OPEN ACCESS

Haya: The Saudi Journal of Life Sciences

Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Cognito-Motor and Neurobehavioural Action of Lutein on Acetylcholinesterase (AchE) in Sodium Azide - Induced Cognitive Dysfunction in Male Mice

Itoro Etim Udoudo[1], Chike CPR[1], Austin. A. Ajah[1*]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Nigeria

DOI: https://doi.org/10.36348/sjls.2025.v10i10.011 | **Received:** 04.10.2025 | **Accepted:** 24.11.2025 | **Published:** 26.11.2025

***Corresponding author:** Austin. A. Ajah
Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Nigeria

Abstract

Many studies have reported that lutein have positive effects in different clinical conditions, thus ameliorating cognitive function, decreasing the risk of cancer, and improving measures of cardiovascular health. This study investigates the potential neuroprotective effects of lutein, a carotenoid with antioxidant properties against sodium azide-induced amnesia in mice. A total of thirty (30) healthy mice weighing 19g - 35g were used for this study. The mice were acclimatized and divided into six groups (n= 5 per group) which are; Group 1 (Control group), Group 2 (Sodium azide only), Group 3(Sodium azide + 20mg/kg of lutein), Group 4 (Sodium azide + 40mg/kg of lutein), Group 5 (Sodium azide + 60mg/kg of lutein), Group 6 (Sodium azide + Donepezil). Motor deficits were assessed using behavioral tests like Barnes maze test, Hand grip test, Rotarod test and Y maze test. While biochemical analysis was performed to evaluate oxidative stress markers, inflammatory cytokines and neurotransmitters. The results demonstrate that the mice treated with sodium azide only had a significantly higher latency compared to the control group. This suggests that sodium azide negatively impacted spatial learning and memory as indicated by the increased time taken to find the target. Lutein, especially at 40mg/kg and 60mg/kg as well as Donepezil (the standard drug) has neuroprotective effects against the spatial learning and memory deficits induced by sodium azide in mice. This study shows that sodium azide induced both motor symptoms such as agnosia and non-motor symptoms such as impaired memory, oxidative stress in mice and lutein an antioxidant carotenoid possesses a dose dependent increase in learning ability and cognitive functions and decrease in oxidative stress.

Keywords: Cognito - Motor, Neurobehavioural, Lutein, Acetylcholineterase Enzymes (AchE), Sodium Azide.

INTRODUCTION

Memory loss, learning disability and neurobehavioral dysfunction are the main symptoms of neurodegenerative disorders like amnesia and Alzheimer disease (AD) is one of such conditions (Kumar *et al.*, 2024; Matthews, 2015). These deficits cause a major impairment in the daily functioning and pose a major challenge to individuals, their families, and health-care services across the globe (Aza *et al.*, 2022). Experimental models for cognitive dysfunction study are crucial in understanding the mechanism of neurodegeneration and to test possible therapeutic strategies (Rapaka *et al.*, 2022). This research is based on the usage of sodium azide, a strong neurotoxin and cytochrome c oxidase (complex IV) inhibitor in the electron transport chain (ETC) in the mitochondrial membrane (Yuwen *et al.*, 2000; Tat *et al.*, 2021). Sodium azide also creates oxidative stress, neuroinflammation and subsequent cognitive and motor impairments in rodents by disrupting mitochondrial functioning, a process that resembles features of Alzheimer disease-like pathology (Weinstock & Shoham, 2004). The model can thus be of great use in explaining neurophysiologic processes underlying cognitive impairment and in assessing neuroprotective agents with the ability of preventing such phenomena (Singla *et al.*, 2022).

