RESEARCH ARTICLE

Garlic synthetic complex ameliorates oxidative stress, improves spatial working memory, and exhibits anxiolytic potential in scopolamine-induced neurotoxicity in Wistar rats

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ABSTRACT

Objective: This study aimed to investigate the antioxidant effect and spatial memory enhancement of the garlic synthetic complex (GSC) on scopolamine-induced neurotoxicity in male Wistar rats.

Method: Fifty-six adult rats (180±20 kg) were randomly assigned to seven groups (n=8). Groups 1 and 3 were orally treated with distilled water and GSC, respectively. Groups 2, 4–7 received 1 mg/kg intraperitoneal scopolamine for one week. On the eighth day, groups 5–7 were administered GSC at doses of 100 mg, 200 mg, and 300 mg/kg, respectively, for three weeks. Group 4 received 5 mg/kg donepezil for three weeks. Y-maze and Open-field tests were used to evaluate the effects on cognitive and motor functions. At the end of the treatment, the rats were sacrificed, and their brains were harvested for further studies, employing standard biochemical and histological techniques.

Results: Scopolamine-induced motor function impairment and anxiolytic behaviors were observed, characterized by a reduced time of movement, horizontal and vertical activities, the number of rearings, time spent in the center square, and an increase in resting time. Additionally, it induced cognitive impairment by decreasing the percentage of spontaneous and correct alternations, the number of entries, and spatial working memory in the Y-maze test. Scopolamine also induced oxidative stress by significantly elevating Malondialdehyde (MDA) levels but did not affect Superoxide Dismutase (SOD) levels. Histologically, the cerebral cortex presented with dilated vasculature, neuronal loss, and vacuolated cytoplasm with scopolamine treatments. Meanwhile, GSC, comparable to Donepezil, improved these changes in a dose-dependent manner.

Conclusion: GSC demonstrated its ameliorative effect on scopolamine-induced neurodegeneration by decreasing lipid peroxidation, improving spatial working memory, and regenerating cortical neurons in the cerebral cortex of rats.

Keywords: Cerebral cortex, cognitive impairment, neurodegeneration, scopolamine, garlic synthetic complex

INTRODUCTION

The cerebrum's outermost layer, known as the cerebral cortex, comprises six layers of nerve cells, housing approximately 14–16 million neuronal cells. The four-lobed cortex plays a crucial role in cognition, memory, interpreting sensations, voluntary body movement, speech, and language (1). However, the degeneration of cortical neurons leads to impairments seen in diseases such as

Alzheimer's, Parkinson's, Huntington's, and other neurodegenerative diseases (ND) associated with the cerebral cortex (2). These diseases generally involve neurodegeneration, which includes the passive and continuous loss of neuronal structures and functions. Unfortunately, neurodegeneration is irreversible and results in cell death (3). Other associated clinical presentations for ND include basic mechanisms like oxidative stress, inflammation, impairment of the ubiquitin-proteasome-autophagy system, and the formation of misfolded proteins, which are all hallmarks of neurodegenerative disease (4).

Neurodegeneration has long been studied in animal models by inducing these animals with neurotoxins capable of activating cellular mechanisms similar to neurodegenerative disease. Some toxins include scopolamine, 1-Methyl-4-phenyl pyridinium, and lipopolysaccharide. Scopolamine is an antimuscarinic drug used to manage and treat post-surgery nausea, vomiting, and motion sickness (5). It is an antimuscarinic agent that inhibits cholinergic transmission, induces oxidative stress, and causes the formation of misfolded proteins (6) and has been tagged as a pharmacological Alzheimer's disease (AD) model (7). Studies have explored neurophysiological variations linked with scopolamine injection resembling those noticed in AD (8). One such study focused on reconstructed Electroencephalogram (EEG) sources using Low-Resolution Electromagnetic Tomography (LORETA) (9) and observed brain activity fluctuations with scopolamine treatment, primarily in the precuneus. These neurophysiological and cognitive alterations, along with the inhibition of the cholinergic pathway and induction of oxidative stress, make scopolamine an adequate approximation to simulate the changes in brain activity that occur in AD (10).

