Unraveling the therapeutic potential of ginsenoside compound Mc1 in Alzheimer's disease: Exploring the role of AMPK/SIRT1/NF-κB signaling pathway and mitochondrial function

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Abstract

Background. Alzheimer's disease (AD) is a disabling neurodegenerating disorder characterized by chronic neuroinflammation, cognitive impairment and memory loss. Current treatment options for AD offer limited benefits, underscoring the urgent need for alternative therapeutics. Despite the promising effects of ginsenosides in neurodegenerative diseases, the therapeutic potential of ginsenoside compound Mc1 (GCMc1) in AD remains to be thoroughly investigated.

Objectives. This study aimed to investigate the therapeutic potential of GCMc1 in rats with AD and to elucidate the molecular mechanisms responsible for its effects.

Materials and methods. Alzheimer's disease was induced in Sprague Dawley rats through a single intracerebro-ventricular injection of amyloid-beta (Aβ)1-42 peptide. The animals were divided into 5 groups: a control group and 4 AD subgroups, with or without receiving 10 mg/kg of GCMc1 and/or 100 μg/kg of compound C intraperitoneally (ip.). Behavioral tests, mitochondrial function, inflammatory cytokines, and proteins expression were evaluated using the Morris water maze (MWM) test, fluorometry, enzyme-linked immunosorbent assay (ELISA), and immunoblotting techniques, respectively.

Results. Treatment with GCMc1 improved cognitive function, reduced hippocampal Aβ accumulation, and suppressed interleukin (IL)-1β, IL-10 and tumor necrosis factor alpha (TNF-α) levels. Ginsenoside compound Mc1 reduced mitochondrial reactive oxygen species (ROS) levels and membrane depolarization, increased adenosine triphosphate (ATP) levels, upregulated the expression of AMPK, PGC-1α and SIRT1 proteins, and downregulated the nuclear factor-kappa-B (NF-κB) expression. Importantly, co-administration of compound C, an AMPK inhibitor, attenuated the beneficial effects of GCMc1, suggesting the involvement of AMPK pathway in mediating GCMc1's neuroprotective effects.

Conclusions. We showed that GCMc1 confers substantial neuroprotection in rats with AD by modulating the AMPK/SIRT1/NF-κB signaling pathway. These findings highlight the potential of GCMc1 as a promising therapeutic agent for AD treatment.

Key words: neuroprotection, neuroinflammation, Alzheimer's disease, cognitive dysfunction, ginsenosides

Background

Alzheimer's disease (AD) is a neurological degenerating disorder that negatively affects the lives of a large population in the world.[1] It is characterized by an impairment in cognitive tasks, memory loss and behavioral abnormalities.[1] Amyloid beta (Aβ) plaques accumulation, neurofibrillary tangles formation and chronic neuroinflammation are considered as the main pathophysiological features of AD.[1,2] Several brain regions play pivotal roles in AD, with the hippocampus and neocortex being particularly affected.[3] The hippocampus, a vital structure for memory consolidation and spatial navigation, is among the first brain regions to exhibit significant damage in AD.[4] Neuronal loss and synaptic dysfunction within the hippocampus contribute to memory impairments commonly observed in AD patients.[4] Additionally, the neocortex, encompassing regions responsible for higher-order cognitive functions such as reasoning and language, experiences progressive degeneration in AD.[5] This degeneration disrupts the intricate neural networks that underlie complex cognitive processes, leading to deficits in reasoning, language and executive function.[6] Neurotransmitters also play a critical role in AD-related cognitive impairments.[7] Acetylcholine, a neurotransmitter involved in memory and learning processes, experiences substantial depletion in different regions of brain in AD. The loss of cholinergic neurons and the subsequent reduction in acetylcholine levels contribute to cognitive decline. Furthermore, excitotoxicity resulting from excessive glutamate release and imbalances in GABAergic neurotransmission can disrupt neural network stability and lead to neuronal damage and cognitive deficits in AD.[7,8] With an aging population, the frequency of AD is increasing, making it a major community health concern.[9] Despite significant efforts to develop effective treatments for AD, there are still no reliable treatments for this debilitating condition, and available therapies only provide modest benefits. Therefore, there is a need to explore alternative approaches for AD treatment.

It has been recently reported that the AMP-activated protein kinase (AMPK) signaling pathway is substantially involved in the regulation of energy metabolism, cellular stress response and neuroinflammation in AD.[10,11] AMP-activated protein kinase is a master regulator of energy homeostasis in cells. In the context of AD, energy metabolism is disrupted, and brain cells struggle to produce and utilize energy efficiently. Activation of AMPK helps restore the energy balance by enhancing processes like glucose uptake and mitochondrial biogenesis, preventing neuronal dysfunction and cell death.[12] In addition, chronic neuroinflammation is a hallmark of AD, and it plays a complex role in disease progression.[13] By inhibiting the activation of proinflammatory pathways, such as nuclear factor-kappa B (NF-κB), AMPK can help reduce the production of inflammatory molecules and cytokines and mitigate the damaging effects of neuroinflammation in AD.[14]

It has been shown that natural compounds, such as ginsenosides, have potential therapeutic benefits in AD.[5,16] Ginsenosides are a class of bioactive compounds found in the roots of ginseng, which have been shown to have neuroprotective effects in several types of neurodegenerative disorders.[6] Among these ginsenosides, ginsenoside compound Mc1 (GCMc1) has shown promising results in improving oxidative, apoptotic and inflammatory responses in the pathophysiology of different diseases in animal models.[17,18] Ginsenoside compound Mc1 is a deglycosylated ginsenoside that has higher pharmacological absorption, bioavailability and activity than deglycosylated ginsenosides.[17] In a recent study, the potential mechanisms underlying the cardioprotective effects of GCMc1 have been studied in an experimental cardiac injury model.[17] The study found that treatment with this compound improved cardiac function and reduced oxidative stress in the hearts and H9C2 cardiomyocytes under hypoxia/reoxygenation injury. The study also showed that GCMc1 increased the activity of AMPK and inhibited the activation of oxidative stress in vivo and in vitro.[17] Activation of AMPK-related signaling pathway and subsequent surviving proteins like situin-1 (SIRT1) can improve cognitive function, reduce Aβ and tau accumulation, and enhance mitochondrial function in animal model of AD.[11,18]

In addition to its effects on AMPK and downstream targets, GCMc1 may also exert its neuroprotective effects by inhibiting the activity of NF-κB-dependent proinflammatory mediators, which show a critical involvement in the pathogenicity of AD.[19] Proinflammatory cytokines and interleukins (ILs) including IL-1β and IL-6, as well as tumor necrosis factor alpha (TNF-α), have been shown to induce neuronal damage and promote the buildup of Aβ and tau proteins within different regions of the brain.[20] Here, mitochondrial dysfunction is closely linked to the production of neuroinflammation in the pathophysiology of AD.[21] Impaired mitochondrial function leads to energy deficits, oxidative stress, mitochondrial DNA damage, inflammasome activation, and neuronal vulnerability, all of which contribute to the chronic neuroinflammatory state observed in AD. Importantly, beneficial impact of GCMc1 on mitochondrial improvement has been reported in cerebral ischemia/reperfusion injury in hyperlipidemic rats.[22] Therefore, targeting mitochondrial activity and neuroinflammation through the safe and effective therapeutic compounds may be a promising strategy for treatment of AD.

