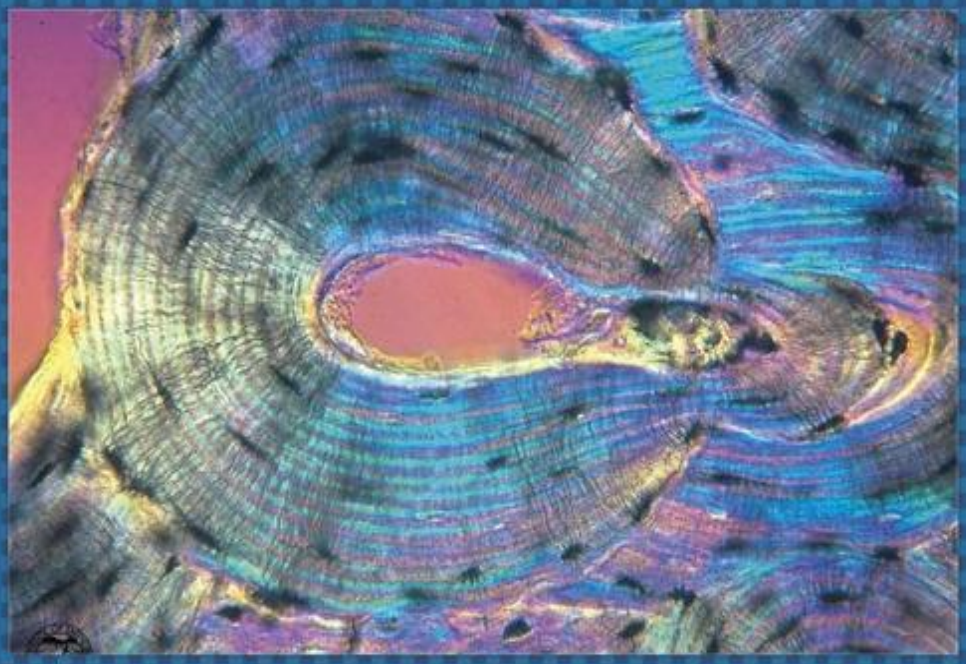
EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
D
HISTOLOGY & HISTOCHEMISTRY



ISSN
2090-0775

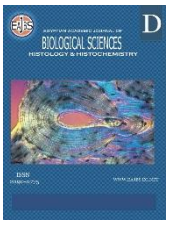
WWW.EAJBS.EG.NET

Vol. 17 No. 1 (2025)

**Citation:** Egypt. *Acad. J. Biolog. Sci. (D-Histology and histochemistry) Vol.17(1) pp59-72(2025)*
**DOI: 10.21608/EAJBSD.2025.423485**

Egypt. Acad. J. Biolog. Sci., 17(1):59-72 (2025)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg



# Evaluation of the Protective Role of Melatonin in The Hippocampus of Adult Male Albino Rat in Primary Adrenal Insufficiency Model: Biochemical, Histopathological and Immunohistochemical study

**Dina A. Badawi*; Reham I. Ali; Salwa E. Mustafa and Hagar A. Hashish**
Anatomy and Embryology Department, Faculty of Medicine, Mansoura University, Egypt
**E-mail** : drdinabadawi@gmail.com

