The Effect Of Iron Citrate Synthesit On Cognitive Functions In Mice

Baiyr Dombaanai[1], Roman Karabanov[2], Shahbaz Baig[3]

[1]Department of Neurosurgery, Burdenko Neurosurgery Institute: Moscow, RU, Place de Longemalle 1, Genève, 1204, Switzerland; [2]Department of European Studies, SPBGU, Saint-Petersberg, Russia, Place de Longemalle 1, Genève, 1204, Switzerland; [3] Department of Community Medicine, Independence Medical College, 177-P, Jinnah Colony, Faisalabad 38000, Punjab, Pakistan
Corresponding Author: Prof. Dr. Shahbaz Baig
Address: Independence Medical College, 177-P, Jinnah Colony, Faisalabad 38000, Punjab, Pakistan
Email: talha_shahbaz557@yahoo.com

Keywords:

iron citrate, synthesit, cognitive functions, spatial memory, eight-arm radial maze, SHK mice.

Abstract

Background: This study evaluated the effect of iron citrate synthesit on cognitive functions in SHK strain mice, focusing on spatial memory and learning efficiency.
Methods and Results: Ten male SHK strain mice (8-10 weeks old, 20-25 g) were randomly divided into two groups of five: a control group and an experimental group. The experimental group received 50 µg/day of iron citrate in drinking water for 6 days. Cognitive functions were assessed using an eight-arm radial maze over a 6-day period, including habituation, training, and testing phases, with a break on day 5 to evaluate long-term memory. Parameters measured included correct and incorrect entries, maze completion time, and memory scores. By day 6, the experimental group showed statistically significant improvement in spatial memory ($p<0.05$), with a mean memory score of -0.19 compared to -0.38 in controls. Maze completion time decreased by 30% (from 322 to 295 seconds), compared to an 18% decrease in the control group (316 to 305 seconds). After the break, the experimental group demonstrated better memory retention (mean correct entries: 3.2 vs. 2.8; $p>0.05$).
Conclusions: Iron citrate Synthesit at 50 µg/day enhances cognitive function in SHK mice, improving spatial memory, learning speed, and retention in maze-based tasks.

Introduction

Iron plays a critical role in central nervous system function, supporting oxygen metabolism, neurotransmitter synthesis, and myelination of nerve fibers (Gao et al., 2025; Khattar et al., 2021; Levi et al., 2024; Möller et al., 2019). Iron deficiency, particularly in early development, is associated with impaired cognitive functions, including memory, attention, and learning ability, as confirmed by numerous studies in animal models and humans. Iron deficiency anemia in children has been linked to long-term cognitive deficits, such as reduced IQ scores and impaired spatial memory (Gutema et al., 2023; Samson et al., 2022; Yeboah et al., 2024). In rodents, iron deficiency disrupts hippocampal neurogenesis, negatively affecting spatial learning (Barks et al., 2021; Bastian et al., 2019; Wang et al., 2019). Studies show that even without anemia, iron deficiency can negatively affect cognition, emphasizing the importance of maintaining proper iron levels for optimal brain function.

Conversely, the effects of iron excess or its targeted supplementation on cognitive performance remain less explored. While iron is essential for brain function, excessive accumulation may lead to oxidative stress and neurotoxicity (Chen et al., 2025). This

underscores the importance of identifying optimal dosages and forms of iron administration to enhance cognition without adverse effects.

In humans, correcting iron deficiency with supplements has shown positive outcomes. For example, Murray-Kolb and Beard demonstrated improvements in attention and short-term memory in women following iron supplementation (MacLean et al., 2023). However, data on iron's impact on spatial memory and long-term learning remain limited, particularly in experimental models.

Synthesit iron citrate enhanced hematopoiesis and improved metabolic markers in aged mice and primates, suggesting systemic benefits beyond anemia correction, even in non-deficient models (Kusnir et al., 2024). This opens possibilities for exploring its role in enhancing brain function through improved metabolic support.

The significance of this study lies in the need for a more thorough understanding of iron's role in cognitive processes, which could inform the development of strategies to prevent and address cognitive impairments associated with metabolic imbalances (Chen et al., 2022). Rodent models, such as the eight-arm radial maze, are widely used to assess spatial memory and learning due to their sensitivity to hippocampal function changes (Dalkiran et al., 2022; Gawel et al., 2018; Kohler et al., 2022). These models enable the investigation of effects potentially applicable to humans, given the similarity in underlying neural mechanisms.

