Kerbala Journal of Veterinary Medical Sciences Issue (3), Volume (1), (2025)

Hippocampal Oxidant / Antioxidant Tissue Levels: An Effect Associated with Age in Male Rat

Doaa Saeed Abdullah[1], Sinaa J. Al-Bazii [2], Wefak Al bazi [3]

[1]Department of Anatomy, College of Veterinary Medicine, University of Kerbala, Karbala,Iraq
[2]College of Education for Pure Sciences, University of Kerbala, Karbala,Iraq
[3]College of Applied Medical Sciences, University of Kerbala, Karbala,Iraq
Corresponding authors: doaa.saeed@s.uokerbala.edu.iq
sinaa.j@uokerbala.edu.iq
wifaq.jbori@uokerbala.edu.iq

Received:29 /8 /2025 **Accepted:** 28/ 9/2025 **Published:** 15/ 12/2025

***Abstract*— The hippocampus plays important role in memory, emotion, learning. The current study looked into the oxidative Stress (Oxidant, Antioxidant) on the Development of the Hippocampus in the albino male rats in various age. Twenty male albino rats were divided four groups: 1-day group, 1-month group,3-month group and adult group, four groups each contained 5 male rats. For biochemical methods use malondialdehyde (MDA), glutathione (GSH) and Superoxide dismutase (SOD) kits. We showed the (MDA) in albino male rats, an increase in levels was observed in the adult group during aging compared to the one-day-old group, which showed a lower level. The (GSH) and (SOD) a decrease in (GSH) and (SOD) levels was observed in the adult group during aging, but in the one-day-old group, a high level of (GSH) and (SOD) was observed.**

***Key word*--- Rat, Hippocampus, oxidative stress**

INTRODUCTION

Buried in medial region of the temporal lobe, the hippocampus is a component of the limbic lobe (1). A well-known curved Structure of grey matter in the brain, the hippocampus extends the whole length of the inferior horn floor in the lateral ventricle. It forms. the pes hippocampus by extending its anterior end. Due to its similarity to a sea horse in a coronal cut view, the structure is dubbed the hippocampus (2) Many developmental brain illnesses selectively affect the hippocampus formation, which has been shown to have a role in learning and memory. Developmental research on this brain area is critical for understanding neurodevelopmental disorders. (3) the region hippocampal formation is a multicomponent of the medial temporal lobe that is engaged in memory dealing out. (4) The short-term memory converts into long-term memory by the hippocampus and solves spatial memory, and recollects the past places experiences. It also plays a crucial role in behavior and emotions (5) the formation of hippocampus (formation hippocampi) includes indusium griseum, longitudinal striae, gyrus fasciolaris, hippocampus proper (cornu ammonis dentate gyrus and subiculum) and portion of the uncus (1–6) the formation of hippocampus has archipallial cortex and progresses alongside the inferomedial surface of the cerebral hemisphere; through development, it tracks the external border of the C-shaped choroidal fissure (7). The proper hippocampus Is subdivided beside its length rendering to the size and density of its major essential pyramidal cells into four distinct regions named Cornu Ammonis (CA1, CA2, CA3, and CA4) With the histochemical preparations, each of these regions consisted of five layers, stratum alveolus, stratum oriens, stratum pyramidale, stratum radiatum and stratum lacunosum-moleculare (8) the hippocampus proper the polymorphic (OL), pyramidal (PL), and molecular (ML)layers(9).

Behavioral studies have proposed that the abnormalities of development in the hippocampal creation after birth may contribute to neurodevelopmental disorders such as Down syndrome, autism, schizophrenia and epilepsy. Understanding the functional maturation and structural development of the hippocampal formation is necessary for insights gaining into both the specific developmental processes and information processing and that may be affected in neurodevelopmental disorders (10).

Glutathione (GSH) is the most plentiful non-protein thiol, and plays vital roles in the system of antioxidant defense and the keep of redox homeostasis in neurons (11). Glutathione (GSH) acting an significant role in protecting neurons against oxidative stress as a chief intracellular antioxidant (12)

Kerbala Journal of Veterinary Medical Sciences Issue (3), Volume (1), (2025)

Glutathione is an essential antioxidant in cells, it mechanism to preserve them from oxygen reactive(13). It encourages follicles and the development and growth of embryos. (14) and it also works on maintenance on the biological level of germ cells (15). Usually, brain tissues anti-oxidant enzymes, such as Superoxide dismutase (SOD) keep brain cells from oxidative damage produced by free radicals, therefore avoiding neurons damage of hippocampus (16). The terminal product of lipid peroxidation is the malondialdehyde (MDA), so, its concentration can reveal the degree of oxidative stress-induced injury in brain tissue (17). The aim of this study is to investigate the Oxidant (MDA)/ Antioxidants (GSH, SOD) changes with the postnatal development of the hippocampus in male rats of various ages.