Objectives

Given the promising effects of GCMc1 on the activity of signaling pathways regulating mitochondrial biogenesis and function, and on reducing NF-κB-dependent prinflammatory cytokines,[17,18,23] we investigated the impact of GCMc1 on AD outcomes and underlying mechanisms in a rat model.

Specifically, we evaluated the cognitive performance, Aβ accumulation, mitochondrial function, and neuroinflammation in rats with AD following treatment with GCMc1. We hypothesized that treatment with this agent will improve AD outcomes in rats by activating AMPK-dependent mitochondrial biogenesis, improving mitochondrial function and inhibiting NF-κB-dependent inflammatory cytokines. This study will provide valuable insights into the underlying therapeutic benefits of GCMc1 in AD and the mechanisms underlying its neuroprotective effects.

Materials and methods

Animal care and ethics statement

Male Sprague Dawley rats weighing 250–300 g were used in this study. The animals were kept under a controlled temperature of 22–24°C in a housing room with a 12 h light and 12 h dark cycle and given water and standard rat chow ad libitum. All experimental protocols and animal care were conducted in accordance with the guidelines and regulations of the National Institutes of Health (NIH) for the care and use of laboratory animals (2011, 8th ed., National Academy of Sciences, USA) and approved by the local Institutional Animal Care and Use Committee of the Wuhan University of Science and Technology, China (approval No. 20230501001).

Experimental design and sample size calculation

The animals were randomly assigned into 5 groups of 6 rats (total number of rats – 30). The 1st group was considered as the control group and received a vehicle (saline) solution. The 2nd group received 5 μg/μL Aβ peptide to induce AD-like symptoms. The 3rd group received Aβ peptide and 10 mg/kg of GCMc1.[17,18] The 4th group received Aβ peptide and then 100 μg/kg compound C, as AMPK inhibitor. The 5th group received Aβ peptide and then compound C plus GCMc1. The drugs were administered daily by intraperitoneal (ip.) injection for 10 days, starting 1 day after Aβ administration. Every day before each GCMc1 injection, AMPK inhibitor-receiving rats were given ip. injection of compound C (dissolved in dimethyl sulfoxide (DMSO) with the dosage of 100 μg/kg/day). The control and AD model groups were injected with the same volume of DMSO and vehicle. We performed a sample size assessment using PS calculator software, v. 3.1.2 (Informer Technologies, Inc, Los Angeles, USA), with assumptions of the 5% alpha level, 95% power and the standard deviation (SD) of 35% of the means, which indicated a sample size requirement of 5 rats for each experimental group. To ensure robustness, we included 1 additional rat in each group, resulting in a final sample size of 6 rats per experimental group.

Induction of AD model

The AD modeling was induced by a single intra-cerebro-ventricular injection of Aβ (1–42) peptide (5 μg/μL) (Sigma-Aldrich, St. Louis, USA), as previously described.[24] The stereotaxic surgery was performed under anesthesia using ip. injection of a combination of ketamine (65 mg/kg) and xylazine (15 mg/kg). The coordinates for creating holes in the skull were set as follows: 0.8 mm posterior to bregma, 1.5 mm lateral to the sagittal suture and 3.2 mm ventral to the surface of the skull. After fixation and recovery time, an injection of 5 μg/μL of the Aβ peptide solution was administered in the lateral ventricles of both hemispheres using Hamilton 26-gauge syringe. In the control group, a comparable procedure was followed with the injection of normal saline.

Behavioral tests

Behavioral (cognitive) tests were conducted in a Morris water maze (MWM) after drug administration and before surgery.[25] The MWM was a round pool with a diameter of 150 cm and a depth of 50 cm filled with water (24 ±1°C) made opaque with nontoxic white paint. The pool was virtually divided into 4 quadrants and a 10 × 10 cm hidden platform was located 1 cm below the water superficial level in the center of one quadrant. The rats underwent training for 5 days (4 trials for each day) to locate the hidden platform using distal cues. On the 6th day, the hidden platform was disconnected from the pool and the rats swam for 60 s in the pool to search the platform (probe trial). The following parameters were recorded: escape latency (time from start of search to finding the platform), time spent in the target quadrant (in which the platform was located at the time of training) and discrimination index (DI).

Tissue collection and preparation

After behavioral tests, the animals were anesthetized with ketamine (65 mg/kg) and xylazine (15 mg/kg), and the brains of animals were rapidly and carefully removed from their skulls immediately after dislocation of the neck. After locating the hippocampi within the brain (they are usually recognizable due to their distinct shape and location), the hippocampi were carefully removed from the brain tissue using scalpels. Once isolated, the hippocampi were immediately placed in a storage container and then stored at –80°C for further analysis. During tissue processing, the tissues were placed in the radioimmunoprecipitation assay (RIPA) buffer containing protease and phosphatase inhibitors and homogenized. The homogenates were centrifuged at 10,000 × g for 10 min at 4°C. Then the supernatants were collected for biochemical analysis.

Mitochondrial function indices

The following mitochondrial function indices were estimated in fresh hippocampal samples after extracting mitochondrial pellets using a commercial extraction kit (Thermo Fisher Scientific, Waltham, USA).

Measurement of mitochondrial membrane potential

The membrane potential of freshly obtained mitochondrial samples was assessed using a fluorescent dye (JC-1; Thermo Fisher Scientific).[26] Hippocampal tissues were homogenized in mitochondrial isolation buffer (10 mM Tris-HCl, 320 mM sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM phenylmethylsulfonyl fluoride (PMSF) pH 7.4) on ice, and centrifuged at 1,000 × g for 10 min at 4°C. The mitochondrial fraction was obtained from the supernatant by centrifugation at 10,000 × g for 10 min at 4°C. The isolated mitochondria were suspended in 1 mL of JC-1 buffer (10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 150 mM NaCl, 5 mM glucose, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4) containing 10 µM JC-1 dye and incubated at 37°C for 30 min. The fluorescent intensity of JC-1 was detected using a fluorescence spectrophotometric reader (PerkinElmer LS55; PerkinElmer, Waltham, USA) with excitation/emission wavelength of 490/530 nm for the green monomers and 540/590 nm for the red aggregates.

Measurement of mitochondrial ROS levels

Mitochondrial reactive oxygen species (ROS) levels were estimated by means of dichloro fluorescein diacetate (DCFDA) assay (Thermo Fisher Scientific).[27] Briefly, the hippocampal tissues were homogenized in isolation buffer and centrifuged at 600 g for 10 min at 4°C. The mitochondrial pellet was re-suspended in DCFDA containing buffer and incubated at 37°C for 30 min. Then, their fluorescent intensities were detected using the fluorescence spectrophotometric reader at excitation/emission wavelength of 485/535 nm.

