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Research Article

Scopolamine-induced brain injury and lipid peroxidation in Wistar rats ameliorated by garlic oil supplementation

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ABSTRACT

Background: Scopolamine induced toxicity has been linked to impaired learning and memory, lipid peroxidation, oxidative stress and impairment of cholinergic transmission resulting in brain injury; which are synonymous with features of neurodegenerative diseases such as Alzheimer's disease. Garlic oil has been found to be a possible therapeutic intervention. Its antioxidant and anticholinesterase activity were evaluated in scopolamine-induced brain toxicity in Wistar rats.

Methods: The animals were grouped into seven groups (n=5). Group I (Normal Control) was given 1mL/kg distilled water for 14days P.O, Group II was treated with 90 mg/kg garlic oil for 14days P.O while Group III was treated with 2 mg/kg scopolamine IP daily for 14 days. Group IV was co-administered garlic oil (90mg/kg) orally and scopolamine 2mg/kg IP daily for 14 days. Group V had garlic oil orally at 90mg/kg for 7 days after 14 days of scopolamine. Group VI had donepezil at 3mg/kg orally co-administered with scopolamine at 2mg/kg IP daily for 14 days, Group VII 3mg/kg donepezil was administered orally for 7 days after 14 days of scopolamine. Elevated plus maze (EPM), was used to assess memory and learning. Thereafter, the rats were anesthetized using diethyl ether and the frontal cortices of the brain were harvested, homogenized and centrifuged. The supernatant was used to assay for dopamine, and malondialdehyde (MDA). The frontal cortices of the animals' brains were subjected to histomorphological analyses.

Results: Scopolamine significantly decreased ($p<0.05$) learning and memory while increasing MDA levels. Brain dopamine levels, and transfer latency in EPM were significantly reduced ($p< 0.05$) and neurodegenerative changes were seen in the brain. All these were significantly reversed in the garlic-oil treated groups.

Conclusions: According to this study, garlic oil has antioxidant and anticholinesterase activities and may have therapeutic benefits against scopolamine-induced toxicity and diseases related to loss of memory.

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1. Introduction

The Amaryllidaceae family includes garlic (*Allium sativum* L.), which is grown all over the world and has many health advantages. In the 1500s garlic was used as medicine to treat a variety of epidemics, including cholera, influenza, typhus, and dysentery (Wang et al., 2018). Garlic's remarkable bioactive compounds, like organic sulfides, are primarily responsible for its therapeutic effects (Lestari *et al*., 2022). Garlic has been found to possess antioxidant, antidiabetic, and antihyperlipidemic properties and has also been found to enhance memory and lessen age-related cognitive disorders (Tesfaye, 2021).

A volatile oil made from garlic is called garlic oil. It is typically made through the maceration or steam distillation of cloves of garlic and includes a range of sulfides such as diallyl disulfide (DAD) and dially trisulfide (DAT) (Dehariya *et al*., 2021).

Memory impairment is becoming a common problem today especially with the increase in life stresses (Lin et al., 2021). Scopolamine, a tropane alkaloid, found in plants of the Solanaceae family has been used to induce memory impairment in experimental animals (Kohnen-Johannse *et al*., 2021). Increased oxidative stress in the brain, as evidenced by an increase in malondialdehyde (MDA) concentration—a detrimental consequence of reactive oxygen species—is linked to memory disorders caused by scopolamine (Akbarian et al., 2022).

This research sought to investigate the effect of garlic oil as a possible ameliorative agent for scopolamine induced memory loss especially because such studies are quite limited in literatures.

2. Methodology

A total of 35 Male Wistar rats weighing between 125-250g were used for this research. The animals were purchased from the Animal House, College of Health Sciences, Benue State University, Makurdi-Nigeria. Ethical clearance (CHS REC No. CREC/DIS/004) for the use of animals for experiment was obtained from the ethical committee in the College of Health Sciences, Benue State University, Makurdi. They were divided randomly into seven (7) groups of five (5) animals each and were housed in polypropylene cages with beddings of wood chips in the Animal House of College of Health Sciences, Benue State University under standard environmental temperature of 23 ± 2°C, humidity 55± 15% and 12h light/ dark cycle. They were fed with standard rat pellet diet manufactured and supplied by Olam chikun and ultima Poultry Feeds Nigeria Limited; and water, *ad libitum*. They were allowed to acclimatize to the laboratory environment for one week before the commencement of the experiment.

