Many studies have shown that the steamed root of *Rehmannia glutinosa* (SRG), which is widely used in the treatment of various neurodegenerative diseases in the context of Korean traditional medicine, is effective for improving cognitive and memory impairments. The purpose of this study was to examine whether SRG extracts improved memory defects caused by administering scopolamine (SCO) into the brains of rats. The effects of SRG on the acetylcholinergic system and proinflammatory cytokines in the hippocampus were also investigated. Male rats were administered daily doses of SRG (50, 100, and 200 mg/kg, i.p.) for 14 days, 1 h before scopolamine injection (2 mg/kg, i.p.). After inducing cognitive impairment via scopolamine administration, we conducted a passive avoidance test (PAT) and the Morris water maze (MWM) test as behavioral assessments. Changes in cholinergic system reactivity were also examined by measuring the immunoreactive neurons of choline acetyltransferase (ChAT) and the reactivity of acetylcholinesterase (AchE) in the hippocampus. Daily administration of SRG improved memory impairment according to the PAT, and reduced the escape latency for finding the platform in the MWM. The administration of SRG consistently significantly alleviated memory-associated decreases in cholinergic immunoreactivity and decreased interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) mRNA expression in the hippocampus. The results demonstrated that SRG had a significant neuroprotective effect against the neuronal impairment and memory dysfunction caused by scopolamine in rats. These results suggest that SRG may be useful for improving cognitive functioning by stimulating cholinergic enzyme activities and alleviating inflammatory responses.

**Keywords:** Scopolamine, memory, cholinergic neurons, proinflammatory cytokines, *Rehmannia glutinosa*

Alzheimer’s disease (AD), which is characterized by a progressive decline in cognitive functioning due to the degeneration of the cholinergic nervous system, is one of the most common forms of dementia [1]. Cholinergic deficits are a major neuropathological feature associated with memory loss and have been closely correlated with the severity of cognitive dysfunction associated with AD [12].

Scopolamine (SCO), a blocker of muscarinic acetylcholine (ACh) receptors, induces the dysregulation of cholinergic activity and the impairment of memory functioning, resulting in deficits in the learning, acquisition, and short-term retention of spatial memory tasks [9, 30]. The damage caused by repeated SCO-induced reductions in cholinergic activity has been hypothesized to play a role in reducing hippocampal volume, which has often been associated with AD [34]. AD has also been correlated with the loss of cholinergic neurons and decreases in the levels of acetylcholine (ACh) and choline acetyltransferase (ChAT) [19]. Lesions in these pathways result in decreased ACh release and thus cause learning and memory dysfunction [24]. Until now, inhibition of acetylcholinesterase (AchE) has served as a strategy for the treatment of AD, senile dementia, ataxia, and Parkinson’s disease. The drugs approved for the AD therapy act by counteracting the acetylcholine deficits, providing symptomatic relief, improving cognitive functioning, and enhancing the acetylcholine levels in the brain [4, 13].

Inflammation, as well as cholinergic neuronal degeneration, may play a critical role in the pathogenesis of the degenerative changes and cognitive impairments associated with AD. Proinflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), are upregulated in the AD brain [26]. These cytokines may play a role in several events in the pathological cascade of AD [8].

Although the mechanisms underlying the anti-amnesic effects of most herbal extracts have not yet been fully elucidated, it has been reported that application of herbal extracts or their pharmacological components has improved
memory-related behavior [18] and activated central ACh functioning through the inhibition of AchE and the activation of ACh synthesis in patients with Alzheimer's disease [36].

The steamed root of Rehmannia glutinosa (SRG), known as Sook-Ji-Whang in Korean, has been used in traditional Oriental medicine to treat various cardiovascular, psychostimulant-related, and inflammatory diseases [17]. An aqueous extract from SRG has been reported to inhibit the inflammation-mediated activation of microglia in rats according to the ischemic brain injury model [16, 31]. Several studies have shown that catalpol, an iridoid of SRG, was effective in ameliorating neurodegenerative changes and improving learning and memory [32, 35], suggesting that the anti-inflammatory effects of SRG may derive from its ability to inhibit the degeneration of cholinergic neurons, thereby alleviating deficits in spatial learning ability in the memory-impairment animal model [17].