Measurement of ATP levels

An ATP Bioluminescent Assay Kit (Sigma-Aldrich) was employed to measure the levels of adenosine triphosphate (ATP) in prepared samples.[28] The supernatant of hippocampal tissues was gathered and diluted to 10 µg/µL protein concentration. The diluted supernatants were mixed with the ATP assay reagent and incubated for 5 min at room temperature. The luminescence signal of the resulting solution was detected by means of a luminometer Victor3 (PerkinElmer).

Hippocampal Aβ accumulation and cytokines measurement

The levels of Aβ accumulation and cytokines IL-1β, IL-10 and TNF-α were assayed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource Inc., San Diego, USA), based on the manufacturer's instructions.[29] Briefly, the previously prepared supernatants were incubated with the cytokine-specific antibody for 2 h, followed by the addition of a biotinylated secondary antibody for 1 h. The solution was then incubated with streptavidin-horseradish peroxidase (HRP) for 30 min, and the reaction was visualized using a substrate solution. The absorbances of solutions were detected at 450 nm using an ELISA reader (BioTek Instruments, Winooski, USA). The concentration of protein in the supernatant was measured using the bicinchoninic acid (BCA) Protein Assay Kit (Thermo Fisher Scientific).

Western blotting

After preparing supernatants, an amount of about 30 µg of protein from each sample was separated with sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by an electrophoretic transfer to a nitrocellulose membrane (Bio-Rad, Hercules, USA).[30] The membrane was then placed in a dish containing 5% non-fat dry milk blocking buffer in Tris-buffered saline and 0.1% Tween 20 (TBST) for 1 h. This stage was followed by the incubation of the membranes with primary antibodies against total AMPK, phosphorylated AMPK, SIRT1, PGC-1α, phosphorylated p65-NFκB (1:1500; Cell Signaling Technology (CST), Danvers, USA), and GAPDH (1:1000; CST) in TBST at 4°C. After washing the membranes 5 times with TBST, they were soaked in HRP-conjugated secondary antibody (1:1500; SCT). One hour later, TBST was used again to rinse the membranes, and then the membranes were placed in the vicinity of the enhanced chemiluminescence (ECL) reagents so that the protein bands appeared. The protein bands were visualized by means of a visualizing device (Bio-Rad) and their intensities were quantified through ImageJ software (National Institutes of Health (NIH), Bethesda, USA). After selecting the specific lanes containing the protein bands of interest within the ImageJ, the pixel values of the bands were calculated and the intensities of the protein bands were normalized to the corresponding intensity of the GAPDH band.

Statistical analyses

Data analysis was performed with GraphPad Prism 7 software (GraphPad Software, San Diego, USA). Data were presented as median (interquartile range (IQR)). The differences between groups were analyzed using one-way

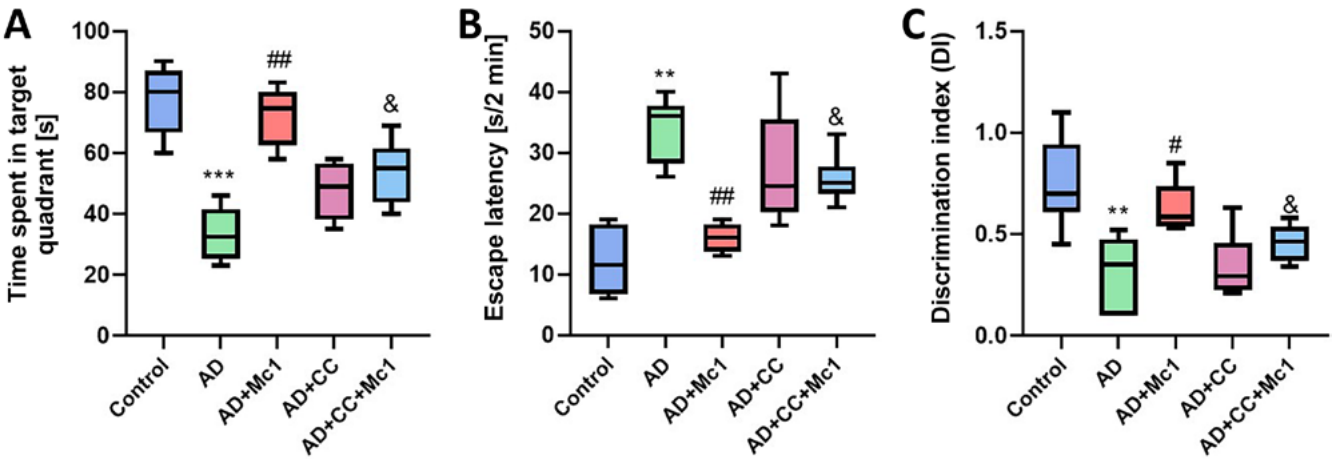

Fig. 1. Effect of ginsenoside compound Mc1 on behavioral cognitive functions in rats with Alzheimer's disease (AD). A. Time spent in target quadrant (in seconds); B. escape latencies (in seconds per 2 min); C. Discrimination index (DI). These cognitive functions were evaluated with the Morris water maze test. Statistical differences were tested using one-way analysis of variance (ANOVA) and Tukey's post hoc test. The box plots represent the median and distribution of the data, where the box edges indicate the interquartile range (IQR; Q1–Q3); n = 6. ** < 0.01, and *** < 0.001 compared to control group; # < 0.05, and ## < 0.01 compared to AD group; & < 0.05 compared to AD+Mc1 group. GCMc1 – ginsenoside compound Mc1; CC – compound C

analysis of variance (ANOVA) and subsequent post hoc with Tukey's test. To ascertain the normality of the dataset, the Shapiro–Wilk test was conducted. Also, Bartlett's test was performed to validate the assumption of homogeneity of variances across groups. A p-value <0.05 was set to determine the statistical significance.

Results

Effect of GCMc1 on AD outcomes

We first evaluated the effect of GCMc1 on cognitive function in rats using the MWM trial. One-way ANOVA analysis revealed that in comparison to the AD group, rats treated with GCMc1 showed significantly shorter escape latencies ($p < 0.01$) and more times spending in the target quadrant ($p < 0.01$), indicating boosted spatial memory and learning. In addition, discrimination index was higher following GCMc1 administration compared with AD group ($p = 0.03$) (Fig. 1A–C). Also, we performed an ELISA assay for Aβ measurement and found that treatment with GCMc1 significantly decreased Aβ accumulation in the hippocampus ($p < 0.01$) compared to the AD group (Fig. 2). However, co-administration of compound C to inhibit AMPK pathway significantly reduced the beneficial impacts of GCMc1 on hippocampal Aβ accumulation as well as spatial learning and memory indices, as evidenced by a significant increase in escape latency ($p = 0.05$), decreased spending times in the target quadrant ($p = 0.03$) and lower discrimination index ($p = 0.04$) in comparison to the AD+Mc1 group (Fig. 1,2). These results suggest that GCMc1 has beneficial effects on AD outcomes and improves learning and memory features in rats, and that these effects are AMPK-dependent.