The Rats were divided into seven groups of five animals each and treated as follows:

Group I (Normal Control) administered 1mL/kg distilled water for 14days P.O.

Group II: Administered 90 mg/kg garlic oil for 14days P.O.

Group III: Administered 2 mg/kg scopolamine IP daily for 14 days (Bihaqi *et al*., 2012).

Group IV: Administered garlic oil orally at 90mg/kg plus scopolamine at 2mg/kg IP daily for 14 days.

Group V: Administered garlic oil orally at 90mg/kg for 7 days after 14 days of scopolamine

Group VI: Administered donepezil at 3mg/kg orally plus scopolamine at 2mg/kg IP daily for 14 days.

Group VII: Administered 3mg/kg donepezil orally for 7 days after 14 days of scopolamine.

All drugs were gotten from a local pharmacy in Makurdi, Benue State. Garlic oil capsule (0.25% w/w) manufactured by Brivosis Soft Cap pvf Ltd, Gujarat, India; Scopolamine injection (10mg/1ml), manufactured by Hanyao Pharmacy China. (NAFDAC Number- A44770), Batch No 200710, Mfg: 07/2020, Expiry Date: 06/2024; donepezil manufactured by Mfg Lic Limited No: 415/84, BN T7638, Expiry Date : 04/2024

2.1. Elevated Plus Maze

The elevated plus maze was locally constructed from wood and painted black. The maze was raised 45 cm above the floor and consisted of two perpendicular open arms (30 cm x 5 cm x 25 cm) and two closed arms (30 cm x 5 cm x 25 cm). It is used for both anxiety and memory studies (Shoji & Miyakawa, 2021). The experiment lasted two days. On the first day each animal was placed at the end of an open arm facing away from the central platform. The time taken for the animal to move from the open arm to the enclosed arm (transfer latency) is an index/parameter of learning. This was repeated on Day 2 to test for memory recall. Each animal was allowed 90s to walk into one of the closed arms. If the animal failed to do that, it was then gently assisted into one of the closed arms and allowed to remain there for 20s to enable it integrate memory. However, if the animal on its own was able to walk into the closed arm, the time to do so was recorded with the aid of a stop watch, if not, it was recorded as 90s. After each trial the maze was wiped

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with a cloth dipped in 70% ethyl alcohol and allowed to dry, this was to obliterate olfactory cues.

2.2. Animal Sacrifice, Craniotomy and Harvest of Tissues

After completing the Elevated plus maze task, all rats were anesthetized with diethyl ether and then sacrificed by cervical dislocation. The frontal cortices of the brain tissues were harvested after craniotomy and fixed in 10% formaldehyde for histology, the remaining brain tissues were gently homogenized with sodium phosphate buffer, centrifuged and supernatants were collected and frozen immediately at -20˚C for biochemical and enzyme assays.

2.3. Histology

The prefrontal cortices of control and experimental groups were sliced and post-fixed overnight in 10% neutral buffered formalin. The tissue was cut at 3 mm thick, and the blocks were embedded in paraffin. Using a rotary microtome, sections of 5 µm thickness were cut. For histopathological study, the sections were stained with hematoxylin and eosin and examined under light microscope for any histopathological changes.

2.4 Dopamine Assay

The Dopamine was determined according to Chatterjee, & Gerlai. (2009) using High precision liquid chromatography (HPLC) of the supernatants from the homogenized centrifuged brain sample of the prefrontal cortices was carried out using a BAS 460 MICROBORE-HPLC system with electrochemical detection. Standard dopamine was used to quantify and identify the peaks on the chromatographs. The detection limits for dopamine were determined by running the known concentrations of dopamine separately in the HPLC system under the set condition.

2.5 Lipid Peroxidation

The MDA, a major secondary product of lipid peroxidation, was spectrophotometrically determined according to Tang et al (2019) by measuring the thiobarbituric acid (TBA) reaction. Each 500 µl of prefrontal cortex of the brain homogenate supernatant had 1 ml of 15% trichloroacetic acid added thoroughly mixed and centrifuged at 3000 rpm for 10 minutes. One milliliter of the supernatant was added to 0.5 ml of 0.7% TBA then the mixture was heated for 60 min at 90 °C. The pink color was obtained, which was measured spectrophotometrically at 532 nm. The results were expressed as micromoles per gram of protein.