Despite considerable research funding for the development of drugs or therapies for the management of ND, there currently needs to be more effective management and therapy available for amelioration and protection against these diseases (11). This scarcity of treatment options has led to an interest in finding complementary means of therapy, with cost-effectiveness and easy accessibility, particularly for rural and sub-urban communities in developing nations. Phytochemical assays of several plant extracts have revealed bioactive compounds capable of treating ND symptoms. These plant extracts include garlic, curcumin, ginkgo, and Melissa officinalis (12,13). The potential therapeutic effects of these plants have sparked interest in using Garlic synthetic extract (GSC) in our study.

The bioactive compounds in garlic have been implicated in antioxidation, immunostimulant effects (14), and anti-inflammation (15). Since antiquity, garlic has been used as a traditional spice for treating various ailments. It was listed in the Egyptian medical papyrus for managing several disorders, such as headaches, tumors, and heart disease (16). In addition, evidence exists for its immunomodulatory role in increasing T-lymphocyte blastogenesis and phagocytosis, influencing cytokine production (17,18). Various aspects of garlic's antioxidant and anti-inflammatory properties have also been studied (19,20). It has been observed that the antioxidant mechanism of a garlic polysaccharide in vitro involves binding to metal ions to minimize the generation of free radicals and directly interacting with distinct free radicals to produce scavenging action. More recently, a clinical study reported that garlic polysaccharide alleviated the development of colitis by inhibiting proinflammatory cytokines and regulating gut microbes (21).

These findings have prompted an investigation into the ameliorative effect of a synthetic garlic complex with alliin, allicin, and s-allyl cysteine as active ingredients on scopolamine-induced neurodegeneration, with an emphasis on the oxidative stress pathway.

METHOD

Ethical Consideration
Ethical clearance was requested and obtained from the University of Medical Sciences Research and Ethics Committee on Animal Use and Care (number: NHREC/TR/UNIMED-HREC-Ondo-St/22/06/21; date: 04.07.2022). All rat experiments were carried out following the "Guidelines on Ethical Treatment of Experimental Animals" described by the European Union directive (Directive 2010/63/EU) and those described by the World Medical Association on "animal use in Biomedical Research."

Drugs and Chemicals Preparation
Alli Ultra 360 mg (synthetic garlic complex) and Donepezil Hydrochloride were purchased from Uche Care Pharmaceutical stores, Ondo, Ondo State. Scopolamine Hydrobromide was obtained from the Department of Pharmacology, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria, and was used to induce neurotoxicity in rats. 200 mg of

Table 1: Experimental protocol

Groups	Administration	Duration
Group A: Control	2 ml distilled water (orally)	**28 days**
Group B	Scopolamine (1 mg/kg) (intraperitoneally)	7 days
Group C	GSC (200 mg/kg) (orally)	28 days
Group D	1 mg/kg Scopolamine (7 days) + 5 mg/kg Donepezil (21 days)	28 days
Group E	1 mg/kg Scopolamine (7 days) + low-dose GSC (100 mg/kg) (21 days)	28 days
Group F	1 mg/kg Scopolamine (7 days) + medium-dose GSC (200 mg/kg) (21 days)	28 days
Group G	1 mg/kg Scopolamine (7 days) + high-dose GSC (300 mg/kg) (21 days)	28 days

Scopolamine hydrobromide was diluted in 1 liter of boiling water, creating a working concentration of 0.2 mg/ml. It was administered at a standard dose of 1 mg/kg (FDA.gov). 5 mg of Donepezil Hydrochloride was diluted in 100 ml of distilled water to create a working concentration of 0.05 mg/ml.

Experimental Design

Fifty-six (56) adult rats (180±20 kg) were procured from the animal house of the University of Medical Sciences, Ondo, Ondo State, Nigeria, and were randomized into seven groups (groups A-G, n=8). Before the experiment, the rats were acclimatized for 14 days under standard environmental conditions and fed with rodent pellets and water ad libidum. Groups A and C were treated with normal saline and GSC only. Groups B and D-G were treated with 1 mg/kg of scopolamine once daily for a week (22). On the eighth day, following scopolamine administration, groups E-G received GSC (100 mg, 200 mg, and 300 mg/kg, respectively, orally) for three weeks. The dosages were modified to suit our experiment based on the 300–1000 mg dose recommendation of Drugs.com (23). Group D was treated with 5 mg/kg donepezil (FDA.gov) (24) as a standard treatment for three weeks. Note that the low, medium, and high doses of GSC were considered 100 mg/kg, 200 mg/kg, and 300 mg/kg, respectively, for easy reference in our manuscript only (Table 1).