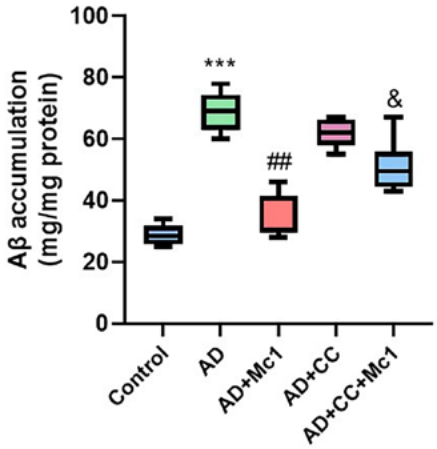

Fig. 2. Effect of ginsenoside compound Mc1 on hippocampal Aβ accumulation in rats with Alzheimer's disease (AD). Aβ accumulation was assessed with enzyme-linked immunosorbent assay (ELISA) method. Statistical differences were tested using one-way analysis of variance (ANOVA) and Tukey's post hoc test. The box plots represent the median and distribution of the data, where the box edges indicate the interquartile range (IQR; Q1–Q3); n = 6. *** < 0.001 compared to control group; ## < 0.01 compared to AD group; & < 0.05 compared to AD+Mc1 group; GCMc1 – ginsenoside compound Mc1; CC – compound C

Effect of GCMc1 on mitochondrial function

We next investigated the effect of GCMc1 on mitochondrial function; the data were analyzed using one-way ANOVA and subsequent Tukey's post hoc tests. Mitochondrial function indices, including mitochondrial ROS levels, mitochondrial membrane potential changes and ATP levels, were measured in the hippocampal tissue samples (Fig. 3A–C). Induction of AD led to a significant rising of mitochondrial ROS level ($p < 0.001$) and declining of mitochondrial membrane potential and ATP levels ($p < 0.01$) in comparison to the control group.

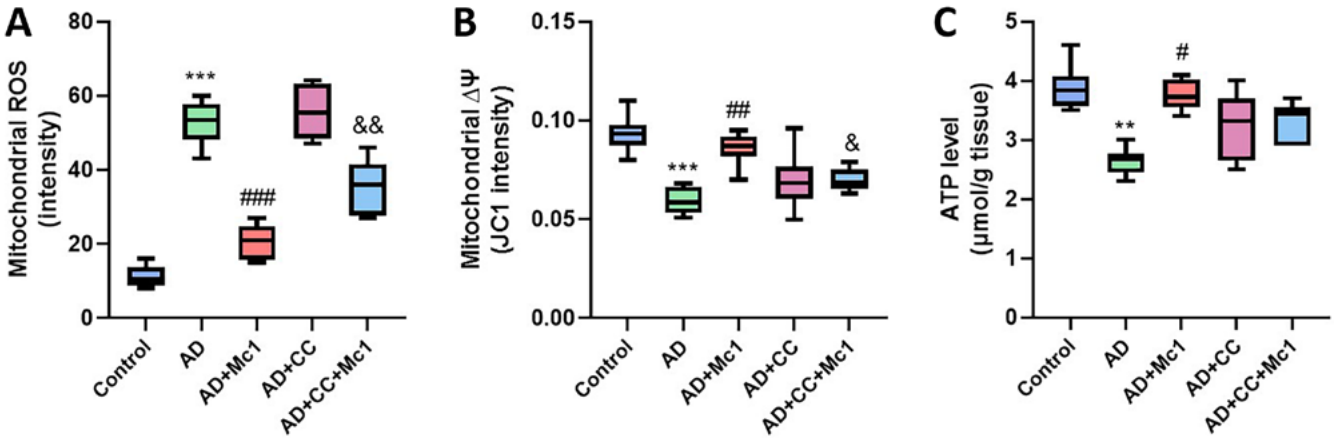

Fig. 3. Effect of ginsenoside compound Mc1 on mitochondrial function indices in rats with Alzheimer's disease (AD). A. Mitochondrial reactive oxygen species (ROS) levels; B. Mitochondrial membrane potential (ΔΨ); C. Adenosine triphosphate (ATP) levels. Mitochondrial ROS and ΔΨ were detected flourometrically and ATP levels were measured with a bioluminescent assay kit. Statistical differences were tested using one-way analysis of variance (ANOVA) and Tukey's post hoc test. The box plots represent the median and distribution of the data, where the box edges indicate the interquartile range (IQR; Q1–Q3); n = 6; ** < 0.01, and *** < 0.001 compared to control group; # < 0.05, ## < 0.01, and ### < 0.001 compared to AD group; & < 0.05 and && < 0.01 compared to AD+Mc1 group. GCMc1 – ginsenoside compound Mc1; CC – compound C

Treatment with GCMc1 in the AD+Mc1 group showed significant improvement in all mitochondrial function parameters compared to the AD group ($p < 0.001$). However, in the AD+CC+Mc1 group, the protective effect of GCMc1 was abolished, as evidenced by a significant overproduction of mitochondrial ROS ($p < 0.01$) and mitochondrial membrane depolarization ($p = 0.05$) compared to the AD+Mc1 group (Fig. 3A, 3B). These results suggest that the activation of AMPK plays a role in the protective effects of GCMc1 on mitochondria in AD.

Effect of GCMc1 on cytokines levels

The levels of IL-1β, IL-10 and TNF-α cytokines were measured in the hippocampal tissue samples to estimate the extent of neuroinflammation. One-way ANOVA demonstrated that the levels of IL-1β and TNF-α were increased and the level of IL-10 was reduced in AD group when compared to the control group ($p < 0.001$) (Fig. 4). Treatment with GCMc1 showed significant reduction of proinflammatory cytokines IL-1β and TNF-α levels and elevation of anti-inflammatory cytokine IL-10 level compared to the AD group ($p < 0.001$ and $p < 0.001$). Conversely, administration of compound-C significantly eliminated the anti-inflammatory effects of GCMc1, so that it amplified the production of IL-1β ($p = 0.04$) and TNF-α ($p = 0.002$) levels and suppressed the production of IL-10 ($p = 0.003$) compared to the AD+Mc1 group ($p < 0.05$). The findings highlight the anti-neuroinflammatory potential of GCMc1 in AD, and the role of AMPK inhibition in partially reversing this effect.