Lutein (a carotenoid) is abundant in green leafy vegetables and in some fruits and has drawn attention due to its anti-inflammatory and antioxidant properties (Ahn & Kim, 2021). Unlike other carotenoids, lutein and its isomer zeaxanthin are the only ones which can cross the blood-brain barrier, accumulate in the neural tissues (including the retina and brain), compose the macular pigment and offer neuroprotection (Stringham, Johnson,

& Hammond, 2019). It has been discovered that the more a person consumes foods that contain lutein, the better the cognitive functioning and the reduced risk of neurodegeneration diseases (Johnson, 2014). Also, the fact that lutein can be found in the brain at various stages of life, including infancy, points to the potential of this compound in cognitive health (Ramirez, 2015). The preclinical experiments have revealed the potential of lutein to decrease oxidative stress, regulate neuroinflammation, and have an antidepressant-like effect, which makes it a promising therapeutic agent in neurological disorders (Zeni, Camargo, & Dalmagro, 2019).

The main issue addressed in this study is how little research has been conducted on the neuroprotective effects of lutein in male mice against cognitive and motor deficits induced by sodium azide, and in particular, how this affects the activity of acetylcholinesterase (AChE) enzyme and subsequent neurobehavioral consequences. AChE is involved in the process of acetylcholine degradation in the synaptic gap, and is vital to the cholinergic neurotransmission, which memory and learning greatly depend upon (Sam & Bordoni, 2023). Impairment of AChE activity is a characteristic feature of cognitive impairment related to neurodegenerative diseases with defective mitochondrial functioning (Chen, Huang, Yang, & Hong, 2022). The presence of sodium azide inhibits the actions of the mitochondrial complex IV hence creating oxidative stress and neuroinflammation, which further suppresses the actions of AChE thus hampering cholinergic signaling leading to cognitive and motoric deterioration (Eiermann *et al.*, 2022; Amer *et al.*, 2024; Fujimura *et al.*, 2002). Even though the antioxidant and anti-inflammatory characteristics of lutein may help it to reverse these effects (Pap *et al.*, 2021; Arab Firozjae *et al.*, 2024), a significant literature gap exists in the context of its efficacy in the context of this particular scenario.

MATERIALS AND METHODS

Animal Model

For this study, thirty (30) healthy mice weighing between were used. The mice were obtained and housed at the animal house of the Faculty of Basic Medical Science for a period of fourteen (14) days before the commencement of the experiment. They were maintained under standard laboratory conditions with a 12-hour light/dark cycle, controlled temperature (22 ± 2°C), and ad libitum access to water and standard laboratory rodent chow.

Ethical Approval

Ethical approval was obtained from the faculty of basic medical science, Abuja campus, University of Port Harcourt. Rat handling and treatment conform to the guideline of the National Research Council (2011) for care and use of laboratory animals.

Chemicals and Reagents

The chemicals and reagents used for this study were purchased from GGI Intl' Nigeria Ltd. located at GGI Place, Plot 8 GGI Crescent, (Opp. Mikab Filling Station), Port Harcourt, Rivers State, Nigeria. The chemicals and reagents include:

Lutein: Lutein a carotenoid with its neuroprotective effect was used as a preventive mechanism for aluminium chloride induced depression. High purity lutein extract was obtained in three different concentrations to create 20mg/kg, 40mg/kg, and 60mg/kg lutein dose groups (Xiong *et al.*, 2022). This was administered orally (O'Brien, 2018);

Sodium azide: Sodium azide, a selective inhibitor of the mitochondrial enzyme cytochrome oxidase, with deficits in memory and long-term potentiation. It was administered intraperitonealy at 5mg/kg;

Donepezil: Donepezil, an acetylcholinesterase inhibitor, served as the standard drug for comparison. It was administered interperitonialy at 5mg/kg;

Sterile Saline: Sterile saline solution was used for dilution of lutein and as a vehicle for drug administered;

Anesthesia: Diethyl ether was used to anesthetize the rats during surgical procedures;

Biochemical assay kits: To measure oxidative stress markers, neurotransmitters and inflammatory cytokines in brain tissue or brain samples, like monoamine neurotransmitter essay kits to check serotonin and dopamine, oxidative stress assay kits to check glutathione;

Apparatus for behavioral tests: this includes the Y maze test and Barnes test;

Surgical Instruments: For stereotaxic surgeries and tissue sampling;

Standard Laboratory Supplies: this includes syringe, needles, feeding syringes, vials and other consumables;

Protective Equipment: Lab coats, hand gloves, facemasks.