**ARTICLE INFO**
**Article History**
Received:18/3/2025
Accepted:20/4/2025
Available:24/4/2025

***Keywords*:**
Melatonin, Primary adrenal insufficiency, Hippocampus, Neurodegenerative disease.

**ABSTRACT**

**Background:** Melatonin has anti-inflammatory, immunomodulatory and anti-apoptotic functions. Hippocampus and dentate gyrus are highly affected by changes in adrenal hormones. **Materials and Methods:** eighteen albino male rats were divided into three groups. *Control group:* six rats' adrenal glands were exposed without manipulation. *Adrenalectomized group (AD):* six rats were adrenalectomized surgically. *Adrenalectomized with hormone replacement plus melatonin (AD+TTT+M):* six rats were adrenalectomized and received Fludrocortisone plus Prednisone as hormone replacement and melatonin. All drugs were given orally from first day after adrenalectomy till 4 weeks. After 4 weeks rats were anaesthetized to be sacrificed then their brains were dissected. One hippocampus was homogenized for malondialdehyde quantitative analysis. Other hippocampus was used for histopathology. **Results:** The cresyl-stained sections of AD group showed disruption and reduction in thickness of CA1, CA3 and dorsal blade of DG, but AD+TTT+M group showed nearly normal thickness with preserved architecture of same regions. Higher magnification of CA1, CA3 and dorsal blade of DG of AD group in synaptophysin stain showed negative immune reaction but AD+TTT+M group showed synaptophysin positive granules across all layers. MDA levels were elevated in AD group, but they were back to normal levels in AD+TTT+M group. Brain weight was reduced in AD group, but it was nearly normal in AD+TTT+M group. Area percentage of Synaptophysin was reduced in all layers in AD group but elevated in AD+TTT+M group. **Conclusion:** adrenal hormones are important for survival of hippocampus neurons and melatonin administration in primary adrenal insufficiency to hormonal supplement may add a neuroprotective effect.

## INTRODUCTION

Adrenal gland hormones have a strong influence on the hippocampus which is rich in both mineralocorticoid and glucocorticoid receptors. Glucocorticoid hormones have an important role in brain development, mood and cognitive functions (de Kloet, 2024). Therefore primary adrenal insufficiency can cause many symptoms including depression, memory loss and other cognitive disfunctions (White *et al.*, 2023).

Hippocampus, which is part of limbic system, helps with various processes related to cognition, various types of memory, learning, motivation, emotional and social processing (Pradip Chauhan *et al.*, 2021).

Melatonin hormone is secreted by the pineal gland. Melatonin receptors are extensively distributed in the nervous system, including the hippocampus. It acts as an endogenous free radical scavenger and commonly as an antioxidant (Feng *et al.,* 2023). Melatonin also inhibits oxidative stress and it was proved in many reports as a neuroprotective and anti-inflammatory agent (Canonico *et al.,* 2024).

This study was designed to investigate the effect of experimentally induced primary adrenal insufficiency on the histological structure of the hippocampus in the adult male albino rat and to evaluate the potential neuroprotective effect of melatonin on hippocampus.

## MATERIALS AND METHODS

**Experimental Animals:**

Eighteen adult male albino rats, weighting 200-250 gm were purchased from Mansoura Experimental Research Centre, Faculty of Medicine, Mansoura University, Egypt. Male rats were preferred in our study because they can handle surgery. They were housed in stainless steel mesh cages with soft wood chips for bedding under controlled condition. The use of animals was after the approval of Mansoura Faculty of Medicine ethical committee and Institutional Research Board (IRB) (code number: MED.MS.23.06.15).

**Experimental Design:**

After two weeks of acclimatization, rats were divided randomly into three groups (Six rats in each group).

**First Group (control) or (sham):** adrenal glands of rats were surgically exposed (skin was incised and then sutured) without manipulation of the glands (sham operation)**. Second Group (adrenalectomized) (AD):** Six rats were exposed to experimental adrenal insufficiency by surgical removal of both adrenal glands under clean aseptic conditions. Rats were anesthetized using intraperitoneal xylazine (15 mg/kg), then hair was shaved from the rat's abdomen and one longitudinal skin incision was done in the midline of abdominal wall**.** The skin was then laterally retracted and both adrenal glands were gently exposed, and vessels were ligated using a silk thread and glands were excised **(**Ibrahim *et al,* 2015).