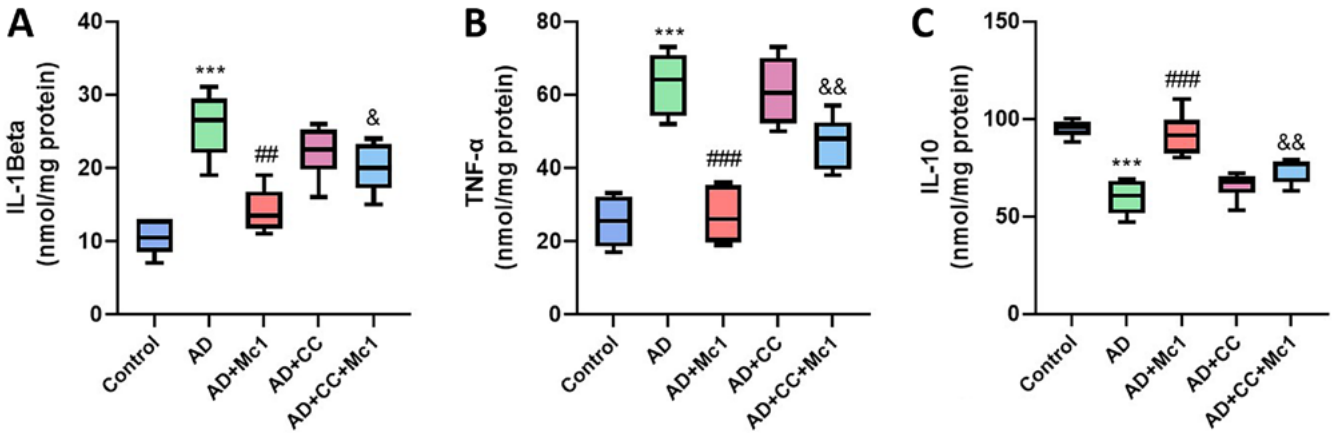

Fig. 4. Effect of ginsenoside compound Mc1 on hippocampal inflammatory cytokines levels in rats with Alzheimer's disease (AD). A. Interleukin-1 beta (IL-1β); B. Tumor necrotic factor alpha (TNF-α); C. Interleukin-10 (IL-10). The cytokines levels were assessed with enzyme-linked immunosorbent assay (ELISA) method. Statistical differences were tested using one-way analysis of variance (ANOVA) and Tukey's post hoc. The box plots represent the median and distribution of the data, where the box edges indicate the interquartile range (IQR; Q1–Q3); n = 6; *** < 0.001 compared to control group; ## < 0.01, and ### < 0.001 compared to AD group; & < 0.05 and && < 0.01 compared to AD+Mc1 group. GCMc1 – ginsenoside compound Mc1; CC: compound C

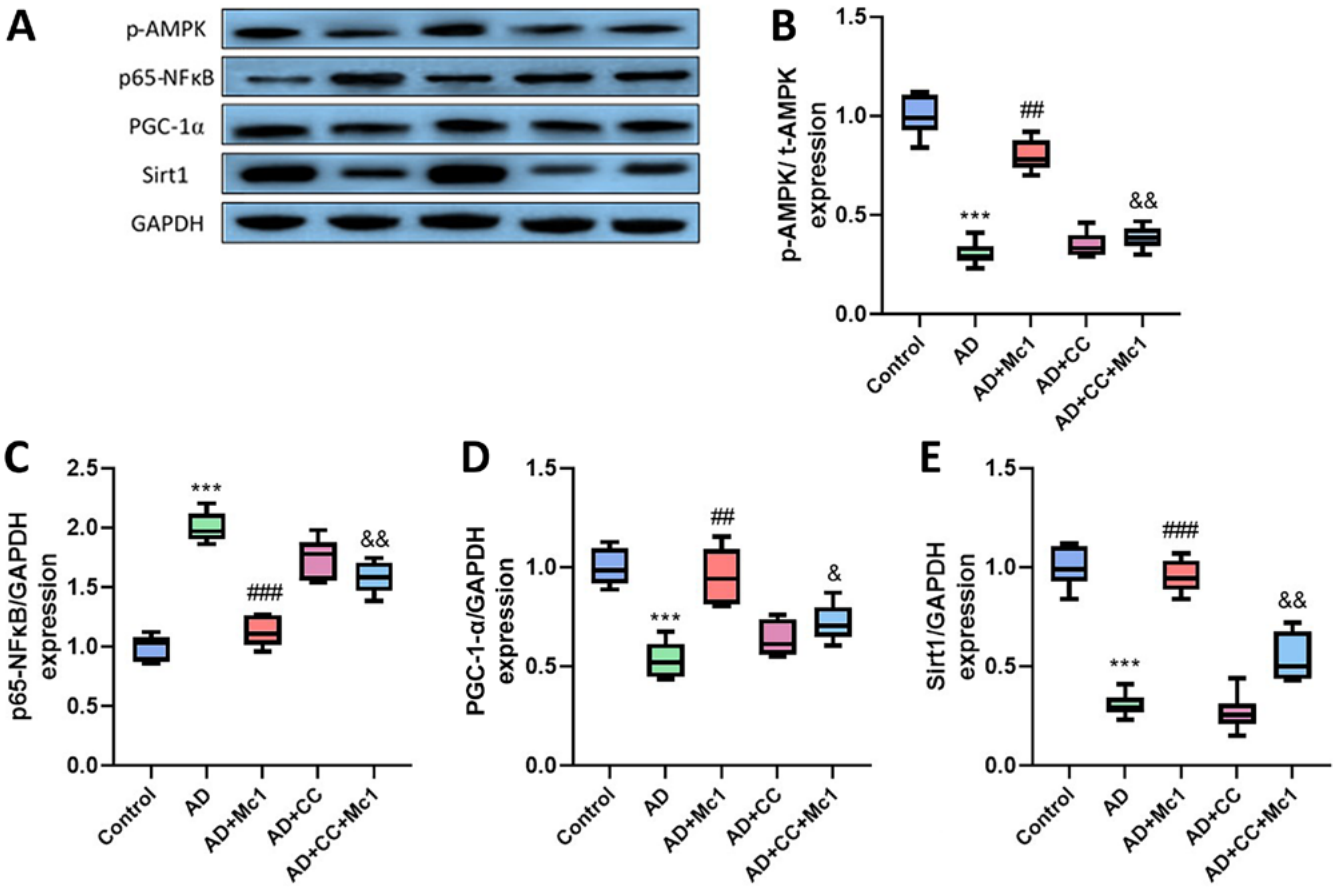

Fig. 5. Effect of ginsenoside compound Mc1 on signaling proteins expression in rats with Alzheimer's disease (AD). A. Western blotting images; B. Phosphorylated AMPK; C. p65-NFkB; D. PGC-1α; E. Sirt1. The expression of proteins was quantified using western blotting technique and subsequent visualization with enhanced chemiluminescence (ECL) reactions. Statistical differences were tested using one-way analysis of variance (ANOVA) and Tukey's post hoc test. The box plots represent the median and distribution of the data, where the box edges indicate the interquartile range (IQR; Q1–Q3); n = 4; *** < 0.001 compared to control group; # < 0.01, and ### < 0.001 compared to AD group; & < 0.05, and && < 0.01 compared to AD+Mc1 group; GCMc1 – ginsenoside compound Mc1, CC – compound C.

Effect of GCMc1 on signaling proteins expression

Analysis of western blot findings with one-way ANOVA between groups showed that the AD group had a significant decrease in phosphorylated AMPK, PGC-1α, Sirt-1, and p65-NFκB compared to the control group ($p < 0.001$) (Fig. 5A–E). Treatment of rats with AD with GCMc1 showed significant upregulation of phosphorylated AMPK ($p < 0.01$) accompanied with higher expression of PGC-1α ($p < 0.01$), and Sirt-1 ($p < 0.001$), and lower expression of p65-NF-κB ($p < 0.001$) compared to the AD group. To explore the role of AMPK pathway activation in the effect of GCMc1 on signaling proteins expression, we measured the expression of proteins after AMPK blockade by compound C. We found that administration of compound C completely suppressed the effect of GCMc1 on AMPK phosphorylation ($p < 0.01$) and partially blocked PGC-1α ($p = 0.05$), SIRT1 and p65-NFκB expression ($p < 0.01$). The findings propose that AMPK pathway activation may be involved in the beneficial effect of GCMc1 on the relevant signaling proteins in rats with AD.