Experimental Design

The study consisted of 30 mice, randomly divided into 6 groups (n=5 per group) as follows:

1. **Control Group (Negative):** Mice received food and water only. Mice in this grouped served as a baseline comparison and did not receive any Sodium azide or Lutein treatment. They were used to assess the natural state of cognition, balance and behavior in mice (Nagat El-Demerdash *et al.*, 2023).

2. **Sodium Azide Only Group (Positive Control):** Mice were administered only sodium azide to induce amnesia. 5mg/kg of sodium azide was administered interperitoneally for 14 days. This group will help establish the negative effects of sodium azide on cognitive function (Rafi *et al.,* 2024).
3. **20mg/kg Dose Group**: Mice in this group received sodium azide and 20mg/kg dose of lutein for 14 days. The purpose is to evaluate the potential beneficial effects of lutein at 20mg/kg dosage on amnesia (Xiong *et al.,* 2022).
4. **40mg/kg Dose Group:** Mice in this group were administered sodium azide and moderate lutien dose of 40mg/kg daily for 14 days. This group's aim is to investigate whether a slightly higher dose of lutein would have more pronounced impact on the neuroprotective function (Xiong *et al.,* 2022).
5. **60 mg/kg Dose Group**: Mice in this group were administered sodium azide and moderate lutien dose of 60mg/kg daily for 14 days. This group's objective is to assess the effects of a higher dose of lutein on the amnestic effect of sodium azide (Chike *et al.,* 2025)
6. **Sodium Azide and Donepezil Group:** Mice in this group received a standard dose of Donepezil and sodium azide (5mg/kg/day). It was administered interperitoneally (Amer *et al.,* 2024).

Drug Administration

The administration was done over a 14-day period (Binawade & Jagtap, 2013).

- **Sodium Azide Administration:** Sodium azide a typical selective inhibitor of the mitochondrial enzyme cytochrome oxidase was used to induce amnesia in mice. The selected dosage (5mg/kg/day, I.P) was based on previous studies that reported memory and cognitive deficits similar to those seen in amnesia (Rafi *et al.,* 2024).
- **Lutein Administration:** Lutein, a carotenoid known for its antioxidant properties was administered orally. The volume of the solution was calculated based on the weight of the mice (He *et al.,* 2011). Lutein was dissolved in 16.3ml of distilled water and stored in bottles. This was kept in a refrigerator (at 4°C) to protect it from light and maintain stability. This solution was stored in light protected vials to prevent degradation. Prior to administration, the capsules were dissolved in distilled water to achieve the appropriate treatment dosage (20mg/kg, 40mg/kg, 60mg/kg respectively (Yoo *et al.,* 2010) for the various lutein treatment groups. The treatment was initiated concurrently with sodium azide administration to assess its neuroprotective effects.
- **Donepezil Administration:** A standard drug with known neuroprotective properties was used as a positive control. It was also administered intraperitoneally to the mice to assess its efficacy in mitigating the effects of sodium azide induced amnesia (Kumar *et al.,* 2023).

Behavioral assessment

Throughout the experimental period, the mice were monitored daily for any adverse effects, including changes in behavior, body weight, anxiety and overall health. Motor dysfunction was evaluated using the Hand grip test while Y maze and Barnes test were used to evaluate memory sharpness and anxiety levels (Fan *et al.,* 2021; Brooks *et al.,* 2012).

Biochemical and Molecular assessments

At the end of the experiment, the mice were euthanized and their brain tissues, blood, kidney heart and liver were collected for histological examination and biochemical analysis. Biochemical assays were conducted to measure oxidative stress marker, inflammatory cytokines, and antioxidant enzyme activities (Marrocco *et al.,* 2017).