Behavioral Test

The effects of scopolamine, GSC, and donepezil on cognitive functions were assessed using open-field and Y-maze tests. Following the experimental protocol, a behavioral test was conducted on the eighth day after scopolamine administration and on days 14, 21, and 28 during GSC administration. The activities of the rats during each test were monitored for 5 minutes by placing a webcam (Logitech C270 HD, USA) above the apparatus, and connected to a PC (Dell Vostro 3400, USA). The apparatus was cleaned with 10% ethanol after each rat session during the experiment to eliminate odor traces.

Open Field Test

The open field test apparatus is a closed-wall square containing 16 smaller squares and a red square in the center. The floors and walls of the apparatus, which are made of wood, measure 50 cm in height, 76 cm in length, and 76 cm in width. The rats were placed in a corner of the apparatus and allowed to explore. The parameters assessed included the number of crossings made, horizontal and vertical activity, the number of rearings, rest and movement time, and the time spent in the center square (25).

Y-Maze Test

The three-armed wooden maze, with an angle of 120 degrees, had a length of 50 cm, a width of 10 cm, and a height of 40 cm. The rats were placed at the center of the maze, and then the sequence and number of arm entries were observed. An actual alternation was recorded when a rat entered all three arms of the maze (i.e., XYZ, XZY, YZX, or ZYX, but not ZYZ, XYX, or YYX). The parameters derived from this test were spontaneous alternation (total number of alternations/number of triads, multiplied by 100) and spatial memory index (% of correct alternation: % of incorrect alternation) (26).

Animal Sacrifice and Sample Collection

At the end of the treatment protocol, the rats were fasted from food and water. After a 24-hour window following the last administration, the rats were sacrificed by cervical dislocation, and the skulls were opened using forceps to expose their brains. The cerebral cortex was excised, weighed using an analytical balance, and fixed in 10% formal saline for histochemical and immunohistochemical studies (27).

Tissue Preparation for Histology

The dissected cerebral tissues were processed using standard manual tissue processing methods. A Slee Medical rotary microtome was used to cut paraffin sections of brain tissues 3–5 μm thick. The Hematoxylin and Eosin (H and E) method was used to display the general histoarchitecture of the cerebral cortex. The method described by Kraeuter and colleagues for H&E was adopted (26). The fixed tissues were cleared and hydrated through descending grades of alcohol (absolute, 95%, and 70%) to remove xylene. The sections were stained in Harris hematoxylin stain for five minutes. After staining, the sections were rinsed in water and briefly differentiated in 1% acid alcohol, then rinsed in water. The sections were then blued under running water for about ten minutes and counterstained in 1% aqueous eosin for two minutes. Following staining, the slides were rinsed in water, dehydrated in several grades of alcohol, and cleared in xylene. A coverslip was used to mount the tissues in Distyrene Plasticizer Xylene (DPX) and placed on a microscopic glass slide for microscopy. The slides were examined on a light microscope for histological changes and subsequently photographed with a Brunel light microscope, 20 megapixels (Brunel SP35 Digital Trinocular).

Biochemical Analysis

Measurement of Lipid Peroxidation

The level of Malondialdehyde (MDA) was measured as described by Ohkawa et al. (28). Malondialdehyde levels were estimated by its reaction with thiobarbituric acid, resulting in a complex compound called a thiobarbituric acid reactive substance (TBARS) that absorbs at 535 nm. The values were expressed as unit/g wet tissue.

Measurement of Superoxide Dismutase Activity

Superoxide dismutase activity was measured according to the procedures followed by Naji et al. (29). A unit of superoxide dismutase was defined as the amount of the Superoxide Dismutase (SOD) enzyme causing a 50% inhibition in the nitro blue tetrazolium (NBT) reduction rate. The values were expressed as unit/g protein.

Data Analysis

All values were expressed as mean±standard error of the mean (SEM). Differences among means were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison tests. Statistical significance was considered at $p < 0.05$. All computations were performed using GraphPad Prism software, version 8.4.3.