2.6. Data analysis

Results obtained from the study were presented as Mean ± SEM. The difference between more groups were determined using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test for multiple comparisons. Differences were considered significant when $P < 0.05$. Data were analyzed using SPSS version 20.0 software. (International Business Machine Corporation

3. Results

3.1. Effect of garlic oil on Transfer latency across Groups by Day 1

The group that received only scopolamine, scopolamine with garlic oil, scopolamine with donepezil, scopolamine with garlic oil post-induction and scopolamine with donepezil post induction had significant ($P < 0.05$) decrease in transfer latency time when compared to the control group, while treatment with only garlic oil and scopolamine with donepezil post induction had significant ($P < 0.05$) increase in transfer latency, relative to rats treated with only scopolamine as shown in Figure 1.

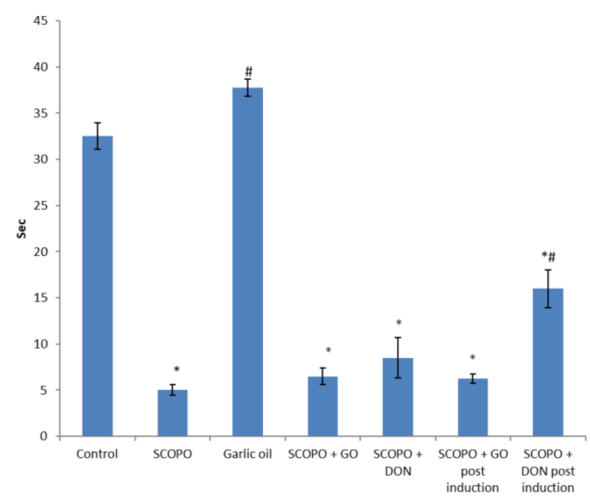

Fig. 1 Figure 1: Elevated Plus Maze Transfer Latency Day 1

N = 4, values express as mean ± SEM. *= significant relative to control at $P < 0.05$, # = significant relative to scopolamine treated at $p < 0.05$. SCOPO = scopolamine, GO = garlic oil, DON = donepezil. N= number of observations per group.

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3.2. Effect of oil on Transfer latency across Groups by Day 2

Figure The time taken to learn and the time taken to recall was significantly (P < 0.05) less in control rats compared to animals that were treated with only scopolamine, scopolamine with garlic oil, scopolamine with donepezil, and scopolamine with donepezil post induction. However, animals treated with only scopolamine had more prolonged transfer latency time followed by groups that received scopolamine with donepezil, scopolamine with donepezil post induction and scopolamine with garlic oil. The groups that received only garlic oil and scopolamine with garlic oil and scopolamine with garlic oil post induction and had a significant (P<0.05) shorter transfer latency time relative to the group that received only scopolamine with a longer transfer latency time. The garlic oil only group had a comparatively faster time to learn (as evidenced by longer transfer time on day 1) and similarly faster memory recall (as also evidenced by shorter transfer latency on day 2) than the group that received scopolamine with garlic oil post induction and scopolamine with garlic oil as shown in Figure 2 below.

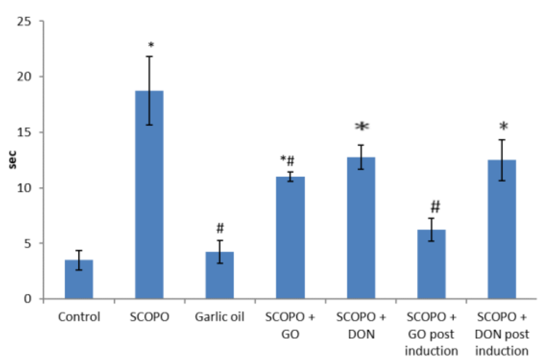

Figure 2: Elevated Plus Maze Transfer Latency Day 2
N = 4, values express as mean ± SEM. *= significant relative to control at P < 0.05, # = significant relative to scopolamine treated at p < 0.05. SCOPO = scopolamine, GO = garlic oil, DON = donepezil. N= number of observations per group.