Biochemical Analysis

AChE was chosen as biomarkers for its relevance to oxidative stress, antioxidant, responses, neurodegeneration and lipid peroxidation which are key factors in amnesia progression (Olufunmilayo *et al.,* 2023). Biochemical assays and immunohistochemical techniques were employed to quantify their levels in brain tissues in each group (Shankar *et al.,* 2009).

Statistical Analysis

All data obtained from the various assessments were subjected to appropriate statistical analyses. Statistical analysis was conducted using GraphPad Analysis software and the results were graphically represented using bar charts, providing a clear visualization of the effects of lutein on various parameters.

Cognitive Tests

Table 1: Effect of Lutein on Barnes Maze Primary Latency, Y-Maze Inflexion Ratio and Y-Maze Percentage Spontaneous Alternation in Sodium Azide - Induced Cognitive

Variables	Groups					
	Control	**Sodium azide only**	**Sodium azide + lutein (20mg/kg)**	**Sodium azide + lutein (40mg/kg)**	**Sodium azide + lutein (60mg/kg)**	**Sodium azide + Donepezil (standard drug)**
Barnes Maze Primary Latency	126.0 ± 3.05	182.8 ± 4.59 **	147.4 ± 15.71	155.8 ± 13.38	127.4 ± 3.53	130.6 ± 6.93

Y-Maze InflexionRatio	111.6 ± 0.93	166.8 ± 3.48*** *	142.6 ± 4.77 **	135.6 ± 4.68 *	122.0 ± 8.13	109.4 ± 2.44
Y-Maze % Spontaneous Alternation	83.88 ± 4.51	61.80 ± 2.87 *	72.60 ± 4.04	73.20 ± 5.95	71.60 ± 5.48	69.10 ± 6.51

Results are presented as Mean ± SEM. N=5

* Indicates statistical significance of Sodium azide and treatments versus the Negative controls * is significant at P < 0.05, **significant at 0.0l, *** significant at 0.001 and **** significant at 0.0001.

Motor Tests

Table 2: Effect of Lutein on Hand Grip Stability Time and Rotarod Stability Time in Sodium Azide - Induced Cognitive Dysfunction in Male Mice

Variables	Groups					
	Control	**Sodium azide only**	**Sodium azide + lutein (20mg/kg)**	**Sodium azide + lutein (40mg/kg)**	**Sodium azide + lutein (60mg/kg)**	**Sodium azide + Donepezil (standard drug)**
Hand Grip Stability Time	19.60 ± 0.68	7.00 ± 0.84****	10.00 ± 0.71 ****	13.00 ± 0.45 ***	18.60 ± 1.63	19.00 ± 0.71
Rotarod Stability Time	24.80 ± 1.46	15.40 ± 1.44 **	31.00 ± 1.73	25.60 ± 1.36	29.60 ± 2.34	26.00 ± 1.52

Results are presented as mean ± SEM. N=5

* Indicates statistical significance of Sodium azide and treatments versus the Negative controls * is significant at P < 0.05, **significant at 0.0l, *** significant at 0.001 and **** significant at 0.0001.

Biochemical/Oxidative Stress Marker

Table 3: Effect of Lutein on Acetylcholinesterase (AChE) Levels in Sodium Azide-Induced Amnesia in Mice

Variables	Group					
	Control	**Sodium azide only**	**Sodium azide + lutein (20mg/kg)**	**Sodium azide + lutein (40mg/kg)**	**Sodium azide + lutein (60mg/kg)**	**Sodium azide + Donepezil (standard drug) .**
AChE	6.94 ± 0.24	4.42 ± 0.18 ****	5.53 ± 0.42 *	6.56 ± 0.27	6.64 ± 0.40	6.70 ± 0.25

Results are presented as mean ± SEM. N=5

* Indicates statistical significance of Sodium azide and treatments versus the Negative controls * is significant at P < 0.05, **significant at 0.0l, *** significant at 0.001 and **** significant at 0.0001.

