide insights into its mechanisms, supporting its promise as a neuroprotective agent. However, further research is needed to fully understand its therapeutic potential for treating neurodegenerative disorders like Alzheimer's. Overall, the study suggests that Phloridzin holds promise as a therapeutic agent for Alzheimer's disease due to its multifaceted effects on memory enhancement, cholinergic function, antioxidant defense, and neuroprotection. However, further research is needed to validate these findings and fully explore the therapeutic potential of Phloridzin in neurodegenerative disorders. Some limitations of the study include using male Wistar rats, which may not fully represent the complexity of Alzheimer's in humans, and employing a fixed dose of Phloridzin. Future research could focus on clinical translation, conducting trials in humans, dose optimization, exploring different dosing regimens, and longitudinal studies to assess sustained neuroprotective effects and disease progression.

Conclusion

Current study investigated the neuroprotective effects of Phloridzin against scopolamine-induced Alzheimer's disease in male Wistar rats. The research found that Phloridzin treated rats significantly decreased AChE activity and lipid peroxidation while elevating antioxidant enzyme activity. Behavioral parameters showed improved cognitive function and memory, as evidenced by decreased ELT and increased TSTQ during the Morris Water Maze test. Histopathological results also revealed a neuroprotective effects of Phloridzin against hippocampal damage induced by scopolamine. Overall, Phloridzin demonstrated promising therapeutic potential for Alzheimer's disease through its effects on memory enhancement, antioxidant defense, cholinergic function, and neuroprotection. Further research is needed to validate these outcomes and explore the full potential of Phloridzin as a treatment for neurological disorders.

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Conflict of interest The authors declare no potential conflicts of interest concerning the research, authorship, and publication of this research article.

List of Abbreviation

MWM: Morris Water Maze

AChE: Acetyl cholinesterase

SOD: Superoxide dismutase

GPx: Reduce glutathione

MDA: Malondialdehyde

TSTQ: Target spent in target quadrant

ELT: Escape latency time

HA: Hippocampus area

GSH: Reduced Glutathione

Ach: Acetylcholine

AD: Alzheimer disease

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