The aim of the present study was to evaluate the ability of SRG to improve learning and memory of rats exposed to repeated scopolamine-induced memory deficits as measured by performance on the passive avoidance test (PAT) and the Morris water maze (MWM) test. Moreover, we also examined how these effects were related to the cholinergic system and to anti-inflammatory effects to elucidate the neural mechanisms underlying the memory-enhancing activity of SRG.

Materials and Methods

Animals

Adult male Sprague-Dawley (SD) rats weighing 260–280 g were obtained from Samtaco Animal Co. (Seoul, Korea). The rats were housed in a limited-access rodent facility with up to five rats per polycarbonate cage. The room controls were set to maintain the temperature at 22°C ± 2°C and the relative humidity at 55% ± 15%. Cages were lit by artificial light for 12 h each day. Sterilized drinking water and standard chow diet were supplied ad libitum to each cage during the experiments. The animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised in 1996, and were approved by the Kyung Hee University Institutional Animal Care and Use Committee. All animal experiments began at least 7 days after the animals arrived.

Preparation of Rehmannia glutinosa Aqueous Extracts

Rehmannia glutinosa (Gaertn.) was purchased from an Oriental drug store (Health Maximum Co., Jcheon, Korea). The classical processing method used to prepare steamed Rehmannia glutinosa is “steaming nine times and drying in the sun nine times” [20]. It was produced by steaming the dried roots for 24 h in the GMP workshop of the factory according to Pharmacopoeia of Korea. A voucher specimen of SRG has been deposited at the herbarium located at the College of Oriental Medicine, Kyung Hee University (Code number KH-SRG01 for SRG). The air-dried and crushed materials were added to distilled water, and extraction was performed by heating for 4 h at 100°C. Then the extract was concentrated with a rotary evaporator (EYELA CCA-1110, Tokyo Rikakikai Co., Tokyo, Japan) and dried with a freeze dryer (EYELA FD-800, Tokyo Rikakikai). The collection rate of the final aqueous extracts was 7.5%.

Experimental Groups

To develop learning and memory deficits, male rats were intraperitoneally injected at 2 mg/kg body weight with scopolamine hydrobromide (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), dissolved in physiological saline solution, once a day for 14 days. Normal animals received saline injections instead of scopolamine as a vehicle. Different rats in an experimental group were subjected to either behavioral testing or immunohistochemistry. The rats were randomly divided into six groups of seven individuals as follows: normal group (NOR group, n=7), the saline-induced plus 200 mg/kg SRG-treated group (SRG group, n=7), the scopolamine-induced and saline-treated group (SCO group as a control, n=7), the scopolamine plus 50 mg/kg SRG group (SRG50+SCO group, n=7), the scopolamine plus 100 mg/kg SRG group (SRG100+SCO group, n=7), and the scopolamine plus 200 mg/kg SRG group (SRG200+SCO group, n=7). The rats were intraperitoneally administered with SRG for 14 days, and SRG was dissolved in 0.9% physiological saline. One hour after SRG administration, all rats except for the NOR group received the scopolamine injection. At the 2nd week after scopolamine injection, rats were subjected to the Morris water maze task. The experimental schedule of all drug administrations and behavioral tests are shown in Fig. 1.

Passive Avoidance Test

All animals were subjected to a passive avoidance test. The test was basically performed according to the step-through method described previously [27]. The Gemini Avoidance System (SD Instruments., San Diego, CA, USA) was used for this experiment. Basically, the step-through passive avoidance apparatus consists of a two-compartment acrylic box with a lightened compartment connected to a darkened one by an automatic guillotine door. Electric shocks was delivered to the grid floor of both compartments, made of stainless steel rods (3 mm diameter) spaced 1 cm apart, by an isolated shock generator (Behbood Pardazo Co., Ghaem, Iran). First, rats were subjected to acquisition test trials in the apparatus. In this trial, rats were placed...
in a lighted compartment for 300 s, and then the guillotine door was opened. Rats have a native preference to the dark environment. Immediately upon entering the dark compartment, the door was closed. The acquisition test recorded the latencies times for entering the dark compartment. After 30 min, rats were again placed in the lightened compartment. After the rats had spontaneously entered the dark compartment, the guillotine door was closed and a mild electrical shock (0.5 mA) was applied for 3 s. Exactly 24 h after the acquisition trial for training, the retention test was performed. The rat was again placed in the lightened compartment and the guillotine door was opened. The retention test measured the latencies times for entering the dark compartment in the same method with acquisition test. The maximum entry latency allowed in the retention test was 180 s [23].