The aim of this study was to assess the effect of iron citrate "Synthesit" on the cognitive functions of SHK strain mice, including spatial memory, learning speed, and long-term memory retention, using an eight-arm radial maze. Additionally, this study sought to draw parallels between our findings and existing data on iron's influence on human cognition.

Methods

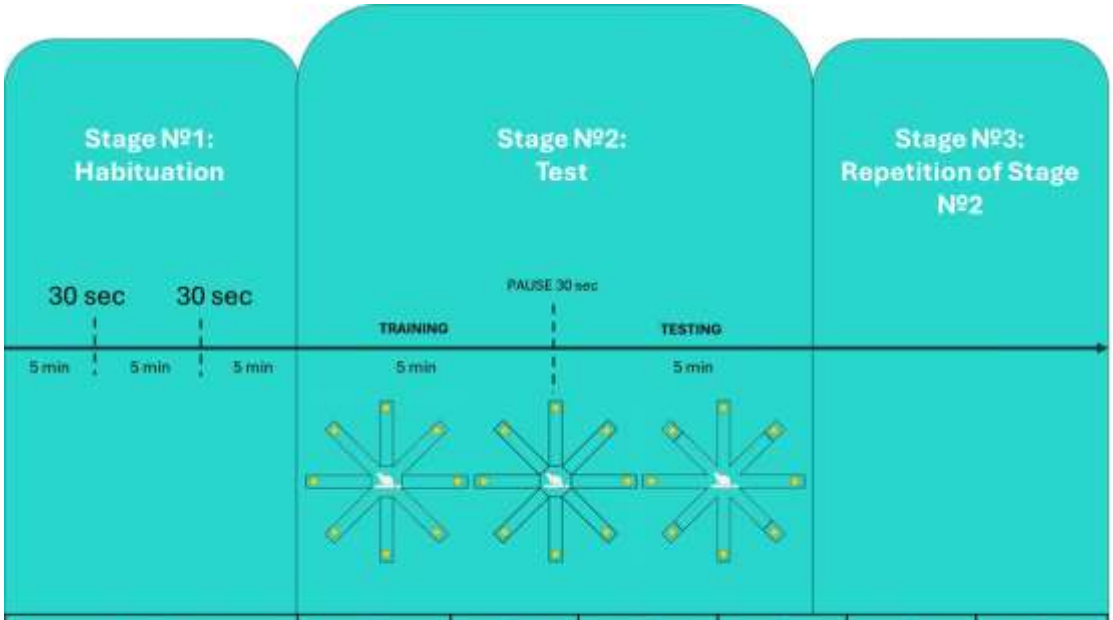
Study Design

Randomized controlled trial with Animals Model was applied and randomly assigned animals to treatment and control groups and compared outcomes between them.

Study sample size

The study involved 10 SHK strain laboratory mice, divided into two groups of 5: a control group (Group 1) and an experimental group (Group 2). All subjects were male, aged 8-10 weeks, with body weights of 20-25 g at the start of the experiment. Mice were housed under standard vivarium conditions: temperature 21±1°C, relative humidity 50-60%, a 12-hour light/dark cycle, and ad libitum access to water and standard feed (Lab Diet 5001). The experimental group received iron citrate "Synthesit" at 50 µg/day per mouse via drinking water for 6 days. The dosage was selected based on mouse metabolism and lifespan, aligning with recommendations by (Beard & Connor, 2003) for studying iron's metabolic effects in rodents. The experiment adhered to ethical standards outlined in the European Community Directive (2010/63/EU) (Lane-Petter, 1950).

Spatial memory and cognitive functions were evaluated using an eight-arm radial maze, consisting of a central platform (20 cm diameter) and eight radial arms (35 cm long, 5 cm wide, 10 cm wall height) (Figure 1). Food rewards (20 mg pellets) were placed in four of the eight arms, with their positions fixed throughout the experiment. The testing protocol was adapted from Richter et al. and Gorina et al. for assessing cognitive functions in rodents (Lebedeva et al., 2024).