Discussion

The current study revealed the beneficial impact of GCMc1 on AD outcomes via an AMPK-dependent manner in a rat model. The results indicated that the administration of this compound ameliorated cognitive impairment, improved mitochondrial function and reduced inflammatory responses in the hippocampus of AD rats. These findings endorse that GCMc1 has potential therapeutic effects on AD by stimulating the AMPK/PGC-1α/SIRT1 signaling pathway and hindering NF-κB-dependent inflammatory cytokines production. In addition, the activation of AMPK by GCMc1 was required for the observed beneficial effects.

Ginsenosides are natural compounds derived from *Panax ginseng*, which has been traditionally used as a medicinal plant for various diseases.[31,32] Emerging reports have demonstrated that GCMc1 has anti-inflammatory, antioxidative and neuroprotective effects.[17,19,23] Our results suggested that that GCMc1 significantly improved cognitive performance in the AD rats as indicated by lower escape latency, higher time spending in target quadrant

and higher discrimination index. These findings are in line with previous reports in which ginsenoside compounds can recover cognitive function in AD model animals.[33] The mitochondrial dysfunction is a hallmark of AD pathology,[34] and our study demonstrated that GCMc1 improved mitochondrial function by reducing ROS production, increasing mitochondrial membrane potential and enhancing ATP production.

There is growing evidence indicating a connection between mitochondrial dysfunction and inflammatory responses in AD.[35] These dysfunctional mitochondria trigger a series of events that result in inflammation within the brain. Inflammatory responses, characterized by the release of proinflammatory mediators, exacerbate neuronal damage and contribute to the progression of AD pathophysiology.[20] Our study showed that GCMc1 repressed the production of IL-1β and TNF-α as proinflammatory cytokines, and amplified the production of IL-10 as anti-inflammatory cytokine in the hippocampus of AD rats. These findings confirm the previous reports regarding that ginsenosides can exert anti-inflammatory effects in AD models.[15,36] The interplay between mitochondrial dysfunction and inflammatory responses forms a vicious cycle, where inflammation further disrupts mitochondrial function, creating a detrimental feedback loop.[37] Intervening in this destructive cycle with GCMc1 could potentially decrease the AD-induced functional and structural changes in brain regions and enhance learning, memory and cognitive functions. Understanding and targeting this interaction may offer potential therapeutic strategies for combating AD.

Neuroplasticity deficits, characterized by synaptic loss and neuronal shrinkage in the prefrontal cortex and hippocampus, significantly contribute to the impairment of learning and memory.[38,39] The accumulation of Aβ within hippocampal neurons has detrimental effects on synaptic plasticity.[40] However, there is currently no available information on the potential impact of GCMc1 in this context. Nonetheless, previous research has suggested that other types of ginsenosides can positively influence these endpoints, resulting in cognitive improvement. For example, ginsenoside Rg1 treatment in experimental models not only increased the sensitivity of triggering synaptic responses and restored long-term potentiation (LTP), but also elevated the expression of proteins associated with synaptic plasticity, including glutamate receptor-1, synaptophysin and postsynaptic density 95 (PSD95).[41] Similarly, ginsenoside Rb1 treatment enhanced LTP and the transmission of glutamatergic and GABAergic signals in the hippocampal CA3 region.[42] This effect was linked to the sequential enhancement of PSD95 and α-synuclein expression in this region. Notably, ginsenoside Rb1 administration restored cholinergic dysfunction and promoted cell survival in the dentate gyrus and hippocampal CA3, indicating that ginsenosides had a positive impact not only on synaptic plasticity but also on the process of neurogenesis.[42,43] It is essential to highlight, however, that there is still a gap in research focusing on the specific mechanisms through which GCMc1 may target synaptic plasticity and neurogenesis to enhance cognitive function in AD.

AMP-activated protein kinase is a key regulator of cellular metabolic activity, and its normal function can improve mitochondrial function and reduce inflammation.[44] Our study demonstrated that GCMc1 activated AMPK and this activation was required for the observed beneficial effects on cognitive function, mitochondrial function and inflammation. Inhibition of AMPK activity by compound C abolished the protective effects of the drug in AD rats. Our results agree with the previous findings demonstrating that AMPK activation by ginsenosides is involved in their beneficial effects on mitochondrial function and inflammation.[45] PGC-1α and Sirt1 are downstream targets of AMPK, and play crucial roles in regulating mitochondrial biogenesis and function.[46] Our study showed that GCMc1 increased the expression of PGC-1α and Sirt1, indicating that improving mitochondrial function is likely due to the activation of the PGC-1α/Sirt1 pathway. In addition, GCMc1 reduced the activity of p65-NF-κB, suggesting that the observed anti-inflammatory property of this compound are intermediated through the inhibition of NF-κB signaling. NF-κB is a transcription molecule that controls various inflammatory cytokines expression, and its activation has been associated with neuroinflammation, neurodegeneration and cognitive impairment in patients with AD.[4] Interestingly, there is emerging evidence suggesting a cross-link among AMPK and NF-κB signalings.[48] The activation of AMPK has been shown to inhibit NF-κB activity, thereby attenuating the inflammatory response. Conversely, NF-κB activation can inhibit AMPK signaling, leading to further disruption of energy metabolism and mitochondrial dysfunction.[48] These findings support the hypothesis that the neuroprotective effects of GCMc1 on AD outcomes may be partly attributed to its ability to activate cerebral AMPK/PGC-1α/Sirt1/NF-κB signaling pathway, leading to the improvement of mitochondrial function and limitation of inflammatory responses in the hippocampus of rats. It is important to note that the exact mechanisms underlying the neuroprotective effects of GCMc1 in AD are still an active area of research. To fully understand its potential therapeutic benefits, further studies are needed to elucidate the contribution of other important mechanisms and mediators responsible for these effects, including the involvement of neurotrophic factors, mitochondrial homeostasis and biogenesis, apoptosis, autophagy, and neurotransmitter regulation.[49–51]

Limitations

There were some limitations to our research. First, we only used male rats in our study and future reseach should explore the effect of ginsenoside on female rats.

Second, we only used 1 AD model here, and similar studies are necessary to investigate the effects of GCMc1 in other AD models that are more similar to human patient's conditions. It is crucial to replicate the study in multiple AD models that better mimic the heterogeneity seen in human patients. Third, the mechanisms responsible for the activation of PGC-1α/Sirt1 pathway independent of AMPK by this compound in AD rats require further investigation.[51,52]

Conclusions

Our study demonstrated that the GCMc1 improved cognitive features and mitochondrial function, and reduced neuroinflammation in AD rats through the activation of AMPK and modulation of the downstream PGC-1α/Sirt1/NF-κB signaling pathway. The findings suggest that GCMc1 has the potential to be a valuable addition to the therapeutic strategy for AD. However, further research, including clinical trials, is needed to confirm GCMc1 translational application in human patients. Clinical studies can provide valuable insights into the GCMc1 safety, dosage and efficacy in a real-world clinical setting. Investigating the long-term effects of this compound on AD is essential to assess its potential as a sustainable treatment option for its clinical use. Finally, given the intricate pathophysiology of AD, the combination of this compound with other pharmacological or non-pharmacological interventions may lead to additive or synergistic effects in enhancing outcomes for individuals with AD.