DISCUSSION

Cognitive Tests - Table 1

- **Barnes Maze:** In Table 1, the sodium azide-only group exhibited significantly higher latency compared to the control group, indicating impaired spatial learning and memory due to sodium azide's neurotoxic effects. Lutein treatment at 20 mg/kg and 40 mg/kg doses reduced latency compared to the sodium azide-only group, suggesting partial neuroprotection. Lutein at 60 mg/kg resulted in the lowest latency among lutein-treated groups, approaching control levels, indicating a dose-dependent neuroprotective effect. Donepezil showed similar efficacy to the 60mg/kg lutein group. These findings align with a study by Leila et al. (2022) which reported that lutein improved spatial memory in a rat model of Alzheimer's disease, reducing latency and errors in the Barnes maze. The results suggest that lutein, particularly at higher doses, mitigates sodium azide-induced cognitive deficits, potentially through its antioxidant and anti-inflammatory properties.
- **Y-Maze - Inflexion Ratio:** The sodium azide-only group had a significantly higher inflexion ratio compared to the control, suggesting disrupted spatial memory. Lutein treatment, particularly at 60mg/kg dose, reduced the inflexion ratio, indicating partial restoration of spatial working memory. However, none of the lutein doses fully restored the ratio to control levels. Donepezil showed comparable effects to the 60mg/kg lutein group.
- **Y Maze - Spontaneous Alternation:** The sodium azide group had the lowest percentage of spontaneous alternation which suggests that sodium azide impaired their spatial memory and learning. The control group had the highest percentage of spontaneous alternation which indicates that they had normal cognitive function. The groups that received sodium azide + lutein and sodium azide + Donepezil had intermediate percentages of spontaneous alternation which implies that lutein and Donepezil partially reversed the negative effect of sodium azide on cogniton. This implies that

lutein has a neuroprotective influence on sodium azide induced amnesia in mice.

Motor Tests - Table 2

- **Hand Grip Stability Test:** Table 2 shows the tabulated representation of the hand grip test and rotarod test. For hand grip test, the control group had a baseline value of 19 secs while the sodium azide only group had a stability time of 7secs. This suggests that sodium azide induced a negative effect on the motor function of the mice. The groups in which sodium azide and lutein were administered had a higher hand grip stability time than the sodium azide only group and this increased across the dosage. This suggests that Lutein was able to reverse the effect of sodium azide on motor function, strength and endurance.
- **Rotarod Stability Test:** The sodium azide group showed the lowest stability time. This suggests that sodium azide impaired the motor coordination and neurological function of the mice. The control group had a higher stability time than the sodium azide only group. The groups that received sodium azide and lutein had a wavering sequence. The sodium azide + lutein at 20mg/kg had the highest stability time, followed by the sodium azide + lutein at 60mg/kg while the sodium azide + lutein at 40mg/kg and sodium azide + Donepezil had the same Rotarod stability time as the control. This suggests that Lutein and Donepezil have the ability to reverse the negative effect of sodium azide on the motor coordination and neurological function of the mice. Very similar research was done with Rotarod test on sodium azide neurotoxicity by Abayomi *et al.*, (2019). The researcher's results were similar as they both demonstrate that sodium azide induces motor impairment and it can be reversed through the use of certain drugs. However, the researcher used vitamin C and E as a corrective measure for sodium azide's negative effects.

Biochemical/Oxidative Stress Marker - AChE (Acetylcholinesterase)

Table 3 shows a graphical representation of the AchE level and its effects on sodium azide induced amnesia in mice. From the table, it can be observed that there was significant decrease in AChE in the sodium azide only group, implying poor cholinergic neurotransmission that is vital in learning and memory. The AChE levels were dose-dependently elevated by lutein treatment, and lutein at 60mg/kg was near the control level, indicating that the cholinergic background has been restored. Donepezil had similar effects with the lutein group receiving 60mg/kg and this signifies the potential of lutein as a neuroprotective agent.

Previous research was done by Singla et al. (2022), also investigating cognitive deficits and neuropathological alterations in sodium azide induced experimental dementia using AchE. However, in this context, there was an increase in AchE activity in the sodium azide only group and a decrease across the other groups.