**Third Group (Adrenalectomized with hormone replacement plus melatonin) (AD+TTT+M):** six rats were adrenalectomized and received Fludrocortisone supplement dose (0.01-0.02 mg/kg/day) plus Prednisone supplement dose (0.5 mg/kg/day) (Lathan, 2018) as hormone replacement. Melatonin was given at a dose of 15 mg/kg (Lee *et al*., 2008). All these drugs were given orally through Oro-gastric tube from the first day after surgery and till the end of the experiment (4 weeks) Hamadi, *et al.,* 2022).

**Histological Analysis:**

At the end of the experiment rats were deeply anaesthetized using intraperitoneal xylazine (15 mg/kg) and ketamine (90 mg/kg) to be sacrificed then their brains were dissected and weighed. The hippocampus was removed and fixed in Bouin's solution then processed for preparation of Cresyl violet stain and Synaptophysin. In synaptophysin staining, some sections were incubated with active Synaptophysin rabbit polyclonal antibody (A6344) at 1:200 dilution and left throughout night at $4^{o}$ c, then sections were incubated with biotinylated goat antipolyvent for 30 mins after washing for three times in PBS for 5 mins (Abdelzaher *et al.,* 2021).

**Biochemical Analysis:**

Portions of hippocampus were homogenized (10% w/v) in ice-cold 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 10 min at 4°C. Malondialdehyde (MDA) marker was detected in the resulting supernatant of hippocampal homogenate in the appropriate kits (Biodiagnostic kits, Giza, Egypt) (Liu *et al.,* (2020).

**Quantitative Analysis:**

Sections were examined using Olympus R digital camera to assess average thickness of the pyramidal layer in CA1 & CA3 and granular layers in dorsal blade of dentate gyrus. Area percentage of Synaptophysin positive regions were measured in five fixed non-overlapping fields. The measurements were done by Image J.

**Statistical Analysis:**

Statistical analysis was carried out through SPSS program version 22, one-way. ANOVA was used to compare between more than two parametric data groups then for multiple comparisons by post-hoc tukey test. $P<0.05$ was considered as significant.

## RESULTS

**Histological Results:**

**1-Cresyl Violet-Stained Sections:**

The hippocampal coronal sections of the control group showed normal C-shaped hippocampus proper which was divided into four areas; CA1, CA2, CA3 and CA4 while the V-shaped dentate gyrus enfolded around CA4. Pyramidal layer of CA1 and CA3 of control group is formed of 3 rows of pyramidal cells. The dentate gyrus had granule layer which was formed of 4–6 rows of granule cells (Fig.1). The adrenalectomized group showed an obvious disruption and reduction in thickness of CA1, CA3 and dorsal blade of DG areas with narrowing of hippocampal sulcus (Fig.2). The coronal section of rat hippocampi in (AD+TTT+M) group showed preserved architecture of CA1, CA3 and dorsal blade of DG regions with normal hippocampal sulcus (Fig.3).

**2-Synaptophysin Stained Sections:**

Higher magnification of CA1, CA3 and dorsal blade of DG regions in the control group with synaptophysin staining revealed that each area had widespread dense positive immune reaction of presynaptic terminals which was seen in the form of coarse beaded granules scattered through the pyramidal and granule cells (Figs.4,5,6). In adrenalectomized group, pyramidal cells of CA1, CA3 and granule cell bodies of the dorsal blade of DG showed negative immune reaction and absence of synaptophysin positive granules reflecting synaptic loss or damage (Figs. 4,5,6). (AD+TTT+M) group showed synaptophysin positive granules across all hippocampal layers indicating a widespread restoration of presynaptic terminals (Figs.4,5,6).