Supplementary data

The Supplementary materials are available at https://doi.org/10.5281/zenodo.10038799. The package includes the following file:

Supplementary File 1. Results of the statistical analysis of project data. The information regarding the results of the statistical analysis for each project's data is presented in this file, organized in accordance with the data order presented in the article.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Breijyeh Z, Karaman R. Comprehensive review on Alzheimer's disease: Causes and treatment. *Molecules*. 2020;25(24):5789. doi:10.3390/molecules25245789
2. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. *Mol Neurodegener*. 2019;14(1):32. doi:10.1186/s13024-019-0333-5
3. Wang X, Michaelis LM, Michaelis EK. Functional genomics of brain aging and Alzheimers disease: Focus on selective neuronal vulnerability. *Curr Genomics*. 2010;11(8):618–633. doi:10.2174/138920210793360943
4. Rao YL, Ganaraja B, Murlimanju BV, Joy T, Krishnamurthy A, Agrawal A. Hippocampus and its involvement in Alzheimer's disease: A review. *3 Biotech*. 2022;12(2):55. doi:10.1007/s13205-022-03123-4
5. Jobson DD, Hase Y, Clarkson AN, Kalaria RN. The role of the medial prefrontal cortex in cognition, ageing and dementia. *Brain Commun*. 2021;3(3):fcab125. doi:10.1093/braincomms/fcab125
6. Menon V, D'Esposito M. The role of PFC networks in cognitive control and executive function. *Neuropsychopharmacology*. 2022;47(1):90–103. doi:10.1038/s41386-021-01152-w
7. Yang Z, Zou Y, Wang L. Neurotransmitters in prevention and treatment of Alzheimer's disease. *Int J Mol Sci*. 2023;24(4):3841. doi:10.3390/ijms24043841
8. Kandimalla R, Reddy PH. Therapeutics of neurotransmitters in Alzheimer's disease. *J Alzheimers Dis*. 2017;57(4):1049–1069. doi:10.3233/JAD-161118
9. Xia X, Jiang Q, McDermott J, Han JJ. Aging and Alzheimer's disease: Comparison and associations from molecular to system level. *Aging Cell*. 2018;17(5):e12802. doi:10.1111/acel.12802
10. Wang X, Zimmermann HR, Ma T. Therapeutic potential of AMP-activated protein kinase in Alzheimer's disease. *J Alzheimers Dis*. 2019;68(1):33–38. doi:10.3233/JAD-181043
11. Assefa BT, Tafere GG, Wondafrash DZ, Gidey MT. The bewildering effect of AMPK activators in Alzheimer's disease: Review of the current evidence. *Biomed Res Int*. 2020;2020:9895121. doi:10.1155/2020/9895121
12. Curry DW, Stutz B, Andrews ZB, Elsworth JD. Targeting AMPK signaling as a neuroprotective strategy in Parkinson's disease. *J Parkinsons Dis*. 2018;8(2):161–181. doi:10.3233/JPD-171296
13. Onyango IG, Jauregui GV, Čarná M, Bennett JP, Stokin GB. Neuroinflammation in Alzheimer's disease. *Biomedicines*. 2021;9(5):524. doi:10.3390/biomedicines9050524
14. Li YQ, Chen Y, Jiang SQ, et al. An inhibitor of NF-κB and an agonist of AMPK: Network prediction and multi-omics integration to derive signaling pathways for acteoside against Alzheimer's disease. *Front Cell Dev Biol*. 2021;9:652310. doi:10.3389/fcell.2021.652310
15. Shi Z, Chen H, Zhou X, Yang W, Lin Y. Pharmacological effects of natural medicine ginsenosides against Alzheimer's disease. *Front Pharmacol*. 2022;13:952332. doi:10.3389/fphar.2022.952332
16. Zheng M, Xin Y, Li Y, et al. Ginsenosides: A potential neuroprotective agent. *Biomed Res Int*. 2018;2018:8174345. doi:10.1155/2018/8174345
17. Hong SH, Hwang HJ, Kim JW, et al. Ginsenoside compound-Mc1 attenuates oxidative stress and apoptosis in cardiomyocytes through an AMP-activated protein kinase-dependent mechanism. *J Ginseng Res*. 2020;44(4):664–671. doi:10.1016/j.jgr.2019.08.006
18. Liu L, Liu C, Fang L. AMPK-SIRT1 pathway dysfunction contributes to neuron apoptosis and cognitive impairment induced by sevoflurane. *Mol Med Rep*. 2020;23(1):56. doi:10.3892/mmr.2020.11694
19. Sun Y, Geng J, Wang D. Cardioprotective effects of ginsenoside compound-Mc1 and *Dendrobium nobile* Lindl against myocardial infarction in an aged rat model: Involvement of TLR4/NF-κB signaling pathway. *Eur J Inflamm*. 2021;19:205873922110005. doi:10.1177/20587392211000577
20. Rani V, Verma R, Kumar K, Chawla R. Role of pro-inflammatory cytokines in Alzheimer's disease and neuroprotective effects of pegylated self-assembled nanoscaffolds. *Curr Res Pharmacol Drug Discov*. 2023;4:100149. doi:10.1016/j.crphar.2022.100149
21. Yoo SM, Park J, Kim SH, Jung YK. Emerging perspectives on mitochondrial dysfunction and inflammation in Alzheimer's disease. *BMB Rep*. 2020;53(1):35–46. doi:10.5483/BMBRep.2020.53.1.274
22. Wang M, Li D. Ginsenoside-Mc1 reduces cerebral ischemia–reperfusion injury in hyperlipidemia through mitochondrial improvement and attenuation of oxidative/endoplasmic reticulum stress. *Arch Biol Sci (Beogr)*. 2022;74(2):159–168. doi:10.2298/ABS220212015W