**Morris Water Maze Test**

**Morris water maze apparatus.** The MWM test was performed using a polypropylene circular pool (painted white internally; 2.0 m in diameter and 0.35 m high). The pool contained water maintained at a temperature of 22 ± 2°C. The water was made opaque by adding 1 kg of skim milk powder. During the MWM test, a platform 15 cm in diameter was located 1.5 cm below the water in one of four sections of the pool, approximately 50 cm from the sidewalls. The pool was surrounded by many cues external to the maze. The pool was divided into four quadrants of equal area. A digital camera was mounted to the ceiling above the pool and was connected to a computerized recording system equipped with a tracking program (S-MART: PanLab Co., Barcelona, Spain), which permitted on- and off-line automated tracking of the paths taken by the rats.

**Hidden platform trial for acquisition test.** The MWM test was initiated on the 8th day after the SRG and SCO administration had commenced. The animals received three trials per day. The rats were trained to find the hidden platform, which remained in a fixed location throughout the test. The trials lasted for a maximum of 180 s, and the time it took to find the submerged platform was recorded each time. The animals were tested three trials per day for 6 days, and they received a 60 s probe trial on the seventh day. Finding the platform was defined as staying on it for at least 4 s before the acquisition time of 180 s ended. If the rat failed to find the platform in the allotted time, it was placed onto the platform for 20 s and assigned a latency of 180 s. Between one trial and the next, water was stirred to erase olfactory traces of previous swim patterns. The entire procedure took seven consecutive days, and each animal had three training trials per day, with a 30- to 40-min inter-trial interval.

**Probe trial for retention test.** For the probe trial, each rat was placed into the water diagonally from the target quadrant, and for 60 s was allowed to search the water from which the platform had been removed. The time (% of total time) spent searching for the platform in the former platform quadrant versus the other three quadrants was measured for each rat.

**Choline Acetyltransferase (ChAT) Immunohistochemistry**

For immunohistochemical studies, the animals were deeply anesthetized with sodium pentobarbital (80 mg/kg, by intraperitoneal injection) and perfused through the ascending aorta with normal saline (0.9%) followed by 300 ml (per rat) of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). The brains were removed, post-fixed overnight, and cryoprotected with 20% sucrose in 0.1 M PBS at 4°C. Coronal sections 30 µm thick were cut through the hippocampus using a cryostat (Leica CM1850; Leica Microsystems Ltd., Nussloch, Germany). The sections were obtained according to the rat atlas of Paxinos and Watson (hippocampus; between bregma -2.6 and -3.6) [25]. The sections were immunostained for ChAT expression using the avidin–biotin–peroxidase complex (ABC) complex method. Briefly, the sections were rinsed three times for 5 min each in PBS and then incubated with primary rabbit anti-ChAT antibody (1:2,000 dilution; Cambridge Research Biochemicals Co., Bellingham, UK) in PBST (PBS plus 0.3% Triton X-100) for 72 h at 4°C. The sections were washed for 5 min in PBS and then incubated for 120 min at room temperature with biotinylated anti-rabbit goat IgG (for the anti-ChAT antibody). Both secondary antibodies were obtained from Vector Laboratories Co. (Burlingame, CA, USA) and diluted 1:200 in PBST containing 2% normal goat serum. To visualize immunoreactivity, the sections were incubated for 90 min in ABC reagent (Vectastain Elite ABC kit; Vector Labs. Co., Burlingame, CA, USA), washed three times for 5 min in PBS, and incubated in a solution containing 3,3′-diaminobenzidine (DAB; Sigma) and 0.01% H2O2 for 1 min. Finally, the tissues were washed in PBS, followed by a brief rinse in distilled water, and mounted individually onto slides. The slides were allowed to air dry and were then cover-slipped. Images were captured using the AxioVision 3.0 imaging system (Carl Zeiss, Inc., Oberkochen, Germany) and processed using Adobe Photoshop (Adobe Systems, Inc., San Jose, CA, USA). The sections were viewed at 200× magnification, and the numbers of cells within 100 × 100 µm2 grids were counted by observers blinded to the experimental groups. Hippocampal area cells were obtained according to the stereotaxic atlas of Paxinos and Watson [25]. The cells were counted in three sections per rat within the hippocampal area.