MATERIALS AND METHODS

The twenty male albino rats were divided 4 groups: 1-day group, 1-month group,3-months group and adult group. Each of the experimental group contain 5 males rats.at the end of the study we measured level of (MDA, GSH, SOD) in the tissue of hippocampus. After taking the sample and placing it in Eppendorf tube, Triton dissolved in 750 microliters of phosphate buffered saline was added with good mixing and protease inhibitor was added. After that, the Eppendorf tube was placed in the Vortex device for 1 to 2 minutes for the strong mixing. After that, we added Triton dissolved in 250 microliters of phosphate buffered saline to become 1000 microliters. Then, we placed the samples again in the Vortex device for 1 to 2 minutes. After that, it was transferred to the centrifuge for 2 to 3 minutes. Then, the liquid was taken from the Eppendorf tube and the sample was placed in another Eppendorf tube. Determination of MDA, GSH, SOD levels. fluid Samples taken were determined by the method of Sandwich ELISA According to study (18).

STATISTICAL ANALYSIS

Graph Pad Prism 8.0 statistical software was used, t-test was used, and $P \leq 0.05$ was chosen as the criterion for significance. Data points were presented as mean ± standard deviation.

RESULT & DISCUSSION

The results of the current study showed a significant Changes in MDA appeared in the tissue of the hippocampus where A significant increase in the brain tissue MDA was observed with increasing age. The results revealed decrease in levels of (MDA) in one-day group and increase in levels from the group to one-month group of males rats, while non significance between one-month group, three-month group and the adult group. (figue1)

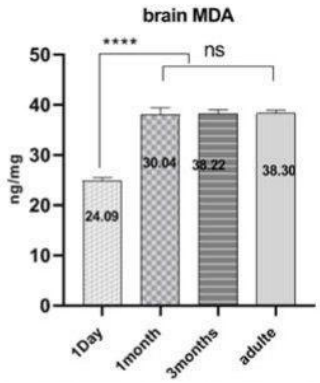

Figure 1. Brain MDA level in different development age rats.

The brain tissue level of GSH significantly decreased in level in hippocampus tissue with development age The results revealed an increase the levels of (GSH) in one-day group and while decrease slightly in levels in one-month age group, three age group and the adult group of male rats, and decrease significance differences in the level of GSH from one-day group to the adult group (figure.2).

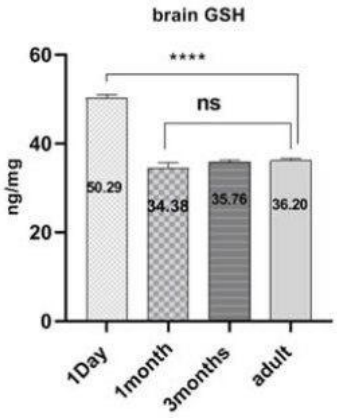

Figure 2. Brain GSH level in different development age rats

The activity of the brain tissue (SOD) in hippocampus tissue of male rats is decrease with aged. The results revealed increase the in one-day group and while decrease slightly in levels in one-month age group, three age group and adult group of male rats, and decrease significance differences in the level of SOD from one-day group to three group (figure.3).

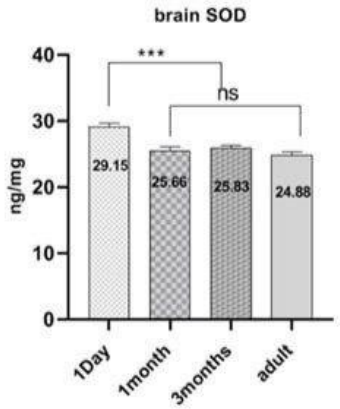

Figure 3. Brain SOD level in different development age rats.