DAY	1	2	3	4	5	6
STAGES	1, 2, 3	2, 3	2, 3	2, 3		2, 3

Figure 1. Diagram of the eight-arm radial maze and experimental stages for assessing cognitive functions in mice

Experiment Process

The experiment comprised three phases:

- **Habituation:** Mice explored the maze without food for 10 minutes to reduce anxiety and adapt to the environment.
- **Training:** Food rewards were placed in four random arms. Each mouse was placed in the maze center for 5-6 minutes (time to locate all rewards), with correct entries (into unvisited arms with food) and incorrect entries (revisits to already explored arms) recorded. The maze was cleaned with 70% ethanol between sessions to eliminate odors.
- **Testing:** Conducted daily for 6 consecutive days, with a break on day 5 to assess long-term memory. Testing occurred twice daily (60-minute interval) to evaluate working memory. Reward locations remained consistent, enabling analysis of memory retention.

Measured Parameters:

The following metrics were recorded:

- Number of correct entries (into new arms with rewards).
- Number of incorrect entries (revisits to explored arms).
- Total entries (sum of correct and incorrect).
- Memory score (P), calculated as:
  P = (correct entries incorrect entries)/total entries
- Maze completion time (seconds) to locate all rewards.
- Change in memory score (ΔP) between trials, calculated as:
  ΔP=(P current trial, P previous trial) / P previous trial * 100%

Data Analysis

Data were analyzed using IBM SPSS Statistics 20.0 (USA). Metrics were grouped by day and mouse, with means and standard deviations calculated for each group. The student's t-test for independent samples was used to compare cognitive performance between groups, with statistical significance set at $p<0.05$.

Results

Analysis of data from the eight-arm radial maze experiment revealed a significant effect of iron citrate "Synthesit" on spatial memory and cognitive abilities in SHK strain mice. During the initial days (days 1-2), both groups exhibited similar performance: high incorrect entries (14-18 in the control group, 15-17 in the experimental group) and low correct entries (2-4 in both). However, from day 3 onward, the experimental group (receiving 50 μg/day of iron citrate) outperformed the control: mean correct entries rose to 4, while incorrect entries declined (e.g., mouse №2 in the experimental group reduced incorrect entries from 15 to 5 by day 6) (Figure 2).

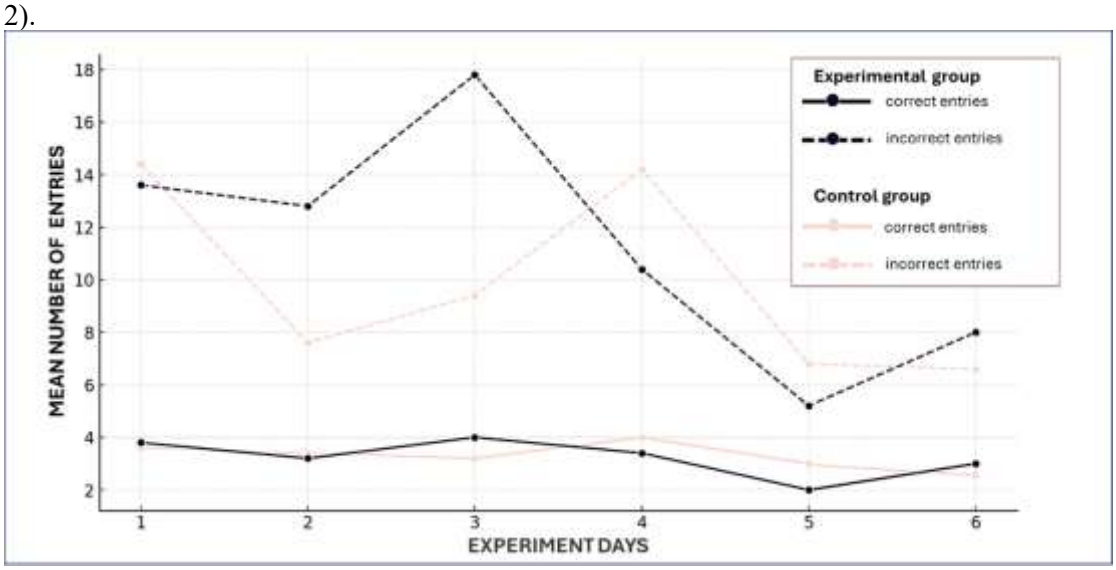


Figure 2. Trends in mean number of correct and incorrect entries in both groups. Solid lines represent correct entries; dashed lines represent incorrect entries

The results in the Figure 3 show a significant difference in memory performance between the experimental and control groups by day 6. The experimental group, which received iron supplementation, demonstrated a higher mean memory score of -0.19, compared to the control group's -0.38. This difference, which was statistically significant ($p<0.05$, Student's t-test), suggests that iron supplementation led to faster learning and improved spatial memory in the mice. The trend in the graph shows that over the 6 days, the experimental group's memory score steadily increased, whereas the control group exhibited more variability and lower overall performance in terms of memory.