RESULTS

Physical Observations

During the experiment, the rats in group A (control) were normal and showed no physical variation. Meanwhile, rats in all groups maintained their appetite throughout the experiment. As for rats in groups B, D-G, the animals became lethargic and less active after the administration of scopolamine. Reduced climbing on the walls of the cages was observed in rats administered scopolamine compared to those in groups A and C. For groups D-G, there was an improvement in activity and decreased lethargy with the continued treatment of GSC, depicting improvement in the general health outcome of the rats.

Effect of Treatment on Motor Function
Open Field Test

Figure 1: The bar charts (A–F) represent the parameters of the open field test, including movement time (A), resting time (B), horizontal and vertical activities (C&D respectively), the number of rearings (E), and time spent in the center square (F). Scopolamine caused motor impairment and anxiolytic behaviors, indicated by low values of all measured parameters in rats compared to the control group ($p < 0.05$). However, GSC comparably improved motor functions by increasing locomotive activities and reducing depression and anxiety (by significantly decreasing resting time) compared to the scopolamine group ($p < 0.05$). GSC also increased the number of rearings and time spent in the center square by the rats, although these increases were not significant compared to those of the control group ($p > 0.05$).

Effect of Treatment on Cognition
Y-Maze Test

Figure 2: The bar charts (A-D) represent parameters assessed during the Y-maze test. Scopolamine caused memory impairment by decreasing values in all parameters of the Y-maze test assessed in the study compared to the control group ($p < 0.01$). The three doses of GSC, comparable to donepezil, improved the values of the measured parameters by causing a significant increase in the percentage of spontaneous alternation and the number of entries (A&C). Only 200 mg of GSC significantly improved right alternations made (B), compared to rats administered only scopolamine. However, there was no significant difference in the spatial memory index (SMI) (D) in the

Figure 1. Open field used to determine the cognitive function of the treated rats.
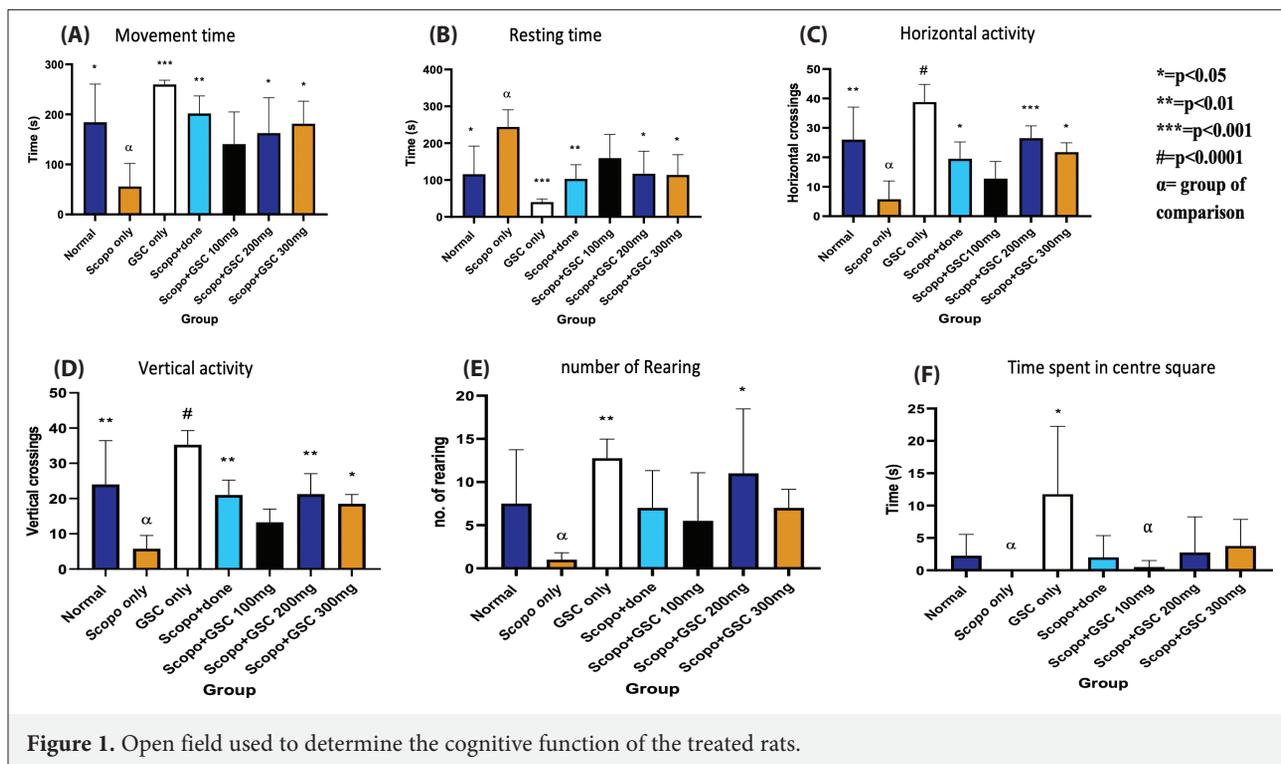
rats treated with all three doses of GSC (p>0.05). It is noteworthy that 200 mg appeared more effective in ameliorating scopolamine-induced neurotoxicity. Our Y-maze test showed that this dose exhibited better potency in vertical and horizontal activities and the number of rearings.