3.3. Effect of garlic oil on brain dopamine levels

Rats that received only garlic oil and the scopolamine with garlic oil groups had significant (P<0.05) elevated dopamine levels compared to scopolamine with donepezil post-induction, scopolamine with garlic oil post induction and scopolamine with donepezil, and scopolamine alone treated rats compared to the control group. The scopolamine-only treated group had significantly (P<0.05) reduced level of dopamine compared with garlic oil alone, scopolamine with garlic oil, scopolamine with donepezil, scopolamine with garlic oil post-induction and scopolamine with donepezil post induction groups as shown in Figure 3 below

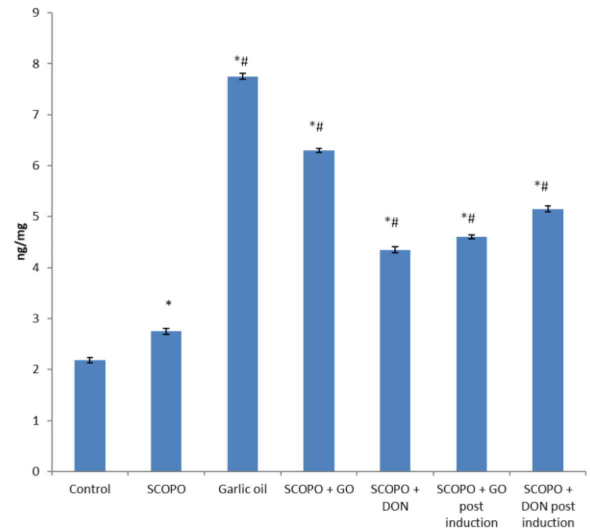

Fig. 3 Effects of dopamine levels in the brain.
N = 4, values expressed as mean ± SEM. *= significant relative to control at P < 0.05, # = significant relative to scopolamine treated at p < 0.05. SCOPO = scopolamine, GO = garlic oil, DON = donepezil. N= number of observations per group.

3.4. Effect of Garlic Oil on Lipid Peroxidation Activity in the Brain Tissue of Scopolamine Treated Rats

The chart in Figure 4 below showed that, the group that received only scopolamine, scopolamine with garlic oil and scopolamine with donepezil had a significant (P<0.05) increase level of Malondialdehyde (MDA) as a measure of lipid peroxidation compared with the control group. The MDA levels of the group that received garlic oil alone, scopolamine with garlic oil and scopolamine with donepezil, scopolamine with garlic oil post-induction and scopolamine with donepezil post induction is significantly (P > 0.05) reduced compared to the scopolamine alone treated rats.

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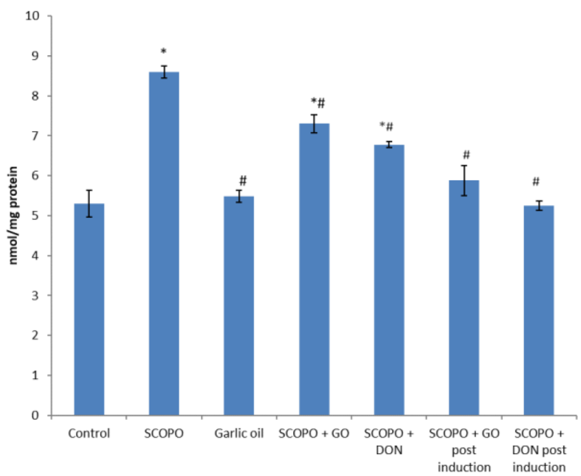

Fig. 4 The Effect of garlic oil on Lipid peroxidation in the brain tissue.
N = 4, values expressed as mean ± SEM. *= significant relative to control at $P < 0.05$, # = significant relative to scopolamine treated at $p < 0.05$. SCOPO = scopolamine, GO = garlic oil, DON = donepezil. N= number of observations per group.

3.5. Histologic sections of the prefrontal cortex of the control and treated groups

Figure 4.11 presents an observation of the normal histological architecture of the prefrontal cortex, delineating an outer white matter predominantly comprised of neuronal axons lacking neuronal cell bodies, juxtaposed with an inner grey matter hosting abundant neuronal cell bodies. Notably, no indications of neurodegenerative changes are discernible.

In Figure 4.12, a depiction of the normal histological features of the prefrontal cortex elucidates a conspicuous grey-white matter differentiation, accompanied by mild gliosis. There are no discernible manifestations of neurodegenerative alterations.