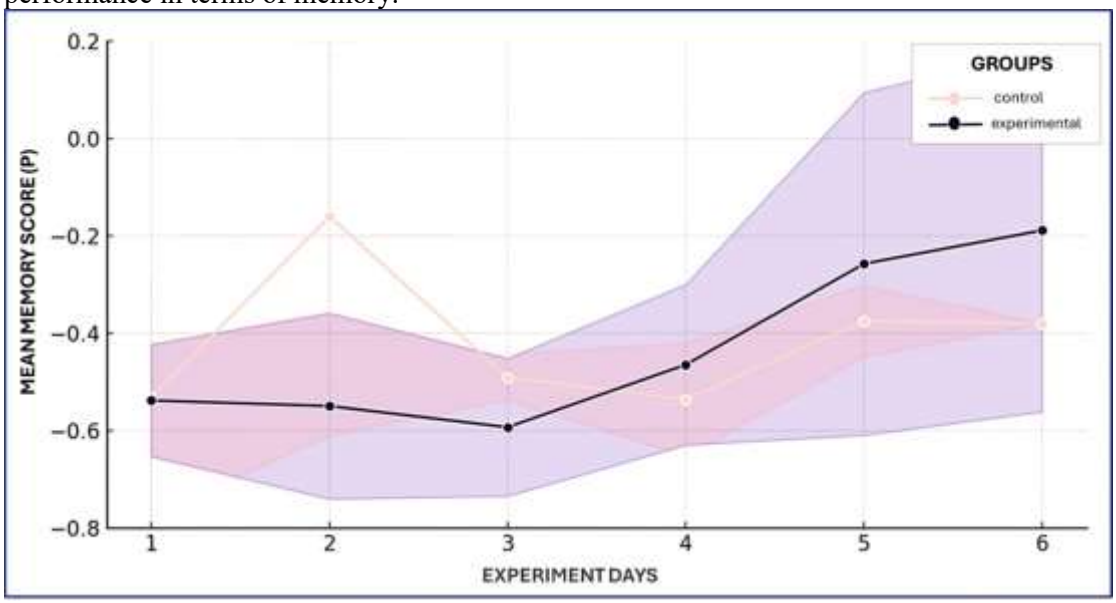


Figure 3. Dynamics of mean memory score changes in group 1 (control) and group 2 (experimental)

Figure 4 illustrates the dynamics of mean maze completion times in both the control and experimental groups throughout the experiment. In the experimental group, the mean completion time decreased from 322 seconds on day 1 to 295 seconds on day 6, reflecting a 30% reduction. In comparison, the control group showed a smaller decrease, from 316 seconds to 305 seconds, an 18% reduction. This greater improvement in the experimental group suggests enhanced spatial orientation and cognitive mapping, supporting the positive effect of iron supplementation on cognitive function.