**Acetylcholinesterase (AchE) Immunohistochemistry**

For AchE histochemistry, the sections were washed in PBS and incubated in a solution with 25 mg of acetylthiocholine iodine for 1 h. The solution was composed of 32.5 ml of 0.1 M sodium hydrogen phosphate buffer (NaH2PO4·H2O, pH 6.0), 2.5 ml of 0.1 M sodium citrate, 5 ml of 30 mM copper sulfate, 5 ml of 5 mM potassium ferricyanide, and 5 ml of distilled water. The color of the mixing solution was green. The densities of stained nuclei of the hippocampal cells were measured using a Scion image program (Scion Co., Frederick, MD, USA). The sections were viewed at 200× magnification, and the numbers of cells within 100 × 100 µm2 grids were counted by observers blinded to the experimental groups. Hippocampal area cells were obtained according to the stereotaxic atlas of Paxinos and Watson [25]. The cells were counted in three sections per rat within the hippocampal area.

**Total RNA Preparation and RT–PCR Analysis**

The hippocampus from each of four rats in each group was isolated. After decapitation, the brain was quickly removed and stored at -80°C until use. The total RNA was isolated from the brain sample using a TRIzol reagent (Invitrogen Co., Carlsbad, CA, USA) and RNA was extracted according to the supplier’s instruction. Complementary DNA was synthesized from total RNA with a reverse transcriptase (Takara Co., Shiga, Japan). Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) mRNA expression levels were determined by the reverse transcription–polymerase chain reaction (RT–PCR). RT–PCR was performed using a PTC-100 programmable thermal controller (MJ Research, Inc., Watertown, MA, USA). The operating conditions were as follows: for glyceraldehydes-3-phosphate dehydrogenase (GAPDH), 30 cycles of denaturation at 95°C for 30 s, annealing at
58°C for 30 s, and extension at 72°C for 30 s; for IL-1β, 27 cycles of
denaturation at 95°C for 30 s, annealing at 51°C for 30 s, and
extension at 72°C for 30 s; for TNF-α, 27 cycles of denaturation at
95°C for 30 s, annealing at 51°C for 30 s, and extension at 72°C for
30 s. All primers were designed using published mRNA sequences
and primer design software (Primer 3; The Whitehead Institute for
Biomedical Research, Cambridge, MA, USA; http://www.genome.
wi.mit.edu) offered through their Web site. The following sequences
were used: for GAPDH, (forward) 5'-ATC CCA TCA CCA CCT
TCC AG-3' and (reverse) 5'-CCT GCT TCA CCA CCT TCT TG-3';
for IL-1β, (forward) 5'-GGC ATA ACA GGC TCA TCT GG-3' and
(reverse) 5'-CAT CAT CCC ACG AGT CAC AG-3'; for TNF-α,
(forward) 5'-AAT GGC ATG GAT CTC AAA GA-3' and (reverse)
5'-GGT ACA TGG GCT CAT ACC AG-3'. The PCR products were
separated on 1.2% agarose gels and stained with ethidium bromide,
after which the density of each band was evaluated using an image-
analyzing system (i-Max, CoreBio System Co., Seoul, Korea).
Complementary DNA expression levels were determined by calculating
the relative density of each IL-1β or TNF-α band to GAPDH.

Statistical Analysis
All measurements were performed by an independent investigator
blinded to the experimental conditions. Results in figures are expressed
as mean ± standard error of means (SE). Differences within or
between normally distributed data were analyzed by analysis of
variance (ANOVA) using SPSS (Version 13.0; SPSS, Inc., Chicago,
IL, USA) followed by Tukey’s post-hoc test. Data were analyzed
via a separate one-way ANOVA followed by Tukey’s post hoc test.
Vertical bars indicate SE.