Figure 4.13 portrays a histologic cross-section of the prefrontal cortex unveiling escalated neuronal loss, vacuolated neuronal cytoplasm, and a sparse population of actively present microglia cells. Remarkably, pronounced edema and vascular congestion, coupled with perivascular edema, collectively suggest notable neurodegenerative transformations. Figure 4.14 exhibits a histologic section of the prefrontal cortex manifesting moderate neurodegenerative changes, characterized by the diminishment of neuronal cell bodies, karyolysis, pyknosis, and the congestion of capillaries alongside perivascular edema. Additionally, vacuolization within neuronal cell bodies substantiates the ongoing neurodegenerative processes.

In Figure 4.15, a histologic depiction of the prefrontal cortex delineates moderate neuronal cell bodies displaying cytoplasmic vacuolization, alongside a variable yet notable presence of neuronal cell bodies exhibiting spiculated morphology. Figure 4.16 illustrates a histologic section of the prefrontal cortex demonstrating attenuated neurodegenerative changes typified by the reduction of neuronal cell bodies, karyolysis, pyknosis, and the presence of perivascular edema. Additionally, vacuolization within neuronal cell bodies is discerned.

Finally, Figure 4.17 presents a histologic section of the prefrontal cortex showcasing moderate neuronal cell bodies characterized by actively spiculated cytoplasm. A subset of neuronal cell bodies exhibits pyknotic nuclei with perinuclear clearing, concomitant with the presence of perivascular edema.

4. Discussion

This study was designed to assess the effect of garlic oil on learning and working memory in scopolamine treated Wistar rats using EPM. This EPM is a useful tool for assessing learning and memory in animal models (Abi et al., 2020). Also, histopathological and biochemical studies were done to assess their role in the changes that may be associated with learning and memory in the experimental rats.

In elevated plus maze, the group that received only garlic oil had shorter transfer latency when compared with the other treated groups. This implies that, garlic oil treated rats had a comparatively faster time to learn and similarly faster memory recall (evidenced by shorter transfer latency on day 2). This finding is corroborated by a study done using garlic crude extract on mice model which showed that, the extracts ameliorated effect of scopolamine induced memory loss from Aβ-induced cytoxicity in neuron-like PC12 cells (Chen et al., 2020).

Dopamine is important in cognition and different dopamine receptor sub-types contributes to different aspects of learning and memory (Hauser et al., 2019).

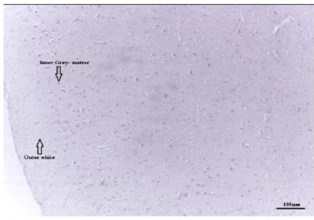

Figure 4.11: Section of the prefrontal cortex (PC) of control group showing normal neuronal tissue (Hematoxylin and Eosin (H&E).

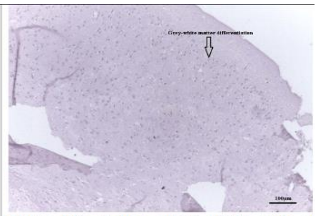

Figure 4.12: Sections of PC of the garlic oil treated group showing layers with normal neuronal cell bodies with gliosis (H&E)

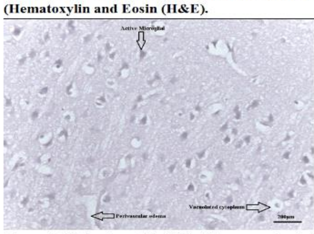

Figure 4.13: Section of the PC of the group treated with scopolamine showing perivascular showing Edema, active microglial cells, and vacuolated cytoplasm stained (H&E).

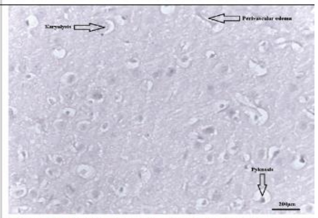

Figure 4.14: Section of PC of the group treated with Garlic oil with Scopolamine showing neuronal loss, perivascular edema, and vacuolization (H&E).

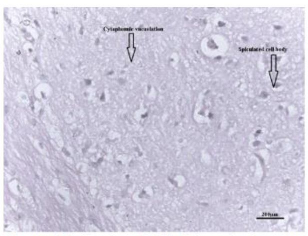

Figure 4.15: Section of PC of Scopolamine post-treatment with Garlic oil group showing moderate neuronal cell bodies with cytoplasmic vacuolization and few neuronal bodies with speculations (H&E).