The current study found significant changes in oxidant / Antioxidants levels hippocampal tissue, increasing in MDA activity with decreasing in antioxidant activity (GSH and SOD) with age. This results in agreement with (19,20,21) respectively. Mitochondria are essential for both health and the development of various diseases, as they are central to oxidative metabolism and the primary location for generating reactive oxygen species (ROS). The modification of cellular functions necessitates redox signaling, and various studies indicate that ROS plays a dual role in cell physiology. Elevated ROS levels beyond normal can cause significant harm to cell and organelle membranes, DNA, and proteins, leading to oxidative stress. Although moderate levels of ROS are essential for the upkeep of various biological processes, such as gene expression (22). Oxidative stress refers to a disparity between the generation and removal of free radicals due to pathological conditions and aging, as the generation of reactive oxygen species (ROS) rises (23,24), which is significant in the pathophysiology of diseases associated with aging. Aging is marked by a slow deterioration of the body's biological functions, which includes metabolic homeostasis (25) Crucial for sustaining the efficiency of life-supporting chemical reactions, metabolic homeostasis depends on a balance between anabolic and catabolic pathways, with the latter being vital for energy generation (26) The gradual rise in mitochondrial ROS generation throughout ageing results in mitochondrial harm and reduced lifespan, which may impair telomeres' repair capabilities, rendering them particularly vulnerable to oxidative damage (27, 28) Conversely, oxidative stress can arise from telomere shortening, linked to aging and a heightened risk of age-related illnesses (28,29) The brain is susceptible to oxidative damage due to its elevated metabolic activity and significant sensitivity to excessive ROS production and tissue harm (30) To safeguard brain tissue from the detrimental impact of ROS and preserve metabolic balance while neutralizing excess ROS, the brain utilizes antioxidants including glutathione, superoxide dismutase, catalase, and vitamins C and E as part of its overall tissue management (31) The primary endogenous antioxidant found in the brain is glutathione (32) As age progresses, GSH levels in the brain are recognized to change in both humans (33) and various animals (34) For instance, in aged mice, impairment of the antioxidant system takes place, linked to reduced mitochondrial GSH (35) and activities of superoxide dismutase in the neocortex (36) along with a reduced GSH/ glutathione disulfide (GSSG) ratio in the cortex, striatum, hippocampus, and cerebellum (37) As mentioned earlier, the authors propose that elevated GSH serves as an adaptive reaction to heightened oxidative stress (OS), and that the level of increase corresponds to the extent of tissue damage in the sensorimotor cortex (38) As time progresses and with higher oxidative stress, the processes that enhance GSH production can be overwhelmed, resulting in reduced GSH levels since GSH is utilized in oxidation and not produced adequately(39) This can initially disrupt enzyme function via reversible oxidation of thiol groups, but it may eventually cause a deeper change in the structure and integrity of biomolecules (40).

CONCLUSION

The study shows that oxidative stress significantly impacts the hippocampus's development and aging in albino male rats. Age-dependent increases in malondialdehyde levels indicate lipid peroxidation, while antioxidant markers decline with aging, highlighting the importance of maintaining hippocampal integrity and function.

REFERENCES

1) Tatu, L., & Vuillier, F. (2014). Structure and vascularization of the human hippocampus. *Frontiers of neurology and neuroscience*, *34*, 18-25.doi:10.1159/000356440 .
2) Moser, E. I., Roudi, Y., Witter, M. P., Kentros, C., Bonhoeffer, T., & Moser, M. B. (2014). Grid cells and cortical representation. *Nature Reviews Neuroscience*, *15*(7), 466-481.
3) Abdelrahim, E. A., & Eltony, S. A. (2011). Postnatal development of the hippocampal formation in male albino rats: histological, immunohistochemical, and morphometric studies. Egyptian Journal of Histology, 34(2), 346-364
4) Lavenex, P., Banta Lavenex, P., & Amaral, D. G. (2006). Postnatal development of the primate hippocampal formation. *Developmental neuroscience*, *29*(1-2), 179-192.
5) Chauhan, P., Jethwa, K., Rathawa, A., Chauhan, G., & Mehra, S. (2021). The anatomy of the hippocampus. Exon Publications, 17-30
6) Ten Donkelaar, H. J., Tzourio-Mazoyer, N., & Mai, J. K. (2018). Toward a common terminology for the gyri and sulci of the human cerebral cortex. *Frontiers in Neuroanatomy*, *12*, 93.doi:10.3389/fnana.2018.00093.
7) Dekeyzer, S., De Kock, I., Nikoubashman, O., Vanden Bossche, S., Van Eetvelde, R., De Groote, J., ... & Achten, E. (2017). "Unforgettable"–a pictorial essay on anatomy and pathology of the hippocampus. *Insights into imaging*, *8*(2), 199-212.doi:10.1007/s13244-016-0541-26
8) Mazher, K. M., & Hassan, R. M. (2021). Histological, histochemical, and immunohistochemical studies of

hippocampus in male New Zealand rabbits. The Anatomical Record, 304(2), 393-399.