The Effect of Treatment on Malondialdehyde and Superoxide Dismutase Activity in the Cerebral Cortex

Figure 3 illustrates the oxidative stress levels in the cerebral cortex of male Wistar rats. From charts A&B, it is apparent that scopolamine significantly induces oxidative stress in the cerebral cortex (p<0.05) by increasing MDA levels in the Scopolamine-only group compared to the control (normal saline) and GSC-only groups (A). However, rats treated with 200 mg and 300 mg of GSC alongside scopolamine showed significantly decreased oxidative stress levels compared to those treated solely with scopolamine. Although 100 mg GSC led to a decrease in MDA level, this difference was not statistically significant when compared to treatment with donepezil (p>0.05). Surprisingly, scopolamine did not cause any significant change in SOD level in the cerebral cortex of rats compared to the control (B). However, a dose of 100 mg of GSC significantly (p<0.01) increased SOD level compared to the other treatment and control groups.

Effect of Treatment on the Histology of the Cerebral Cortex

Figure 4 presents the photomicrographs of the cerebral cortices. 4A shows normal cortical lamina and stroma, with neuronal cells appearing healthy. Photomicrograph 4B (scopolamine-group) shows mildly dilated capillaries in the cortex, cytoplasmic vacuolations, and neuronal loss compared to the control group. However, photomicrographs of rats treated with donepezil and varying doses of GSC indicate improvement in the damage caused by scopolamine treatment compared to rats treated solely with scopolamine.

DISCUSSION

Neurodegeneration is a global issue with an increasing annual prevalence despite a wealth of research on drugs and other therapies to improve management and find a cure (30). The cerebral cortex and its cortical component, the hippocampus—located on the temporal lobe of the cerebral cortex—is a complex structure responsible for learning and memory. It is the earliest and most severely affected structure in neurological diseases such as Alzheimer's and Parkinson's (31).