CONCLUSION

This study shows that lutein has a significant neuroprotective effect against cognitive and motor deficits induced by sodium azide in male mice. Sodium azide affected spatial learning, memory, and motor coordination, with performance in the Barnes maze, Y-maze, hand grip, and rotarod tests being severely impaired, as well as decreased AChE levels, which points to oxidative stress and cholinergic dysfunction. Treatment with lutein, especially at a dose of 60 mg/kg, was able to improve cognitive and motor abilities as well as the biomarkers levels significantly, and it was comparable in effect to donepezil. The results indicate that lutein offsets the neurotoxic actions of sodium azide through the improvement of antioxidant defense system and recovery of cholinergic activity, which is a promising dietary supplement to neurodegenerative diseases accompanied by oxidative stress and cognitive impairment. The dose dependent effect of lutein highlights its therapeutic potential which should be explored both in preclinical and clinical studies.

REFERENCES

- Abayomi, T. A., Tokunbo, O. S., Adebisi, B. T., Fadare, M. U., Gbadamosi, I. T., Akinwale, J. O., Sawyer, E. A., & Arogundade, T. T. (2019). *Neurobehavioral assessment of the impact of vitamins C and E following acute exposure to sodium azide-induced neurotoxicity*. *4*, 15–20. https://doi.org/10.5281/zenodo.2577031
- Ahn, Y. J., & Kim, H. (2021). Lutein as a Modulator of Oxidative Stress-Mediated Inflammatory Diseases. *Antioxidants*, *10*(9), 1448. https://doi.org/10.3390/antiox10091448
- Amer, A. S., Ali, E. H. A., Zahra, M. M., & Sabry, H. A. (2024). Mesenchymal stem cell-derived exosomes modulate the COX-IV pathway via inhibition of amyloidogenesis and mitoprotection in sodium azide- Alzheimer model in rats. *Scientific African*, *25*, e02274. https://doi.org/10.1016/j.sciaf.2024.e02274
- Arab Firozjae, A., Shiran, M. R., Ajami, A., Farzin, D., & Rashidi, M. (2024). Lutein improves remyelination by reducing of neuroinflammation in C57BL/6 mouse models of multiple sclerosis. *Heliyon*, *10*(20), e39253. https://doi.org/10.1016/j.heliyon.2024.e39253
- Aza, A., Gómez-Vela, M., Badia, M., Begoña Orgaz, M., González-Ortega, E., Vicario-Molina, I., & Montes-López, E. (2022). Listening to families with a person with neurodegenerative disease talk about their quality of life: integrating quantitative and qualitative approaches. *Health and Quality of*