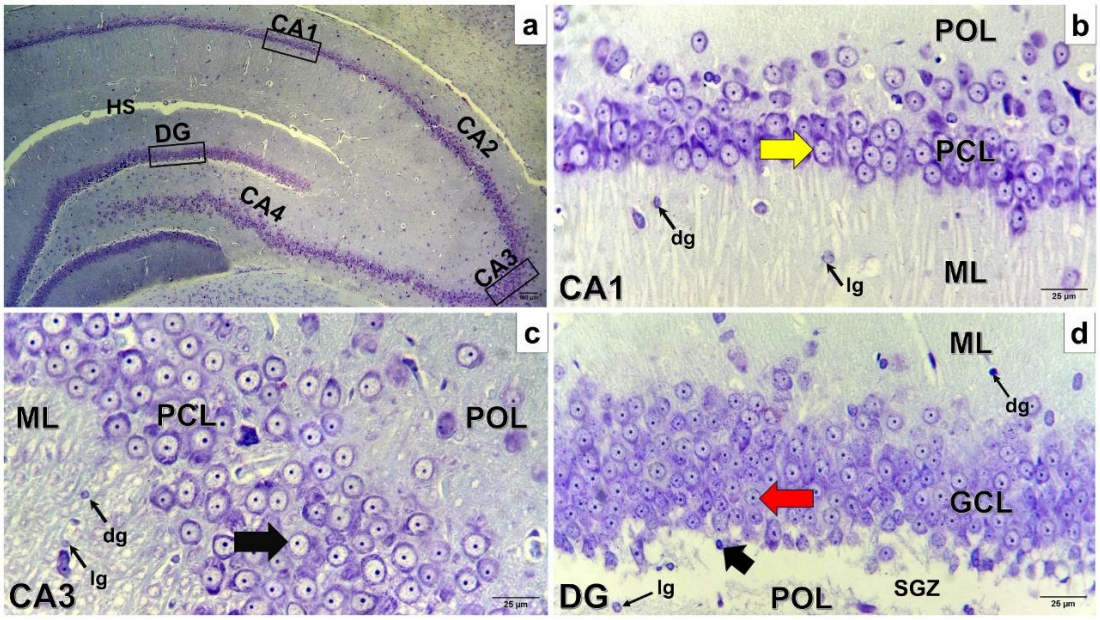



**Fig.1:** a) A photomicrograph of coronal sections in control rat hippocampus shows normal 4 parts of Cornu Ammonis CA1, CA2, CA3 and CA4 and it is separated from dentate gyrus (DG) by hippocampal sulcus (HS) (Cresyl violet X40). b, c) Higher magnification of the squared area of CA1 and CA3 regions shows well-defined three layers; polymorphic layer (POL), pyramidal cell layer (PCL) and molecular layer (ML). PCL, which is formed of closely packed pyramidal neurons in 3-4 rows, appears with vesicular nuclei, prominent nucleoli, and scanty cytoplasm (yellow& black arrows). The POL and ML displays deeply (dg- small black arrow) and lightly stained glial cells (lg- small black arrow). d) A higher magnification of the squared area of the dorsal blade dentate gyrus (DG) shows also well-defined three layers; ML, granule cell layer (GCL) and POL. The GCL reveals aggregation of rounded to oval granule cell bodies (red arrow). (Cresyl violet X400).