9) Abdelrahim, E. A., & Eltony, S. A. (2011). Postnatal development of the hippocampal formation in male albino rats: histological, immunohistochemical, and morphometric studies. *Egyptian Journal of Histology*, *34*(2), 346-364.

10) Lavenex, P., Banta Lavenex, P., & Amaral, D. G. (2006). Postnatal development of the primate hippocampal formation. *Developmental neuroscience*, *29*(1-2), 179-192.

11) Aoyama, K. (2021). Glutathione in the Brain. International journal of molecular sciences, 22(9), 5010.

12) .Higashi, Y., Aratake, T., Shimizu, T., Shimizu, S., & Saito, M. (2021). Protective role of glutathione in the hippocampus after brain ischemia. International journal of molecular sciences, 22(15), 7765

13) Rani, V., Deep, G., Singh, R. K., Palle, K., & Yadav, U. C. (2016). Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life sciences*, *148*, 183-193.

14) Ashibe, S., Miyamoto, R., Kato, Y., & Nagao, Y. (2019). Detrimental effects of oxidative stress in bovine oocytes during intracytoplasmic sperm injection (ICSI). *Theriogenology*, *133*, 71-78.

15) Nishihara, T., Matsumoto, K., Hosoi, Y., & Morimoto, Y. (2018). Evaluation of antioxidant status and oxidative stress markers in follicular fluid for human in vitro fertilization outcome. *Reproductive medicine and biology*, *17*(4), 481-486.

16) Al-Amin, M. M., Akhter, S., Hasan, A. T., Alam, T., Nageeb Hasan, S. M., Saifullah, A. R. M., & Shohel, M. (2015). The antioxidant effect of astaxanthin is higher in young mice than aged: a region specific study on brain. *Metabolic brain disease*, *30*(5), 1237-1246.

17) Chen, J., Bi, Y., Chen, L., Zhang, Q., & Xu, L. (2018). Tanshinone IIA exerts neuroprotective effects on hippocampus-dependent cognitive impairments in diabetic rats by attenuating ER stress-induced apoptosis. Biomedicine & Pharmacotherapy, 104, 530-536

18) Zahra, K., Patel, S., Dey, T., Pandey, U., & Mishra, S. P. (2021). A study of oxidative stress in cervical cancer-an institutional study. Biochemistry and biophysics reports, 25, 100881.)

19) Leutner, S., Eckert, A., & Müller, W. E. (2001). ROS generation, lipid peroxidation and antioxidant enzyme activities in the aging brain. Journal of neural transmission, 108, 955-967

20) Venkateshappa, C., Harish, G., Mahadevan, A., Srinivas Bharath, M. M., & Shankar, S. K. (2012). Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer's disease. Neurochemical research, 37, 1601-1614.

21) Onodera, K., Omoi, N. O., Fukui, K., Hayasaka, T., Shinkai, T., Suzuki, S., ... & Urano, S. (2003). Oxidative damage of rat cerebral cortex and hippocampus, and changes in antioxidative defense systems caused by hyperoxia. Free Radical Research, 37(4), 367-372

22) Giorgi, C., Marchi, S., Simoes, I. C. M., et al (2018). Mitochondria and Reactive Oxygen Species in Aging and Age-Related Diseases. International review of cell and molecular biology, 340, 209–344. https://doi.org/10.1016/bs.ircmb.2018.05.006

23) Chakrabarti S, Munshi S, Banerjee K, Thakurta IG, Sinha M and Bagh MB (2011) Mitochondrial dysfunction during brain aging: role of oxidative stress and modulation by antioxidant supplementation. Aging Dis 2, 242–256.

24) Andr_es CMC, P_erez de la Lastra JM, Juan CA, Plou FJ and P_erez-Lebe~na E (2022) Chemistry of hydrogen peroxide formation and elimination in mammalian cells, and its role in various pathologies. Stress 2, 256–274.