RESULTS

Effect of SRG on the Passive Avoidance Test
To determine whether the SRG promotes the recovery of
memory dysfunction using the PAT, we administrated SRG
to the scopolamine-induced memory impairment model.
Rats in all groups were subjected to acquisition trials
without electric challenge, to make sure there were no
physiological defects (i.e., motor function) or intrinsic cognitive
impairments prior to the scopolamine-induced impairments.
The time for acquisition trials, indicated by the latencies
times for entering the dark compartment, was not significantly
different in all groups [F(5,41)=1.153, p=0.978] (Fig. 2).

After the acquisition trials, the SRG effect on the retention
latency, indicated by the latencies times for entering the
dark compartment, was measured 24 h after applying electric
shock in the dark box of the PAT test. In the retention, it
was shown that the SRG100+SCO group (p<0.05) and
SRG200+SCO group (p<0.05) had significantly increased
latencies to enter the dark compartment for retention as
compared with those of the SCO group (F[5,41]=4.177,
p<0.01). This study indicates that scopolamine injection
severely impaired long-term memory, and the SRG treatment
significantly attenuated the scopolamine-induced memory
deficit in the PAT test.

Effect of SRG on the Water Maze Test
The effect of SRG treatment on the swimming time to
reach the submerged platform is illustrated in Fig. 3. The
NOR group rapidly learned the location of the submerged
hidden platform and reached it within 20 s on day 6 of the
trials. The SRG-treated rats (200 mg/kg) also showed a
reduction in the escape latency through the training period.
The SCO group showed a marked retardation in escape
latency, probably due to memory deficits resulting from
scopolamine-induced impairment of learning and memory.
Analysis of the training data by repeated-measures ANOVA
showed that the escape latency differed significantly among
the groups when the times were averaged over all the
sessions [F(5,36)=8.055, p<0.001]. During the experiment,
the latency to escape diminished over time [F(5,180)
=145.996, p<0.001]; however, there was no significant
interaction between experimental groups and time [F(25,180)
=0.598, p=0.935]. The Tukey’s post hoc test revealed that
rats in both the SRG100+SCO and SRG200+SCO groups
had significantly reduced swimming latency compared
with subjects in the SCO group (SRG100+SCO group:
p<0.05 on day 6; SRG200+SCO group: p<0.05 on day 4,
5, and 6; Fig. 3A). The SCO group was not significantly
different from other groups in terms of mean swimming
speed, as calculated by dividing the total swim distance
by the latency [F(5,36)=0.128, p=0.985] (Fig. 3C). Total
distance traveled in each group was closely associated with
escape latency in this task (data not shown). Based on these

Fig. 2. Effect of SRG on the latencies to enter the dark compartment
for the acquisition trial and for the retention test during the PAT
test. *p<0.01 vs. the NOR group; #p<0.05 vs. the SCO group.

Vertical bars indicate SE.
results, the 200 mg/kg SRG-treated rats showed greater improvements in acquisition during the hidden platform trial and reached the platform quicker than the scopolamine-treated rats.

To examine the spatial memory of rats, performance in the probe trial on day 7 was analyzed by comparing the percentage of time spent swimming to the expected position of the platform (Fig. 3B). The times spent swimming around was significantly reduced in the rats that swam to the target area where the platform had been located [F(5,41)=5.278, p<0.01]. The repeated administration of SCO severely impaired spatial performance in the MWM (p<0.01). Rats in the 200 mg/kg SRG-treated (p<0.05) groups spent more time around the platform area than did those in the SCO group. The SCO group was not significantly different from other groups in terms of mean swimming speed, as calculated by dividing the total swim distance by the latency [F(5,36) =0.128, p=0.985] (Fig. 3C). Total distance traveled in each group was closely associated with escape latency in this task (data not shown). Based on these results, the 200 mg/kg of SRG-treated rats showed greater improvements in acquisition during the hidden platform trial and reached the platform quicker than the scopolamine-treated rats. SRG treatment significantly attenuated the scopolamine-induced deficit of learning and memory demonstrated in the water maze. This study indicates that the swimming latency in rats receiving SRG administration was higher than that of scopolamine-induced deficit rats used as controls. Thus, SRG-treated rats showed a significant amelioration in the memory retention test, because they spent more time in the quadrant where the platform was formerly located and swam over the former location of the platform more frequently.