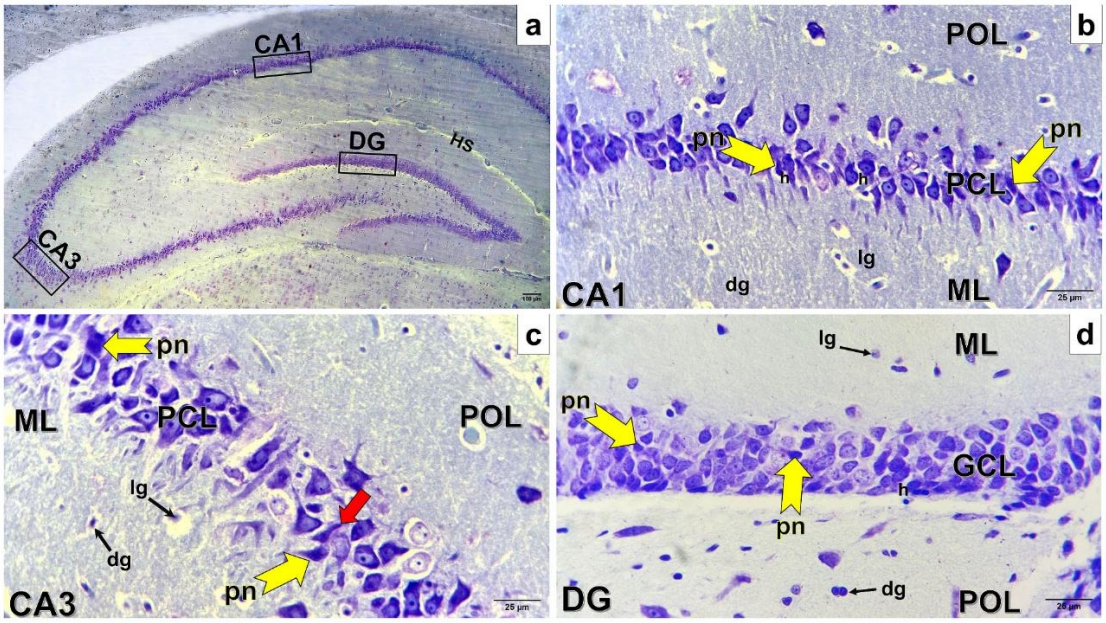



**Fig.2:** a) A photomicrograph of coronal sections in AD rat hippocampus shows obvious narrowing in hippocampal sulcus (HS) (Cresyl violet X40). b, c) Higher magnification of the squared area of CA1 and CA3 regions reveals that pyramidal cells of PCL are disarranged and loosely packed, they appear dark, shrunken and having pyknotic nuclei (pn) with pericellular haloes (h) (yellow arrows). Lightly and deeply stained glial cells nuclei can be noticed in POL and ML (lg and dg- small black arrow). d) A higher magnification of the squared area of the dorsal blade DG shows dark shrunken granule cell bodies having pyknotic nuclei (pn) with pericellular haloes (h) (yellow arrows) (Cresyl violet X400).