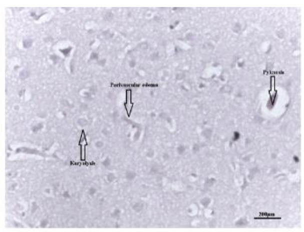

Figure 4.16: Section of PC of the group treated with donepezil and scopolamine showing reduced neurodegenerative changes with neuronal cell bodies, karyolysis, pyknosis with perivascular edema, and vacuolization of neuronal cell bodies (H&E).

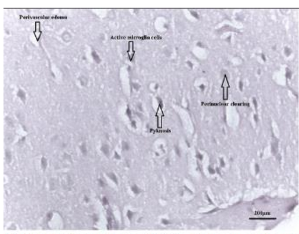

Figure 4.17: Sections of PC of the Scopolamine post-treatment with Donepezil Group showing numerous neuronal cell bodies with speculated cytoplasm, pyknotic nuclei with perinuclear clearing, and perivascular edema H&E..

Rats that received only garlic oil and the scopolamine with garlic oil groups had significant ($P<0.05$) elevated dopamine levels compared to scopolamine with donepezil post induction, scopolamine with garlic oil post induction and scopolamine with donepezil, and scopolamine alone treated rats compared to control group. While the scopolamine only treated group had significantly ($P<0.05$) reduced level of dopamine compared with garlic oil alone, scopolamine with garlic oil, scopolamine with donepezil, scopolamine with garlic oil post induction and scopolamine with donepezil post induction groups. This is in line with study by Speranza et al (2021) which implies that, dopamine receptor 1(D1), is very important in enhancing neuro-plasticity and different aspects of learning and memory including, incentive and extinction learning, reversal and spatial learning This is consistent with Wagatsuma et al (2018), which showed that, the neuromodulator input from Locus coeruleus (LC) to CA3 is crucial for the formation of a persistent memory in the hippocampus. due to Dopaminergic signaling from LC which is an important source to enhance memory of novelty. This also agrees with a recent study, which showed that dopamine consolidates memory (Sharp et al., 2020). Thus, the groups with elevated levels of dopamine had good performance compared with the groups with reduced dopamine levels.

Lipid peroxidation can be described generally as a process under which oxidants such as free radicals attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs). Scopolamine-induced memory disorders are associated with increased oxidative stress in the brain (Kim et al., 2021) characterized by an increased malondialdehyde (MDA) concentration, which is a harmful effect of reactive oxygen species (Mohammad et al., 2023). Since the brain is composed of lipids, the effect of reactive oxygen species (ROS) leading to lipid peroxidation may directly lead to destruction of brain tissue. The MDA levels of the group that received garlic oil alone, scopolamine with garlic oil and scopolamine with donepezil, scopolamine with garlic oil post induction and scopolamine with donepezil post induction were significantly reduced compared to the scopolamine alone treated rats.

This is in line with the study by Singh et al (2022) which showed a collapse in the brain antioxidant defense system characterized by a higher MDA level and lower catalase and glutathione activity in the scopolamine-treated group. This also agreed with the work by Balakrishnan et al (2023) which showed that, scopolamine induces memory impairment, decreases the activities of anti-oxidative enzymes such as GPx, and increases levels of lipid peroxidant, MDA, in the brain. Thus the antioxidant properties of garlic oil and donepezil ameliorated neuronal injury and free radicals generated by scopolamine in the brain reducing level of MDA. Study done by Huang et al (2023) showed that garlic essential oil has high antioxidant activity and this may be due to diallyl sulphide, Diallyl trisulfide and Diallyl disulfide, and diallyl polysulfides, which exhibit lipid peroxidation, reduce ischemic/reperfusion damage, and reduce oxidative stress, thereby protecting DNA from free radical–induced damage and mutations. Khan et al. (2020) also showed that garlic essential oil exerted its antioxidant activity by affecting the activities of Superoxide dismutase, Catalase, and glutathione S-transferase and reducing the content of lipid peroxidation and MDA by studying the toxicity of garlic essential oil on amine-coated and spherical Ag-NPs in freshwater rohu *Labeo rohita*.