Life Outcomes, *20*(1). https://doi.org/10.1186/s12955-022-01977-z

- Binawade, Y., & Jagtap, A. (2013). Neuroprotective Effect of Lutein Against 3-Nitropropionic Acid–Induced Huntington's Disease–Like Symptoms: Possible Behavioral, Biochemical, and Cellular Alterations. *Journal of Medicinal Food*, *16*(10), 934–943. https://doi.org/10.1089/jmf.2012.2698
- Brooks, S. P., Trueman, R. C., & Dunnett, S. B. (2012). Assessment of motor coordination and balance in mice using the rotarod, elevated bridge, and footprint tests. *ResearchGate*, *2*, 37–53. https://www.researchgate.net/publication/284087171_Assessment_of_motor_coordination_and_balance_in_mice_using_the_rotarod_elevated_bridge_and_footprint_tests
- Chen, Z.-R., Huang, J.-B., Yang, S.-L., & Hong, F.-F. (2022). Role of Cholinergic Signaling in Alzheimer's Disease. *Molecules*, *27*(6), 1816. https://doi.org/10.3390/molecules27061816
- Chike , C. P. R., Austin-Ajah, G., & Ajah, A. (2025). Evaluation of Antioxidant Enzyme Activity and Oxidative Stress Markers in Male Wistar Rats Following Scopolamine-Induced Depression and Lutein Treatment. *Sch Bull*, *11*(7), 124–130. https://doi.org/10.36348/sb.2025.v11i07.002
- Eiermann, N., Stoecklin, G., & Jovanovic, B. (2022). Mitochondrial Inhibition by Sodium Azide Induces Assembly of eIF2α Phosphorylation-Independent Stress Granules in Mammalian Cells. *International Journal of Molecular Sciences*, *23*(10), 5600. https://doi.org/10.3390/ijms23105600
- Fan, Y., Han, J., Zhao, L., Wu, C., Wu, P., Huang, Z., Hao, X., Ji, Y., Chen, D., & Zhu, M. (2021). Experimental Models of Cognitive Impairment for Use in Parkinson's Disease Research: The Distance Between Reality and Ideal. *Frontiers in Aging Neuroscience*, *13*. https://doi.org/10.3389/fnagi.2021.745438
- Fujimura, T., Kobayashi, H., Suzuki, T., Sato, I., & Hara, S. (2002). Neurotoxicity in sodium azide poisoning. *Chudoku Kenkyu : Chudoku Kenkyukai Jun Kikanshi = the Japanese Journal of Toxicology*, *15*(3), 281–288. https://pubmed.ncbi.nlm.nih.gov/12415870/
- He, R.-R., Tsoi, B., Lan, F., Yao, N., Yao, X.-S., & Kurihara, H. (2011). Antioxidant properties of lutein contribute to the protection against lipopolysaccharide-induced uveitis in mice. *Chinese Medicine*, *6*(1), 38. https://doi.org/10.1186/1749-8546-6-38
- Johnson, E. J. (2014). Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan. *Nutrition Reviews*, *72*(9), 605–612. https://doi.org/10.1111/nure.12133
- Kumar, A., Sharma, S., & Gupta, V. (2023). *Donepezil*. National Library of Medicine; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK513257/
- Kumar, A., Tsao, J. W., Sidhu, J., & Goyal, A. (2024, February 12). *Alzheimer Disease*. National Library of Medicine; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK499922/
- Marrocco, I., Altieri, F., & Peluso, I. (2017). Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. *Oxidative Medicine and Cellular Longevity*, *2017*, 6501046. https://doi.org/10.1155/2017/6501046
- Matthews, B. R. (2015). Memory Dysfunction. *CONTINUUM: Lifelong Learning in Neurology*, *21*(3), 613–626. https://doi.org/10.1212/01.con.0000466656.59413.29
- Nagat El-Demerdash, Pan, T., Choi, O., Saraswati, M., Koehler, R. C., Robertson, C. L., & Savonenko, A. (2023). Importance of Control Groups for Evaluating Long-Term Behavioral and Cognitive Outcomes of Controlled Cortical Impact in Immature Rats. *Journal of Neurotrauma*. https://doi.org/10.1089/neu.2021.0376
- National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals, 8th edition. National Academies Press*. https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf
- Nazari, L., Komaki, S., Salehi, I., Raoufi, S., Golipoor, Z., Kourosh-Arami, M., & Komaki, A. (2022). Investigation of the protective effects of lutein on memory and learning using behavioral methods in a male rat model of Alzheimer's disease. *Journal of Functional Foods*, *99*, 105319. https://doi.org/10.1016/j.jff.2022.105319
- O'Brien, S. (2018, July 11). *Lutein and Zeaxanthin: Benefits, Dosage and Food Sources*. Healthline. https://www.healthline.com/nutrition/lutein-and-zeaxanthin
- Olufunmilayo, E. O., Gerke-Duncan, M. B., & Holsinger, R. M. D. (2023). Oxidative Stress and Antioxidants in Neurodegenerative Disorders. *Antioxidants*, *12*(2), 517. https://doi.org/10.3390/antiox12020517
- Pap, R., Pandur, E., Jánosa, G., Sipos, K., Agócs, A., & Deli, J. (2021). Lutein Exerts Antioxidant and Anti-Inflammatory Effects and Influences Iron Utilization of BV-2 Microglia. *Antioxidants*, *10*(3), 363. https://doi.org/10.3390/antiox10030363
- Rafi, H., Rafiq, H., & Farhan, M. (2024). Agmatine Improves Oxidative Stress Profiles in Rat Brain Tissues Induced by Sodium Azide. *Current Chemical Biology*, *18*(3), 129–143. https://doi.org/10.2174/0122127968308662240926114002
- Ramirez, M. (2015). Why lutein is important for the eye and the brain. *OCL*, *23*(1), D107. https://doi.org/10.1051/ocl/2015027
- Rapaka, D., Adiukwu, P. C., & Bitra, V. R. (2022). Experimentally induced animal models for cognitive