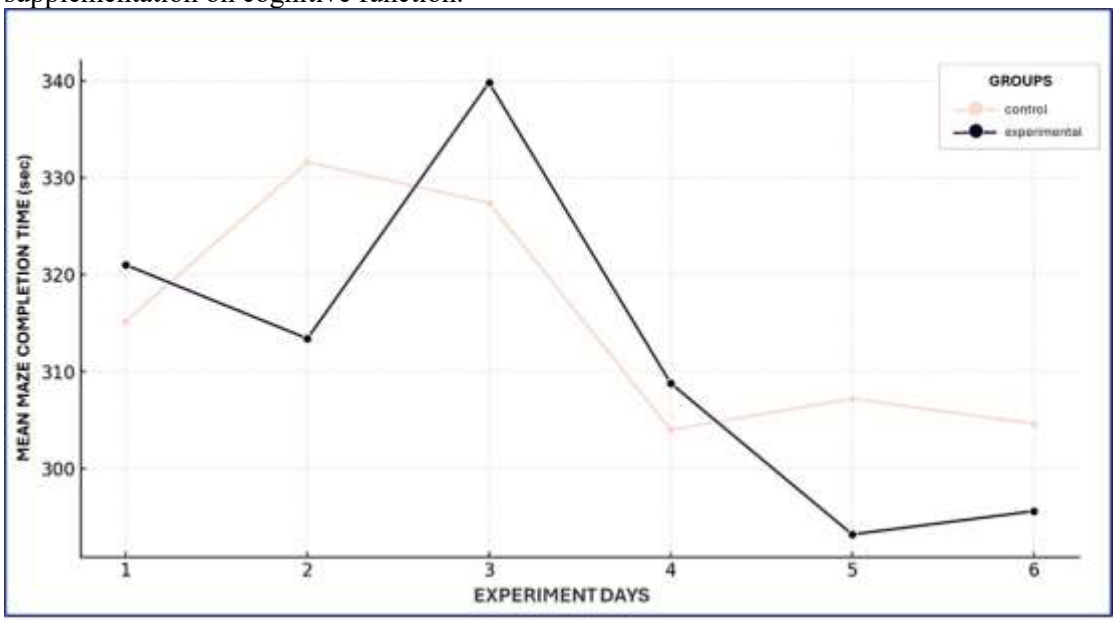


Figure 4. Dynamics of mean maze completion time in group 1 (control) and group 2 (experimental)

Discussion

The current study on the effects of iron citrate "Synthesit" on cognitive function in mice highlights that, following a 5-day break, the experimental group demonstrated better long-term memory retention, as evidenced by a higher number of correct entries (mean: 3.2) compared to the control group (mean: 2.8). This finding supports the hypothesis that iron, a crucial component of brain metabolism, enhances neural connections that underpin long-term memory. Additionally, it aligns with previous research showing that iron deficiency impairs cognitive function in rats. In a rat model of iron deficiency, hippocampal neuronal ultrastructural damage was observed to persist into adulthood, even after early-life iron supplementation (Barks, 2020). Extrapolating these results to humans necessitates further investigation; however, existing data partially support our findings. For instance, a study in humans demonstrated enhanced attention and memory in women following the correction of iron deficiency anemia (Pivina et al., 2019).

In current study, iron supplementation not only improved the number of correct entries but also decreased performance variability. Specifically, the maze completion time in the experimental group decreased by 30% (from 322 to 295 seconds), suggesting an enhanced spatial orientation and cognitive efficiency, compared to the 18% reduction observed in the control group (from 316 to 305 seconds). This more substantial reduction in maze completion time suggests that iron supplementation improves cognitive processing speed, which is consistent with improved memory retention. These results are consistent with another findings that iron supplementation, when administered at appropriate doses and developmental stages, can enhance cognitive performance, particularly memory and processing speed. These benefits are likely mediated by upregulation of neurotransmitter systems crucial for learning and memory. However, the positive effects depend on avoiding both deficiency and overload, and may vary with age and sex (Shete et al., 2024). Previous findings suggest iron citrate (Synthesit) may influence hematologic and immune markers, potentially supporting red blood cell production and reducing chemotherapy-induced neuropathy in cancer patients (Kusnir & Baig,

2024). Such systemic effects could indirectly support improved brain oxygenation and metabolic activity, contributing to cognitive enhancement.

The current study shows that while iron supplementation can enhance cognitive function, excessive iron can be toxic. A separate study linked high doses of iron to neurotoxic effects (D'Mello & Kindy, 2020). In current study, the dosage of 50 µg/day was specifically adjusted for mouse metabolism and did not result in any noticeable side effects over the 6-day period. However, another animal study indicated that higher doses of ferric citrate (50-100 mg/kg) led to hippocampal iron accumulation, neuroinflammation, and impaired cognitive and motor functions. These effects were associated with proteomic changes that impacted iron metabolism proteins, such as the transferrin receptor and DMT1 (divalent metal transporter 1) (Cui et al., 2024). In a dyslipidemic patient, Synthesit initially reduced cholesterol levels, followed by a slight rebound, highlighting potential short-term lipid-lowering effects and the need for further long-term evaluation (Patrik & Shahbaz, 2024). This suggests Synthesit may also influence lipid metabolism, which is increasingly recognized as a factor in neurodegenerative conditions and cognitive decline.

The small sample size (10 mice) reduced statistical power. These results are supported by multiple large-scale human trials and animal studies from recent years demonstrating that iron supplementation improves various cognitive domains, particularly memory and intelligence. These converging findings underscore that prenatal iron supplementation, customized to maternal hemoglobin levels and baseline iron stores, contributes to improved cognitive functioning in children at the age of 4 (Iglesias-Vázquez et al., 2023).