25) MacDonald, M. E., & Pike, G. B. (2021). MRI of healthy brain aging: A review. *NMR in Biomedicine*, *34*(9), e4564. .https://doi.org/10.1002/nbm.4564.

26) Pflug, K. M., Lee, D. W., & Sitcheran, R. (2021). NF-κB-inducing kinase rewires metabolic homeostasis and promotes diet-induced obesity. *bioRxiv*. https://doi.org/10.1101/ 2021.08.26.457753.

27) Richter T and von Zglinicki T (2007) A continuous correlation between oxidative stress and telomere shortening in fibroblasts. Exp Gerontol 42, 1039–1042.

28) von Zglinicki T (2002) Oxidative stress shortens telomeres. Trends Biochem Sci 27, 339–344.

29) von Zglinicki T (2000) Role of oxidative stress in telomere length regulation and replicative senescence. Ann N Y Acad Sci 908, 99–110.

30) Grimm, A., & Eckert, A. (2017). Brain aging and neurodegeneration: from a mitochondrial point of view. *Journal of neurochemistry*, *143*(4), 418-431. https://doi.org/10.1111/jnc.14037.

31) Dwivedi, D., Megha, K., Mishra, R., & Mandal, P. K. (2020). Glutathione in brain: overview of its conformations, functions, biochemical characteristics, quantitation and potential therapeutic role in brain disorders. *Neurochemical research*, *45*(7), 1461-1480. https://doi.org/10.1007/s11064-020-03030-1.

32) Bottino, F., Lucignani, M., Napolitano, A., Dellepiane, F., Visconti, E., Rossi Espagnet, M. C., & Pasquini, L. (2021). In vivo brain GSH: MRS methods and clinical applications. *Antioxidants*, *10*(9), 1407.

33) Emir, U. E., Raatz, S., McPherson, S., Hodges, J. S., Torkelson, C., Tawfik, P., ... & Terpstra, M. (2011). Noninvasive quantification of ascorbate and

glutathione concentration in the elderly human brain. *NMR in Biomedicine*, *24*(7), 888-894.

34) Zhu, Y., Carvey, P. M., & Ling, Z. (2006). Age-related changes in glutathione and glutathione-related enzymes in rat brain. *Brain research*, *1090*(1), 35-44. https://doi.org/ 10.1016/j.brainres.2006.03.063

35) Shi, C., Xiao, S., Liu, J., Guo, K., Wu, F., Yew, D. T., & Xu, J. (2010). Ginkgo biloba extract EGb761 protects against aging-associated mitochondrial dysfunction in platelets and hippocampi of SAMP8 mice. *Platelets*, *21*(5), 373-379. https://doi.org/10.3109/ 09537100903511448.

36) Wang, J., Lei, H., Hou, J., & Liu, J. (2015). Involvement of oxidative stress in SAMP10 mice with age-related neurodegeneration. *Neurological Sciences*, *36*(5), 743-750. https://doi.org/ 10.1007/s10072-014-2029-5.

37) Rebrin, I., Forster, M. J., & Sohal, R. S. (2007). Effects of age and caloric intake on glutathione redox state in different brain regions of C57BL/6 and DBA/2 mice. *Brain research*, *1127*, 10-18.

38) Hupfeld, K. E., Hyatt, H. W., Alvarez Jerez, P., Mikkelsen, M., Hass, C. J., Edden, R. A. E., ... & Porges, E. C. (2021). In vivo brain glutathione is higher in older age and correlates with mobility. *Cerebral cortex*, *31*(10), 4576-4594. https://doi.org/ 10.1093/cercor/bhab107

39) Detcheverry, F., Senthil, S., Narayanan, S., & Badhwar, A. (2023). Changes in levels of the antioxidant glutathione in brain and blood across the age span of healthy adults: A systematic review. NeuroImage. Clinical, 40, 103503. https://doi.org/10.1016/j.nicl.2023.103503

40) Freitas, I., Boncompagni, E., Tarantola, E., Gruppi, C., Bertone, V., Ferrigno, A., ... & Vairetti, M. (2016). In situ evaluation of oxidative stress in rat fatty liver induced by a methionine-and choline-deficient diet. *Oxidative Medicine and Cellular Longevity*, *2016*(1), 9307064.