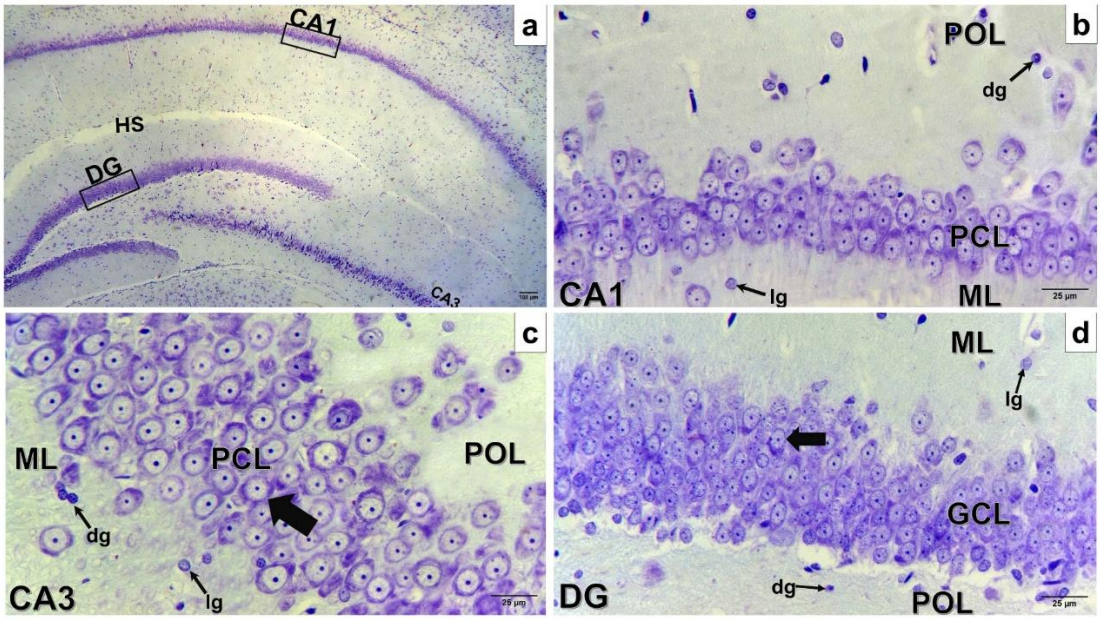



**Fig.3:** a) A photomicrograph of coronal sections in AD plus hormone and melatonin treated rat hippocampus group demonstrates preserved architecture of CA1, CA3 and DG with normal hippocampal sulcus (Cresyl violet X40). b, c) Higher magnification of the squared area of CA1 and CA3 regions shows regularly arranged pyramidal cell bodies in PCL. POL and ML display both deeply and lightly stained nuclei of glial cells (lg and dg- small black arrow). d) A higher magnification of the squared area of the DG shows some apparently normal granule cell bodies (black arrow) (Cresyl violet X400).

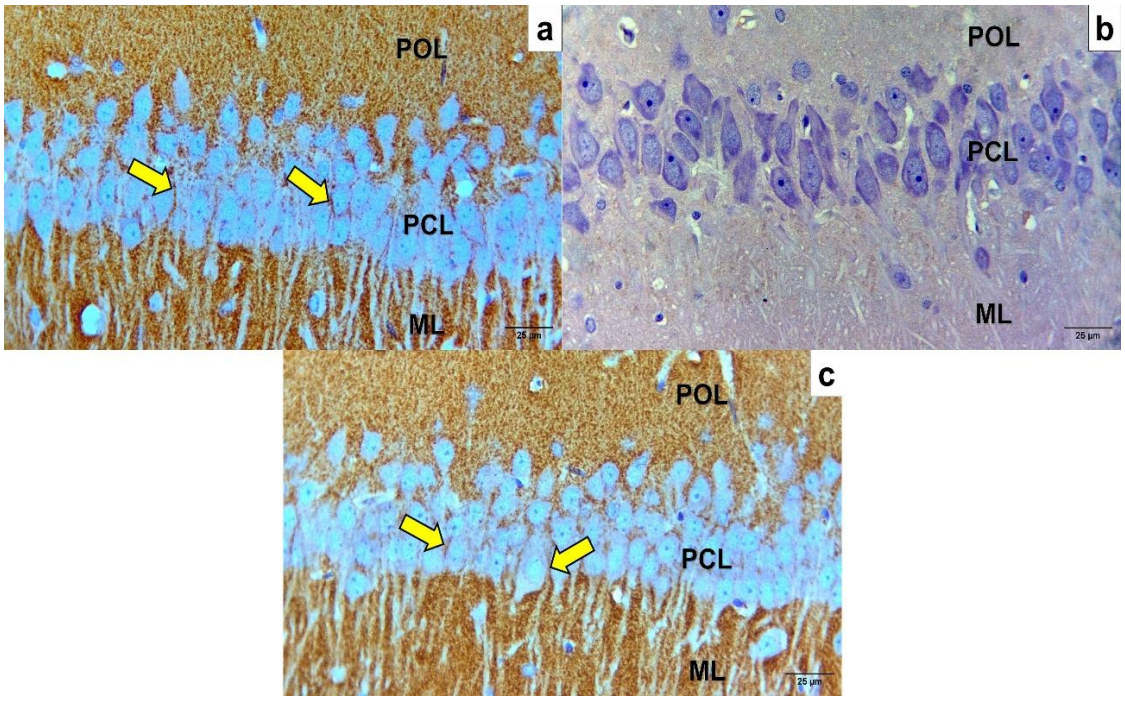



**Fig.4**: **a, c)** A photomicrograph of coronal section of rat hippocampus CA1 in control (a) and melatonin groups (c). The synaptophysin staining highlights the presynaptic terminals that appear as small, punctate dots and clusters which are distributed all over the area reflecting intact synaptic network (yellow arrows). **b)** A photomicrograph of coronal section of AD rat hippocampus CA1 demonstrates negative synaptophysin stain (synaptophysin immune-stain X400).