Scopolamine is implicated in neurodegenerative disorders by causing a reduction in the activation of cells in the cerebral cortex and hippocampus (32). This research investigated the potential of GSC to alleviate
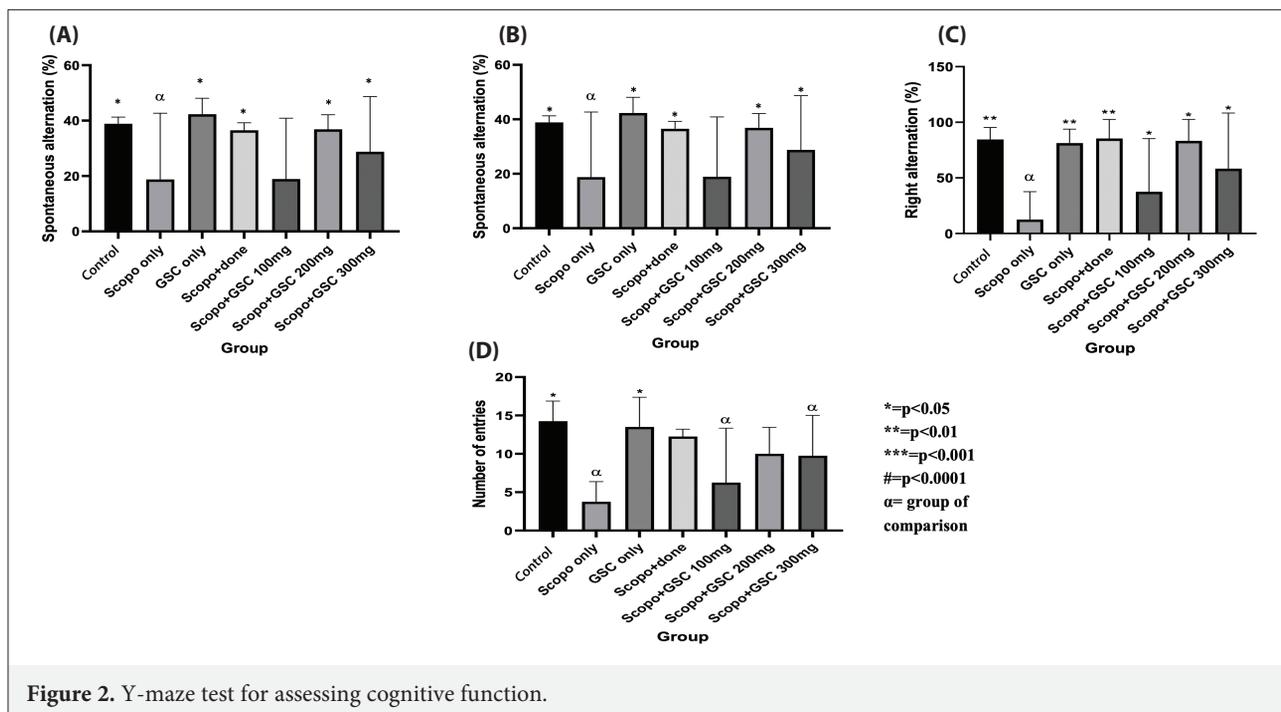
Figure 2. Y-maze test for assessing cognitive function.

scopolamine-induced Alzheimer's-like symptoms in the cerebral cortex. Our open-field test revealed that scopolamine significantly impaired locomotive function in the rats by reducing the time spent before movement, horizontal and vertical activities, and time spent in the center square compared to the animals in the control group. We observed a notable amelioration of these physiological features upon treatment with 100, 200, and 300 mg of GSC. Scopolamine also induced anxiety by decreasing rearing and exploratory behavior (Fig. 1). Whimbey & Denenberg (33) noted that rearing is an emotional behavior associated with stress and anxiety.

Consequently, based on the reduction of the above parameters, rats displaying decreased motor function are considered less active and display lower ambulatory activity (34). We observed a significant improvement after treatment with different doses of GSC compared to the control and animals treated with scopolamine. However, donepezil treatment yielded better results than the three GSC doses. This finding suggests that while GSC could reverse damage related to navigation in the brain, prompting the rats to show increased exploratory behavior, its effect is not comparable to that of donepezil treatment. Therefore, GSC may not serve as a standalone treatment for motor impairment and anxiety. Our results align with previous observations that donepezil reduced anxiety and depression (35,36), thus suggesting the anti-anxiolytic potential of GSC in rats.

Moreover, the Y-maze test evaluated whether GSC could restore or enhance spatial learning and short-term memory impaired by scopolamine in rats. According to Kraeuter and colleagues, rats with intact spatial working memory will remember a previously visited arm in the maze and exhibit a lower tendency to enter a familiar arm (26). The parameters for our Y-maze test include the percentage of spontaneous alternation, spatial memory index, number of entries made, and the percentage of right alternation. Our study revealed that scopolamine significantly impaired cognitive function by decreasing spontaneous alternation, right alternation, and spatial memory index (Fig. 2-Charts a, b, and d).