dysfunction and Alzheimer's disease. *MethodsX*, *9*, 101933. https://doi.org/10.1016/j.mex.2022.101933

- Sam, C., & Bordoni, B. (2023, April 10). *Physiology, Acetylcholine*. PubMed; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557825/
- Shankar, G. M., Leissring, M. A., Adame, A., Sun, X., Spooner, E., Masliah, E., Selkoe, D. J., Lemere, C. A., & Walsh, D. M. (2009). Biochemical and immunohistochemical analysis of an Alzheimer's disease mouse model reveals the presence of multiple cerebral Aβ assembly forms throughout life. *Neurobiology of Disease*, *36*(2), 293–302. https://doi.org/10.1016/j.nbd.2009.07.021
- Singla, R. K., Dhonchak, K., Sodhi, R. K., Babu, M. A., Madan, J., Madaan, R., Kumar, S., Sharma, R., & Shen, B. (2022). Bergenin ameliorates cognitive deficits and neuropathological alterations in sodium azide-induced experimental dementia. *Frontiers in Pharmacology*, *13*. https://doi.org/10.3389/fphar.2022.994018
- Stringham, J. M., Johnson, E. J., & Hammond, B. R. (2019). Lutein across the Lifespan: From Childhood Cognitive Performance to the Aging Eye and Brain. *Current Developments in Nutrition*, *3*(7). https://doi.org/10.1093/cdn/nzz066
- Tat, J., Heskett, K., Satomi, S., Pilz, R. B., Golomb, B. A., & Boss, G. R. (2021). Sodium azide poisoning: a narrative review. *Clinical Toxicology*, *59*(8), 683–697. https://doi.org/10.1080/15563650.2021.1906888
- Weinstock, M., & Shoham, S. (2004). Rat models of dementia based on reductions in regional glucose metabolism, cerebral blood flow and cytochrome oxidase activity. *Journal of Neural Transmission*, *111*(3), 347–366. https://doi.org/10.1007/s00702-003-0058-y
- Xiong, K., Zhao, Y., Hu, S., Ma, A., & Ma, Y. (2022). Dose-Response Relationship Between Oral Lutein Intake and Plasma Lutein Concentration: A Randomized Controlled Trial. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.924997
- Yoo, J. H., Shanmugam, S., Thapa, P., Lee, E.-S., Balakrishnan, P., Baskaran, R., Yoon, S.-K., Choi, H.-G., Yong, C. S., Yoo, B. K., & Han, K. (2010). Novel self-nanoemulsifying drug delivery system for enhanced solubility and dissolution of lutein. *Archives of Pharmacal Research*, *33*(3), 417–426. https://doi.org/10.1007/s12272-010-0311-5
- Yuwen, L., Yi, L., Cunxin, W., Songsheng, Q., & Fengjiao, D. (2000). Microcalorimetric studies of the inhibition of sodium azide on the mitochondrial metabolism of fish liver tissue. *Thermochimica Acta*, *351*(1-2), 51–54. https://doi.org/10.1016/s0040-6031(00)00415-9
- Zeni, A. L. B., Camargo, A., & Dalmagro, A. P. (2019). Lutein prevents corticosterone-induced depressive-like behavior in mice with the involvement of antioxidant and neuroprotective activities. *Pharmacology Biochemistry and Behavior*, *179*, 63–72. https://doi.org/10.1016/j.pbb.2019.02.004