Study Limitation

The limitations of this study include a small sample size (10 mice), which restricts the generalizability of the findings, as well as the lack of baseline biochemical markers, limiting the ability to draw causal inferences regarding cognitive improvements. The short study duration (6 days) and individual behavioral variability further limit the findings. Additionally, the lack of mechanistic insights and potential confounding factors, such as diet and environmental stressors, require further exploration.

Conclusion

This study demonstrated that iron citrate "Synthesit" at 50 µg/day enhances cognitive functions in SHK strain mice, including spatial memory and learning speed, as confirmed by eight-arm radial maze testing. The experimental group exhibited more correct entries (P = -0.19 vs. -0.38 in controls, $p<0.05$), reduced maze completion time (30% decrease), and better retention post-break. These findings suggest iron positively influences memory and learning-related neural processes, consistent with data on iron deficiency correction. The dosage was optimal, with no adverse effects observed, though the lack of biochemical markers and short duration limit mechanistic insights. Individual behavioral variability requires further analysis. Overall, the results highlight the potential of iron citrate "Synthesit" for cognitive enhancement, but larger, longer-term studies with biochemical analysis are needed to confirm its therapeutic value.

FINDINGS:

1. The experimental group, receiving 50 µg/day of iron citrate "Synthesit," showed statistically significant improvement in spatial memory ($p<0.05$): mean memory score by day 6 was -0.19 vs. -0.38 in the control group.
2. Maze completion time in the experimental group decreased by 30% (322 to 295 seconds), vs. 18% in the control group (316 to 305 seconds), indicating more efficient spatial orientation ($p>0.05$).
3. Post-break long-term memory was better preserved in the experimental group (mean correct entries: 3.2 vs. 2.8 in controls) ($p>0.05$).

Acknowledgements

Not applicable

Funding
No funding was received

Authors' contributions
Baiyr Dombaanai conceptualized the study and wrote the manuscript. Roman Karabanov conducted the experiments, and collected and analyzed the data. Shahbaz Baig provided statistical support, contributed to data interpretation, and offered critical

Disclosures
The authors declare no conflicts of interest.

References

1. Möller HE, Bossoni L, Connor JR, Crichton RR, Does MD, Ward RJ, et al. Iron, Myelin, and the Brain: Neuroimaging Meets Neurobiology. Trends in Neurosciences. 2019;42(6):384-401.
2. Levi S, Ripamonti M, Moro AS, Cozzi A. Iron imbalance in neurodegeneration. Molecular Psychiatry 2024 29:4. 2024;29(4):1139-52.
3. Gao Q, Zhou Y, Chen Y, Hu W, Jin W, Zhou C, et al. Role of iron in brain development, aging, and neurodegenerative diseases. Annals of Medicine. 2025;57(1):2472871-.
4. Khattar N, Triebswetter C, Kiely M, Ferrucci L, Resnick SM, Spencer RG, Bouhrara M. Investigation of the association between cerebral iron content and myelin content in normative aging using quantitative magnetic resonance neuroimaging. NeuroImage. 2021;239:118267-.
5. Gutema BT, Sorrie MB, Megersa ND, Yesera GE, Yeshitila YG, Pauwels NS, et al. Effects of iron supplementation on cognitive development in school-age children: Systematic review and meta-analysis. PLOS ONE. 2023;18(6):e0287703-e.
6. Samson KLI, Fischer JAJ, Roche ML. Iron Status, Anemia, and Iron Interventions and Their Associations with Cognitive and Academic Performance in Adolescents: A Systematic Review. Nutrients. 2022;14(1):224-.
7. Yeboah FA, Bioh J, Amoani B, Effah A, Senu E, Mensah OSO, et al. Iron deficiency anemia and its association with cognitive function among adolescents in the Ashanti Region - Ghana. BMC Public Health. 2024;24(1):1-12.
8. Barks AK, Liu SX, Georgieff MK, Hallstrom TC, Tran PV. Early-Life Iron Deficiency Anemia Programs the Hippocampal Epigenomic Landscape. Nutrients 2021, Vol 13, Page 3857. 2021;13(11):3857-.
9. Bastian TW, von Hohenberg WC, Georgieff MK, Lanier LM. Chronic Energy Depletion due to Iron Deficiency Impairs Dendritic Mitochondrial Motility during Hippocampal Neuron Development. Journal of Neuroscience. 2019;39(5):802-13.
10. Wang Y, Wu Y, Li T, Wang X, Zhu C. Iron metabolism and brain development in premature infants. Frontiers in Physiology. 2019;10(APR):428141-.
11. Chen L, Shen Q, Liu Y, Zhang Y, Sun L, Ma X, et al. Homeostasis and metabolism of iron and other metal ions in neurodegenerative diseases. Signal Transduction and Targeted Therapy 2025 10:1. 2025;10(1):1-48.
12. MacLean B, Sholzberg M, Weyand AC, Lim J, Tang G, Richards T. Identification of women and girls with iron deficiency in the reproductive years. International Journal of Gynecology & Obstetrics. 2023;162(S2):58-67.
13. Kusnir P, Bulgin D, Baig S. Iron citrate Synthesit: impact on blood parameters in non-iron deficient models. Edelweiss Applied Science and Technology. 2024;8(4):2331-47.
14. Chen Z, Yang H, Wang D, Sudfeld CR, Zhao A, Xin Y, et al. Effect of Oral Iron Supplementation on Cognitive Function among Children and Adolescents in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis. Nutrients. 2022;14(24):5332-.
15. Dalkiran B, Açıkgöz B, Dayı A. Behavioral Tests Used in the Evaluation of Learning and Memory in Experimental Animals. Journal of Basic and Clinical Health Sciences. 2022;6(3):938-45.
16. Kohler J, Mei J, Banneke S, Winter Y, Endres M, Emmrich JV. Assessing spatial learning and memory in mice: Classic radial maze versus a new animal-friendly automated radial maze allowing free access and not requiring food deprivation. Frontiers in Behavioral Neuroscience. 2022;16:1013624-.
17. Gawel K, Gibula E, Marszalek-Grabska M, Filarowska J, Kotlinska JH. Assessment of spatial learning and memory in the Barnes maze task in rodents—methodological consideration. Naunyn-Schmiedeberg's Archives of Pharmacology 2018 392:1. 2018;392(1):1-18.