Percentage alternation is related to exploratory behavior and cognitive function impairment (37). Furthermore, a decreased spontaneous alternation percentage indicates an impairment of short-term memory (38). However, treating the rats with 100, 200, and 300 mg of GSC demonstrated a recovery comparable to the control group. Additionally, our findings reveal that 200 mg of GSC compared favorably with donepezil in potency for spontaneous alternation due to very close values. The reason why this dose of GSC was more potent remains to be discovered, necessitating further research to determine why this dose should be the optimal standard dose of treatment. However, treatment with 100 mg GSC did not lead to significant brain repair compared to donepezil treatment.
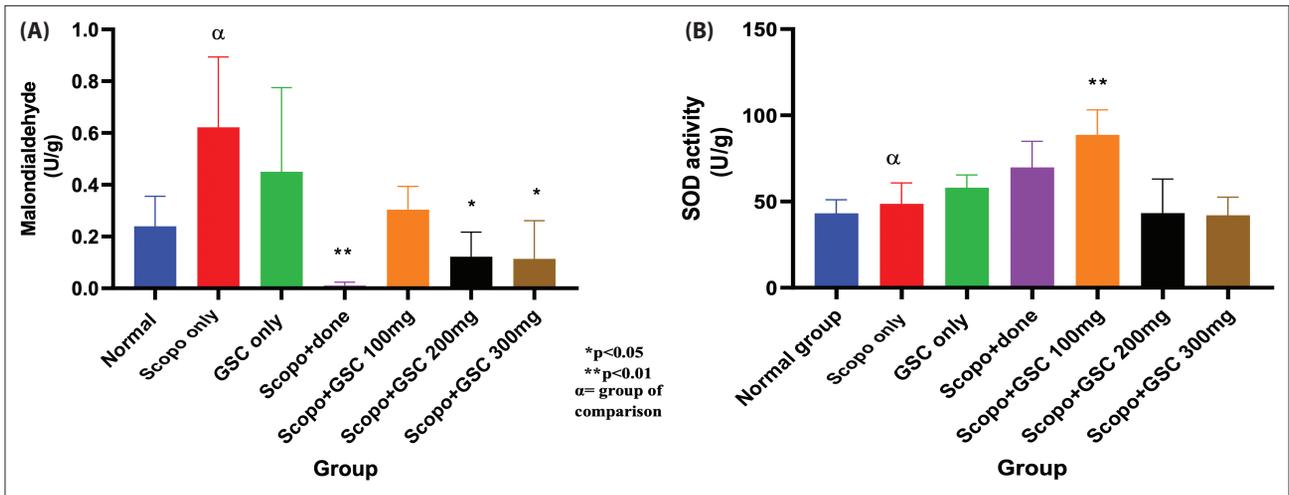
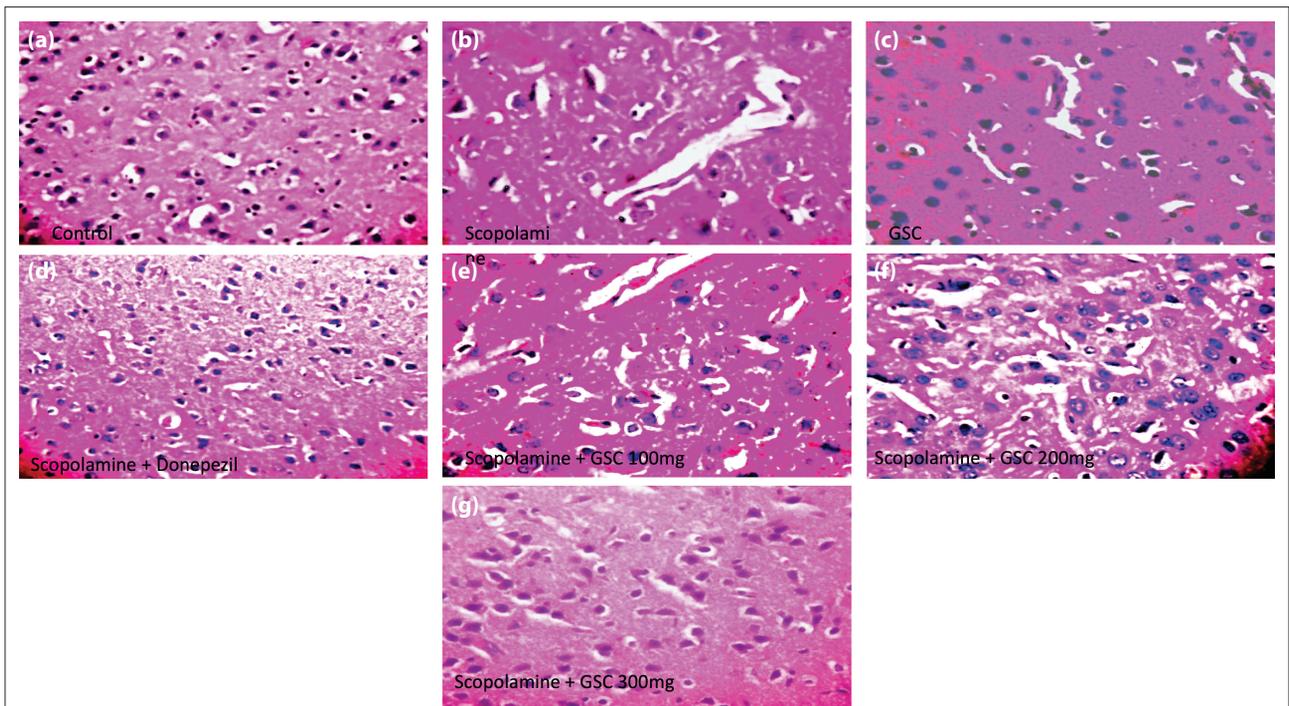**Figure 3.** Oxidative stress markers assayed for the treated rats.