**Effect of SRG on the Central Cholinergic System**

**ChAT Immunohistochemistry**

Following the behavioral tasks, brain tissue samples from the subjects were analyzed using immunohistochemistry to

![Fig. 3](image-url) **Fig. 3.** Time to escape (latency) during acquisition trials of hidden platform (A), probe trial (B), and swim speed (C) during the Morris water maze test.

Data were analyzed using a repeated-measures ANOVA followed by Tukey’s post-hoc test. *p < 0.05 and **p < 0.01 vs. the NOR group; *p < 0.05 vs. the SCO group. Vertical bars indicate SE.

![Fig. 4](image-url) **Fig. 4.** Representative photographs showing the distribution of choline acetyltransferase and acetylcholinesterase reactive cells in the hippocampus of the NOR-ChAT-CA1 (A), SCO-ChAT-CA1 (B), SRG200+SCO-ChAT-CA1 (C), NOR-ChAT-CA3 (D), SCO-ChAT-CA3 (E), SRG200+SCO-ChAT-CA3 (F), NOR-AchE (G), SCO-AchE (H), and SRG200+SCO-AchE (I) groups. Sections were cut coronally at 30 µm. The scale bar represents 50 µm.
investigate the effect of SRG administration on neurons loss due to scopolamine-induced memory impairment. ChAT immunoreactivity analyses in the CA1 and CA3 areas of the hippocampus are shown in Fig. 4. The brains of the SCO group showed significant neurons loss in the CA1 area of the hippocampus, as compared with the NOR group (p<0.01). Comparison of the numbers of ChAT-immunoreactive neurons using one-way ANOVA revealed a significant difference among the groups. The number of ChAT-immunoreactive neurons in hippocampal area CA1 was 31.83±3.62 (100.0±6.52%) in the NOR group, 30.83±3.03 (96.86±9.50%) in the SRG group, 18.14±2.40 (56.99±7.53%) in the SCO group, 21.59±1.95 (67.83±6.11%) in the SRG50+SCO group, 23.70±2.28 (74.46±7.15%) in the SRG100+SCO group, and 29.07±2.32 (91.33±7.28%) in the SRG200+SCO group [F(5,149)=5.172, p<0.001]. The number of ChAT-immunoreactive neurons significantly increased in hippocampal region CA1 in the SRG200+SCO group (p<0.05), as compared with the SCO group (Fig. 5).

The number of ChAT-immunoreactive neurons in hippocampal area CA3 was 25.13±1.81 (100.0±7.24%) in the NOR group, 22.73±3.79 (90.48±15.07%) in the SRG group, 13.48±1.54 (53.64±6.12%) in the SCO group, 18.30±2.23 (72.82±8.86%) in the SRG50+SCO group, 22.33±1.65 (88.89±6.56%) in the SRG100+SCO group, and 24.44±2.01 (97.29±8.00%) in the SRG200+SCO group [F(5,149)=2.941, p<0.05]. The number of ChAT-immunoreactive neurons significantly increased in hippocampal region CA3 in the SRG200+SCO group (p<0.05), as compared with the SCO group (Fig. 5).

Effect of SRG on the Acetylcholinesterase (AchE) Histochemistry

The density of AchE-immunopositive fibers in the CA1 area of the rat hippocampus was significantly increased in the SCO group, as compared with the NOR group (Fig. 4). The AchE-positive neuron density in the CA1 was 13.63±0.51 (100.0±7.42%) in the NOR group, 14.83±0.98 (108.87±7.17%) in the SRG group, 22.00±2.04 (161.47±14.96%) in the SCO group, 18.85±1.66 (138.36±12.19%) in the SRG50+SCO group, 17.04±1.50 (125.04±10.98%) in the SRG100+SCO group, and 15.04±1.33 (110.36±9.73%) in the SRG200+SCO group [F(5,154)=4.789, p<0.001]. The AchE-reactive neuronal activity in the hippocampus area CA1 due to scopolamine-induced memory impairment was significantly restored in the SRG200+SCO group (p<0.05), as compared with the SCO group (Fig. 6).