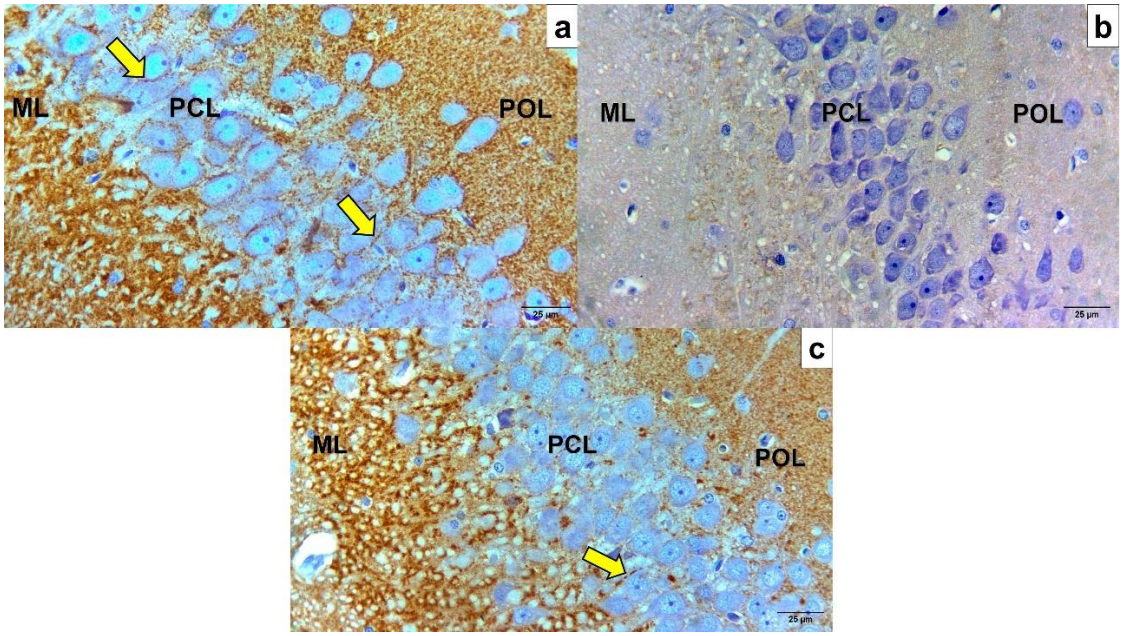



**Fig.5**: **a, c)** A photomicrograph of coronal section of rat hippocampus CA3 in control (a) and melatonin groups (c). There are small dots and clusters which are distributed all over the area reflecting intact synaptic network (yellow arrows). **b)** A photomicrograph of coronal section of AD rat hippocampus CA1 demonstrates negative synaptophysin stain (synaptophysin immune-stain X400).