Figure 4. Sections of cerebral cortex (A-E), x400 (H&E).

Scopolamine significantly decreased the number of entries (Fig. 2c), indicating impaired locomotion and general motor activity. Nonetheless, administering either 100, 200, or 300 mg doses of GSC significantly improved this motor function impairment compared with the control group. Our findings also suggest that donepezil treatment had better efficacy for motor impairment treatment when compared to the three different GSC treatments. These findings align with several studies that have shown the bioactive components of garlic improve spatial working memory in cognitively impaired brains through their neuroprotective effect (13,39). Therefore, we infer that GSC has substantial potential for treating scopolamine-induced cognitive and motor function impairment in rats.

Similarly, the scopolamine-induced oxidative reaction in the cerebral cortex significantly increased the MDA level (Fig. 3a) but did not have a significant impact on SOD (Fig. 3b) compared to the control group animals. On treatment with GSC and donepezil, there was a substantial reduction in the oxidative

stress markers; however, donepezil outperformed the three doses of GSC in its ameliorative effects, primarily at the MDA level. In the group of rats treated with donepezil against scopolamine, the MDA level decreased dramatically, affirming its effectiveness as an antioxidant drug and corroborating the study conducted by Munishamappa et al. (40) on the antioxidant activity of donepezil.

In our study, histological examination revealed that the cerebral cortex (CC) of rats treated with scopolamine had a less dense neuronal population than those in other groups, a clear indication of cellular death induced by the drug. This finding aligns with several studies proving that the administration of scopolamine activates cellular mechanisms resulting in neuronal cell death (41,42). Additionally, cytoplasmic vacuolation accompanying dilated vasculature was observed in scopolamine-treated rats, suggesting an involvement of the endoplasmic reticulum, organelles of the endosomal-lysosomal system, and Golgi apparatus (43). Shubin and colleagues' study on "cytoplasmic vacuolization in cell death and survival" suggested that vacuolization accompanies cell death (44); however, its role in cell death processes remains unclear. Interestingly, treatment with 100, 200, and 300 mg of GSC comparably resolved these negative histological features compared to donepezil treatment. For instance, rats treated with donepezil and varying doses of GSC against scopolamine had reduced cellular loss in the CC compared to those treated solely with scopolamine. This finding indicates that GSC and Donepezil either ameliorated or delayed the cellular neurodegenerative mechanisms activated by scopolamine, which results in cell death.

CONCLUSION

In conclusion, GSC improved cognitive and memory deficits, reduced oxidative stress, and ensured neuronal cell survival in the cerebral cortex of rats induced by scopolamine. We recommend further studies on GSC involving an anti-anxiolytic drug for a comparative treatment.

Contribution Categories		Author Initials
Category 1	Concept/Design	K.A.I.
	Literature review	K.A.I.
	Data analysis/Interpretation	K.A.I., T.A.
Category 2	Drafting manuscript	K.A.I., T.A.
	Critical revision of manuscript	K.A.I.
Category 3	Final approval and accountability	K.A.I.

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