The density of the AchE fibers in the CA3 region of the hippocampus was also markedly increased in the SCO group, as compared with the NOR group (Fig. 4). The AchE neuronal density in the CA3 region was 11.13±0.80 (100.0±4.56%) in the NOR group, 12.17±0.82 (109.36±7.37%) in the SRG group, 18.59±2.66 (167.12±23.95%) in the SCO group, 14.89±2.47 (133.83±22.19%) in the SRG50+SCO group, 14.07±1.40 (126.51±12.60%) in the SRG100+SCO group, and 11.67±0.89 (104.87±7.99%) in the SRG200+SCO group [F(5,154)=2.739, p<0.05]. The AchE-reactive neuronal activity in hippocampus area CA3 due to scopolamine-induced memory impairment was significantly restored in the SRG200+SCO group (p<0.05), as compared with the SCO group (Fig. 6). The densities of AchE-reactive neurons in the SRG200+SCO group were closely similar to that of the NOR group. The effect of SRG
on the density of AchE reactive neurons in hippocampus area CA3 was similar to that in the CA1 region.

**Effect of SRG on Interleukin-1β (IL-1β) and Tumor Necrosis Factor (TNF)-α mRNA Expression Levels in the Hippocampus**

The effect of SRG administration on IL-1β and TNF-α mRNA expression levels in the rats with SCO-induced hippocampus lesions was investigated using RT-PCR analysis (Fig. 7). The IL-1β and TNF-α mRNA expression levels were normalized using GAPDH mRNA as an internal control. Hippocampal IL-1β mRNA expression in the SCO group was significantly increased, as compared with that of the NOR group (p<0.01). The increased expression of IL-1β mRNA in the SCO group was significantly restored in the SRG200+SCO group (p<0.05). The restored levels were similar to that of normal rats in the NOR group. SRG200+SCO group had restored levels similar to the normal rats. The current finding that the

**DISCUSSION**

The present results demonstrated that repeated SCO-induced dementia produced severe deficits in performances on tests of cognitive functioning as well as corresponding signs of neurodegeneration in the brain, including decreased ChAT and increased AchE activities in the hippocampus. Our results showed that administration of SRG significantly improved learning and memory on the PAT and MWM tests and also produced increased ChAT and decreased AchE immunoreactivity in the hippocampal areas associated with the SCO-induced memory impairment of male rats. Additionally, SRG also inhibited the increase in proinflammatory cytokines in the hippocampus of rats with SCO-induced deficits.

Acute and chronic administrations of SCO in animals have transiently produced several deficits in learning acquisition and short-term memory that are considered to be characteristic of cholinergic deficits in AD or senile CNS dysfunction [5]. The well-replicated amnesic effect of SCO has been interpreted as a principal consequence of the blockade of post-synaptic muscarinic M1 transmission, leading to disruption in the functioning of the hippocampus in working memory. Thus, the SCO-induced amnesic model has been widely used to provide a pharmacological model of memory dysfunction for use in screening for agents with potential cognitive-enhancement effects [7].

The PAT is generally used to evaluate treatments for impairments in the three stages of memory: learning acquisition, memory retention, and memory retrieval [21]. This test showed that SCO significantly shortened the step-through latency of the retention trial, demonstrating that the central cholinergic neuronal system plays an important role in learning acquisition and synaptic plasticity. SRG prolonged the step-through latency of the passive avoidance response that had been shortened by SCO. These results suggested that the anti-amnesic effect of SRG on SCO-induced memory impairment may be related to mediation by the cholinergic nervous system.

To confirm the effect of SRG on other types of memory, we performed the MWM test to evaluate spatial learning. When assessing spatial learning and memory using a rat model, the MWM is considered to be advantageous relative to other types of conventional mazes such as the T-maze and the radial-arm maze. Training for spatial memory can be easily achieved across several acquisition trials, and the task does not require strong motivating conditions such as scent, punishment, or food and/or water deprivation. The MWM is a hippocampus-dependent memory task that is frequently used for examining cognitive deficits and demonstrating permanent spatial learning ability and reference memory in rodents [15]. Animals encode spatial working information during the learning step, which then serves to guide future memory retrieval. The current finding that the
memory deficits demonstrated by the SCO-impaired rats in a MWM task were more pronounced than those in the NOR group were consistent with findings from previous studies [18]. During the trial sessions, the SRG group showed significantly reduced escape latency, enhanced cognitive performance, and amelioration of memory deficits associated with SCO.