23. Zhang Y, Xu K, Zhang Y. Ginsenoside-Mc1 reduces ischemia/reperfusion-induced cardiac arrhythmias through activating JAK2/STAT3 pathway and attenuating oxidative/endoplasmic reticulum stress in hyperlipidemic rats. *Turk J Biochem*. 2022;47(4):491–500. doi:10.1515/tjb-2021-0171
24. Cetin F, Yazihan N, Dincer S, Akbulut G. The effect of intracerebroventricular injection of beta amyloid peptide (1–42) on caspase-3 activity, lipid peroxidation, nitric oxide and nos expression in young adult and aged rat brain. *Turk Neurosurg*. 2012;23(2):144–150. doi:10.5137/1019-5149.JTN.5855-12.1
25. Othman MZ, Hassan Z, Che Has AT. Morris water maze: A versatile and pertinent tool for assessing spatial learning and memory. *Exp Anim*. 2022;71(3):264–280. doi:10.1538/expanim.21-0120
26. Sivandzade F, Bhalerao A, Cucullo L. Analysis of the mitochondrial membrane potential using the cationic JC-1 dye as a sensitive fluorescent probe. *Bio Protoc*. 2019;9(1):e3128. doi:10.21769/BioProtoc.3128
27. Dikalov SI, Harrison DG. Methods for detection of mitochondrial and cellular reactive oxygen species. *Antioxid Redox Signal*. 2014;20(2):372–382. doi:10.1089/ars.2012.4886
28. Chu Y, Park J, Kim E, Lee S. Fluorescent materials for monitoring mitochondrial biology. *Materials (Basel)*. 2021;14(15):4180. doi:10.3390/ma14154180
29. Alaaeldin R, Bakkar SM, Mohyeldin RH, Ali FEM, Abdel-Maqsoud NMR, Fathy M. Azilsartan modulates HMGB1/NF-κB/p38/ERK1/2/JNK and apoptosis pathways during renal ischemia reperfusion injury. *Cells*. 2023;12(1):185. doi:10.3390/cells12010185
30. Wang N, Jiang D, Zhou C, Han X. Alpha-solanine inhibits endothelial inflammation via nuclear factor kappa B signaling pathway. *Adv Clin Exp Med*. 2023;32(8):909–920. doi:10.17219/acem/158781
31. Liu CY, Zhou RX, Sun CK, et al. Preparation of minor ginsenosides C-Mc, C-Y, F2, and C-K from American ginseng PPD-ginsenoside using special ginsenosidase type-I from *Aspergillus niger* g.848. *J Ginseng Res*. 2015;39(3):221–229. doi:10.1016/j.jgr.2014.12.003
32. Lu JM, Yao Q, Chen C. Ginseng compounds: An update on their molecular mechanisms and medical applications. *Curr Vasc Pharmacol*. 2009;7(3):293–302. doi:10.2174/157016109788340767
33. Feng H, Xue M, Deng H, Cheng S, Hu Y, Zhou C. Ginsenoside and its therapeutic potential for cognitive impairment. *Biomolecules*. 2022;12(9):1310. doi:10.3390/biom12091310
34. Perez Ortiz JM, Swerdlow RH. Mitochondrial dysfunction in Alzheimer's disease: Role in pathogenesis and novel therapeutic opportunities. *Br J Pharmacol*. 2019;176(18):3489–3507. doi:10.1111/bph.14585
35. Galizzi G, Di Carlo M. Mitochondrial DNA and inflammation in Alzheimer's disease. *Curr Issues Mol Biol*. 2023;45(11):8586–8606. doi:10.3390/cimb45110540
36. Li J, Huang Q, Chen J, et al. Neuroprotective potentials of Panax Ginseng against Alzheimer's disease: A review of preclinical and clinical evidences. *Front Pharmacol*. 2021;12:688490. doi:10.3389/fphar.2021.688490
37. López-Armada MJ, Riveiro-Naveira RR, Vaamonde-García C, Valcárcel-Ares MN. Mitochondrial dysfunction and the inflammatory response. *Mitochondrion*. 2013;13(2):106–118. doi:10.1016/j.mito.2013.01.003
38. Price RB, Duman R. Neuroplasticity in cognitive and psychological mechanisms of depression: An integrative model. *Mol Psychiatry*. 2020;25(3):530–543. doi:10.1038/s41380-019-0615-x
39. Liu Y, Zhou G, Song L, et al. DEAD-Box helicase 17 promotes amyloidogenesis by regulating BACE1 translation. *Brain Sci*. 2023;13(5):745. doi:10.3390/brainsci13050745
40. Ma T, Klann E. Amyloid β: Linking synaptic plasticity failure to memory disruption in Alzheimer's disease. *J Neurochem*. 2012;120(s1):140–148. doi:10.1111/j.1471-4159.2011.07506.x
41. Nie L, Xia J, Li H, et al. Ginsenoside Rg1 ameliorates behavioral abnormalities and modulates the hippocampal proteomic change in triple transgenic mice of Alzheimer's disease. *Oxid Med Cell Longev*. 2017;2017:6473506. doi:10.1155/2017/6473506
42. Qu S, Meng X, Liu Y, Zhang X, Zhang Y. Ginsenoside Rb1 prevents MPTP-induced changes in hippocampal memory via regulation of the α-synuclein/PSD-95 pathway. *Aging*. 2019;11(7):1934–1964. doi:10.18632/aging.101884
43. O'Day DH. Calmodulin binding proteins and Alzheimer's disease: Biomarkers, regulatory enzymes and receptors that are regulated by calmodulin. *Int J Mol Sci*. 2020;21(19):7344. doi:10.3390/ijms21197344
44. Zhang J, Wang Y, Liu X, Dagda RK, Zhang Y. How AMPK and PKA interplay to regulate mitochondrial function and survival in models of ischemia and diabetes. *Oxid Med Cell Longev*. 2017;2017:4353510. doi:10.1155/2017/4353510
45. Huang Q, Gao S, Zhao D, Li X. Review of ginsenosides targeting mitochondrial function to treat multiple disorders: Current status and perspectives. *J Ginseng Res*. 2021;45(3):371–379. doi:10.1016/j.jgr.2020.12.004
46. Cantó C, Auwerx J. PGC-1α, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol*. 2009;20(2):98–105. doi:10.1097/MOL.0b013e328328d0a4
47. Sun E, Motolani A, Campos L, Lu T. The pivotal role of NF-kB in the pathogenesis and therapeutics of Alzheimer's disease. *Int J Mol Sci*. 2022;23(16):8972. doi:10.3390/ijms23168972
48. Abd El-Fattah EE, Saber S, Mourad AAE, et al. The dynamic interplay between AMPK/NFκB signaling and NLRP3 is a new therapeutic target in inflammation: Emerging role of dapagliflozin in overcoming lipopolysaccharide-mediated lung injury. *Biomed Pharmacother*. 2022;147:112628. doi:10.1016/j.biopha.2022.112628
49. Han Y, Liu D, Cheng Y, et al. Maintenance of mitochondrial homeostasis for Alzheimer's disease: Strategies and challenges. *Redox Biol*. 2023;63:102734. doi:10.1016/j.redox.2023.102734
50. Qi Y, Chen L, Shan S, Nie Y, Wang Y. Vitexin improves neuron apoptosis and memory impairment induced by isoflurane via regulation of miR-409 expression. *Adv Clin Exp Med*. 2020;29(1):135–145. doi:10.17219/acem/104556
51. Abubakar MB, Sanusi KO, Ugusman A, et al. Alzheimer's disease: An update and insights into pathophysiology. *Front Aging Neurosci*. 2022;14:742408. doi:10.3389/fnagi.2022.742408
52. Li Y, Hu K, Liang M, et al. Stilbene glycoside upregulates SIRT3/AMPK to promotes neuronal mitochondrial autophagy and inhibit apoptosis in ischemic stroke. *Adv Clin Exp Med*. 2021;30(2):139–146. doi:10.17219/acem/130608