The prefrontal cortex of brain tissues of control group and group that received only garlic oil showed normal neuronal tissue without evidence of neurodegeneration. (Yoo *et al.,* 2014; Xu *et al.,*2019). It was observed that garlic oil enhances learning and memory due its overall neuroprotective effect which may be attributed to its sulfur containing components, potent antioxidant properties hence protecting neural cells from damage. However, it is clearly visible that scopolamine treated group showed more degenerative cells compared with other groups. This is indicated by presence of degenerative changes such as, neuronal loss, ghost cells, vacuolated cytoplasm, karyolysis, congestion of capillaries with perivascular edema and active appearing microglia. Kim *et al* (2021) reported that, the neuroprotective effect of garlic root against apoptosis is due to expression of caspases-3 and bax genes in the brain tissues of Alzheimer's induced rats. The garlic oil with scopolamine treated group showed neurodegenerative changes, compared to the garlic oil with scopolamine post induction and scopolamine with donepezil post induction group which showed reduced neurodegenerative changes evidenced by moderate number of neuronal cell bodies with cytoplasmic vacuolization and few number of active microglia cells. The neurodegenerative changes is due to the damaging effect of scopolamine on the brain tissue, while garlic oil and donepezil has neuroprotective effect on the brain tissue thus ameliorating the damaging effect of scopolamine induced learning and memory loss. This implies that, the combined effects of scopolamine and garlic oil indicates reduction in neuronal changes in the brain tissue. The neuroprotective effect of GO might also be due to its antioxidant properties. This agrees with a similar study by Aydın *et al* (2020) who demonstrated that garlic extract prevents degeneration of the brain's frontal lobe and neuronal death in the hippocampus.

5. Conclusion

In conclusion, this study has shown that garlic oil enhanced learning and memory in scopolamine treated animals. This might be due to its potential to increase brain dopamine levels in treated animals. Furthermore, garlic oil exerted some neuroprotective function by increasing the activity of superoxide dismutase and reducing lipid peroxidation in the brain tissue. Our result also showed that learning and memory was better in garlic oil alone treated rats than in control.

Disclosure

None

References

Abi, I., Adeniyi, S. O., Abi, E. *et al*. (2020). Chronic high fat diet induced weight gain, hyperglycaemia and cognitive impairment in albino mice. *Journal of BioMedical Research and Clinical Practice*, *3*(3), 382-388.

Akbarian, M., Mirzavi, F., Amirahmadi, S. *et al.* (2023). Small Molecule Improves Learning and Memory Function in Scopolamine-Induced Amnesic Mice Model through Regulation of CREB/BDNF and NF-κB/MAPK Signaling Pathway. *Antioxidants*; 12(3):648. https://doi.org/10.3390/antiox12030648

Aydın, S., & Tahmas, K. D. (2020). Antioxidant effect potential of garlic in vitro and real food system: effects of garlic supplementation on oxidation stability and sensory properties of butter. *European journal of lipid science and technology*, *122*(3), 1900261.

Balakrishnan, R., Park, J. Y., Cho, D. Y., Ahn, J. Y., Yoo, D. S. *et al*.(2023). AD−1 Small Molecule Improves Learning and Memory Function in Scopolamine-Induced Amnesic Mice Model through Regulation of CREB/BDNF and NF-κB/MAPK Signaling Pathway. *Antioxidants*. 2023; 12(3):648. https://doi.org/10.3390/antiox12030648

Chatterjee, D. and Gerlai, R. (2009). High precision liquid chromatography analysis of Dopaminergic and serotoninergic responses to acute alcohol exposure in zebra fish. *Behavioral brain research*, *200*(1), 208–213. https://doi.org/10.1016/j.bbr.2009.01.016

Chen, N., Wang, J., He, Y. *et al.* (2020). Trilobatin Protects Against Aβ_{25-35}-Induced Hippocampal HT22 Cells Apoptosis Through Mediating ROS/p38/Caspase 3-Dependent Pathway. *Frontiers in pharmacology*, *11*, 584. https://doi.org/10.3389/fphar.2020.00584

Dehariya, N., Guha, P., Gupta, R. K. *et al.* (2021). Extraction and characterization of essential oil of garlic (Allium sativa L.). *International Journal of Chemical Studies, 9*, 1455-1459.

Feizi, H. and Rajabian, A. (2022). Amelioration of oxidative stress, cholinergic dysfunction, and neuroinflammation in scopolamine-induced amnesic rats fed with pomegranate seed. *Inflammopharmacology*, *30*(3), 1021-1035.

Hauser, T. U., Eldar, E., Purg, N., *et al.* (2019). Distinct roles of dopamine and noradrenaline in incidental memory. *Journal of Neuroscience*, *39*(39), 7715-7721. https://doi.org/10.1016/j.heliyon.2023.e13452.