In terms of the average swim speeds and rest times, indices of motor functioning, the SCO group did not significantly differ from the SRG-treated groups, suggesting that motor impairment was not the primary cause of the poor performance exhibited by the SCO group in the MWM test. Thus, it is evident that SRG administration improved MWM performance by directly enhancing spatial working memory.

Scores on the escape and the spatial probe tests of the MWM test are considered to primarily reflect long-term spatial memory ability. In this study, SRG treatment shortened the escape latency without affecting swimming velocity and extended the time spent swimming in the place where the platform was previously located. In the probe test, administration of SRG lowered the escape latency to levels comparable to those demonstrated by the control group and significantly increased the number of times that the rats crossed the platform. If the animals spent more time and swam longer distances in the pool where the platform had previously been located during the training session, the animals would be demonstrating that they learned from their prior experiences with the MWM test, showing spatial memory improvement [2]. This would indicate that administration of SRG significantly improved long-term spatial memory in rats that had experienced SCO-induced memory impairment.

According to the results from the PAT and MWM tests, SRG ameliorated the SCO-induced deficits in learning and memory. In the present study, we observed SCO-induced cholinergic dysfunction in rats with amnesia. It is likely that the observed memory-related improvements of the SRG-treated rats was associated with the increase in the proinflammatory cytokines IL-1β and TNF-α expression [26]. In the present study, SCO administration caused an increase in the proinflammatory cytokines IL-1β and TNF-α as well as learning and memory deficits. SRG inhibited the increased activation of proinflammatory cytokines such as IL-1β and TNF-α in the SCO-treated rats. Thus, our results suggest that SCO may cause memory dysfunction through a combination of cholinergic neuronal dysregulation and complicated inflammatory action.

The expression and activation of AchE and ChAT regulate the dynamic concentration of ACh in the cholinergic synapses in the brain. Thus, the hippocampal expression of AchE and ChAT and its correlation with the SCO-induced memory impairment rats was examined. SRG also improved cholinergic neurons in the frontalparietal cortex and CA1 region of the hippocampus and continuously induced increases in ChAT and decreases in AchE activities, which eventually resulted in recovery of the entire cholinergic circulation pathway [29]. According to the cholinergic hypothesis, memory impairments in patients with senile dementia are due to a selective and irreversible deficiency in the cholinergic functions of the brain [10]. Thus, cholinesterase inhibitors and ChAT activators may compensate for reduced the ACh levels in brains with AD disease. Previous biochemical and behavioral evidence have indicated that central cholinergic transmission declines as a result of SCO administration [33] and the type of dementia associated with AD [3]. This type of decline has been closely associated with cognitive disturbances. It is likely that the observed amelioration in the deficits in spatial learning capability demonstrated by the SRG-treated rats was associated with the increase release of ACh. We thus propose that the beneficial effects of SRC could be related to increases in central cholinergic functioning [17].

At the same time, many studies have suggested that amyloid β-peptide (25–35)-induced or SCO-induced IL-1β and TNF-α expression is upregulated in AD [6, 11] and that these cytokines may play a role in several events in the pathological cascade of AD [8]. These results suggest that inflammatory reactions may be related to the pathogenesis of the degenerative changes and cognitive impairments [26]. In the present study, SCO administration caused an increase in the proinflammatory cytokines IL-1β and TNF-α as well as learning and memory deficits. SRG inhibited the increased activation of proinflammatory cytokines such as IL-1β and TNF-α in the SCO-treated rats. Thus, our results suggest that SCO may cause memory dysfunction through a combination of cholinergic neuronal dysregulation and complicated inflammatory action.

The present study showed that repeated SCO-induced dementia produced learning and memory deficits as well as associated degeneration of cholinergic neurons and inflammatory reactions in rats, as manifested in their performance on the PAT and MWM. However, administration of SRG extracts attenuated these repeated SCO-induced dementia effects, as indicated by improved cognitive functioning during behavioral tests and increases in the density of cholinergic neurons. Therefore, SRG may possibly be used as an effective agent to prevent cholinergic dysfunction and anti-inflammatory effects such as those observed in AD.
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REFERENCES