18. Beard JL, Connor JR. Iron status and neural functioning. Annual Review of Nutrition. 2003;23(Volume 23, 2003):41-58.
19. Lane-Petter W. Protection of Laboratory Animals. British Medical Journal. 1950;2(4688):1114.
20. Lebedeva A, Gerasimova SA, Yashanova MI, Naumov AV, Ivanov AA, Karchkov DA, et al. A Method for Assessing Working Memory in Rats Using Controlled Virtual Environment. Sovremennye Tehnologii v Medicine. 2024;16(3):12-22.
21. Barks AK. The effect of developmental iron deficiency on gene expression, TET proteins, and DNA hydroxymethylation in the rodent brain A DISSERTATION SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA: University Digital Conservancy; 2020.
22. Pivina L, Semenova Y, Doşa MD, Dauletyarova M, Bjørklund G. Iron Deficiency, Cognitive Functions, and Neurobehavioral Disorders in Children. Journal of Molecular Neuroscience. 2019;68(1):1-10.
23. Shete PA, Ghatpande NS, Varma ME, Joshi PV, Suryavanshi KR, Misar AV, et al. Chronic dietary iron overload affects hepatic iron metabolism and cognitive behavior in Wistar rats. Journal of Trace Elements in Medicine and Biology. 2024;84:127422-.
24. Kusnir P, Baig S. The Neuropathy as an Adverse Effect of Chemotherapy Diminished After Administration of Dietary Supplement with Iron Citrate – Case Study. Futurity Medicine. 2024;3(3).
25. D'Mello SR, Kindy MC. Overdosing on iron: Elevated iron and degenerative brain disorders. Experimental Biology and Medicine. 2020;245(16):1444-73.
26. Cui L, Zhou H, Hao Y, Yang X, Li Z, Gao Y, et al. Effect of ferric citrate on hippocampal iron accumulation and widespread molecular alterations associated with cognitive disorder in an ovariectomized mice model. CNS Neuroscience & Therapeutics. 2024;30(9):e70018-e.
27. Patrik K, Shahbaz B. Effect of the Synthesit Dietary Supplement on Serum Cholesterol Levels. Journal of University Medical & Dental College. 2024;15(4).
28. Iglesias-Vázquez L, Voltas N, Hernández-Martínez C, Canals J, Coronel P, Gimeno M, et al. Importance of Maternal Iron Status on the Improvement of Cognitive Function in Children After Prenatal Iron Supplementation. American Journal of Preventive Medicine. 2023;65(3):395-405.