Huang, L., Liu, Z., Wang, J.*et al.* (2023). Bioactivity and health effects of garlic essential oil: A review. *Food science & nutrition*, *11*(6), 2450–2470. https://doi.org/10.1002/fsn3.3253

Khan, M. S., Qureshi, N. A., Jabeen, F. *et al.* (2020). The role of garlic oil in the amelioration of oxidative stress and tissue damage in rohu Labeo rohita treated with silver nanoparticles. *Fisheries Science*, 86(2), 255–269.

Kim, Y., Kim, J., He, M. *et al.* (2021). Apigenin Ameliorates Scopolamine-Induced Cognitive Dysfunction and Neuronal Damage in Mice. *Molecules*. 26(17):5192. https://doi.org/10.3390/molecules26175192

Lestari, A.R., Batubara, I., Wahyudi, S.T. *et al.* (2022). Bioactive Compounds in Garlic (*Allium sativum*) and Black Garlic as Antigout Agents, Using Computer Simulation. *12*, 1131. https://doi.org/10.3390/life12081131

Lin, Y. F., Wang, L. Y., Chen, C. S. *et al.* (2021). Cellular senescence as a driver of cognitive decline triggered by chronic unpredictable stress. *Neurobiology of stress*, *15*, 100341. https://doi.org/10.1016/j.ynstr.2021.100341

Mohammad, H., Arezoo, R., Mohsen, P., *et al.* (2023). Minocycline alleviated scopolamine-induced amnesia by regulating antioxidant and cholinergic function. *Heliyon journal*, 9 (2):2405-8440

Sharp, M. E., Duncan, K., Foerde, K. *et al.* (2020). Dopamine is associated with prioritization of reward-associated memories in Parkinson's disease. *Brain, a journal of neurology*, *143*(8), 2519–2531. https://doi.org/10.1093/brain/awaa182

Shoji, H., & Miyakawa, T. (2021). Effects of test experience, closed-arm wall color, and illumination level on behavior and plasma corticosterone response in an elevated plus maze in male C57BL/6J mice: a challenge against conventional interpretation of the test. *Molecular brain*, *14*(1), 34. https://doi.org/10.1186/s13041-020-00721-2

Singh, P., Barman, B., Thakur, M. K. *et al.* (2022). Oxidative stress-mediated memory impairment during aging and its therapeutic intervention by natural bioactive compounds. *Frontiers in aging neuroscience*, *14*, 944697. https://doi.org/10.3389/fnagi.2022.944697

Sochor, J., Ruttkay-Nedecky, B., Babula, P., Adam, V., Hubalek, J., & Kizek, R. (2012). Automation of methods for determination of lipid peroxidation. *Lipid peroxidation*.16(5), 234-265

Speranza, L., di Porzio, U., Viggiano, D. *et al.* (2021). Dopamine: The Neuromodulator of Long-Term Synaptic Plasticity, Reward and Movement Control. *Cells*, *10*(4), 735. https://doi.org/10.3390/cells10040735

Tang, Q., Su, Y. W., Xian, C. J. (2019). Determining oxidative damage by lipid peroxidation assay in rat serum. *Bio-protocol*, *9*(12), 3263-3263.

Tesfaye, A. (2021). Revealing the therapeutic uses of garlic (allium sativum) and its potential for drug discovery. *The Scientific World Journal*, *2021*, 8817288. https://doi.org/10.1155/2021/8817288

Wagatsuma, A., Okuyama, T., Sun, C.*et al.* (2018). Locus coeruleus input to hippocampal CA3 drives single-trial learning of a novel context. *Proceedings of the National Academy of Sciences, 115*, 310–316.

Xu, X., Hu, P., Ma, Y., Tong, L., Wang, D., Wu, Y., Chen, Z., & Huang, C. (2019). Identification of a pro-elongation effect of diallyl disulfide, a major organosulfur compound in garlic oil, on microglial process.. *The Journal of nutritional biochemistry*, 78, 108323 . https://doi.org/10.1016/j.jnutbio.2019.108323.

Yoo, D., Kim, W., Nam, S., Yoo, M., Lee, S., Yoon, Y., Won, M., Hwang, I., & Choi, J. (2014). Neuroprotective effects of Z-ajoene, an organosulfur compound derived from oil-macerated garlic, in the gerbil hippocampal CA1 region after transient forebrain ischemia.. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 72, 1-7 . https://doi.org/10.1016/j.fct.2014.06.023