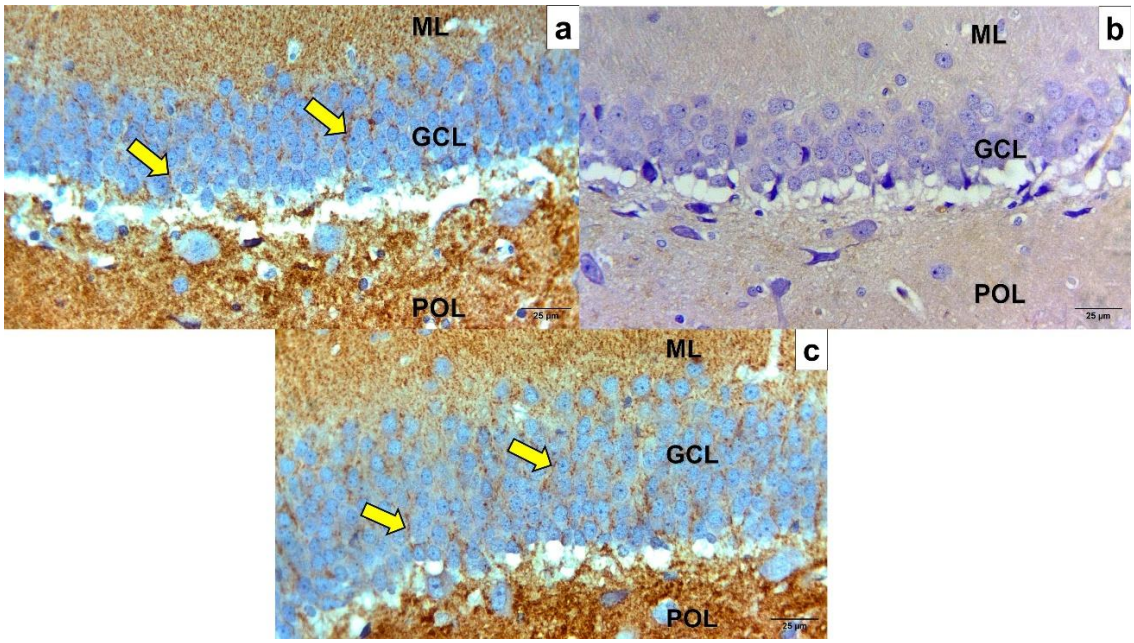


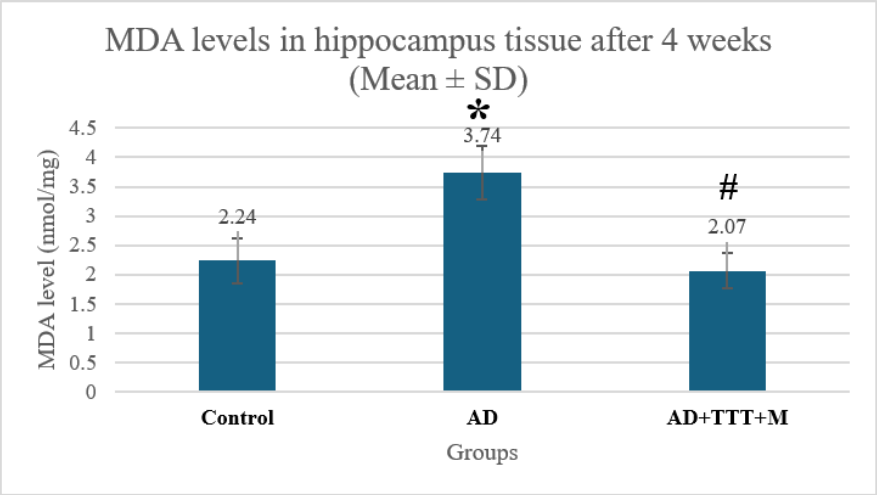

**Fig.6**: **a, c)** A photomicrograph of coronal section of dorsal blade of DG in control (a) and melatonin groups (c). The synaptophysin staining demonstrates the presynaptic terminals that appear as small, punctate dots and clusters which are distributed all over the area reflecting intact synaptic network (yellow arrows). **b)** A photomicrograph of coronal section of AD rat hippocampus DG shows negative synaptophysin stain (synaptophysin immune-stain X400).

**Biochemical Results:**

**1. Oxidative stress markers levels:**

***Serum malondialdehyde (MDA) levels*:**

There was significant elevation in the levels of MDA in adrenalectomized group (3.74 ± 0.45 nmol/mg) when compared to the control group (2.24 ± 0.39 nmol/mg), but there was significant reduction in their levels in (AD+TTT+M) group (2.07± 0.31 nmol/mg) when compared to AD group (P < 0.001) (Histogram.1).




**Histogram.1:** shows the mean MDA levels in hippocampus tissue of different groups 4 weeks after the surgery.

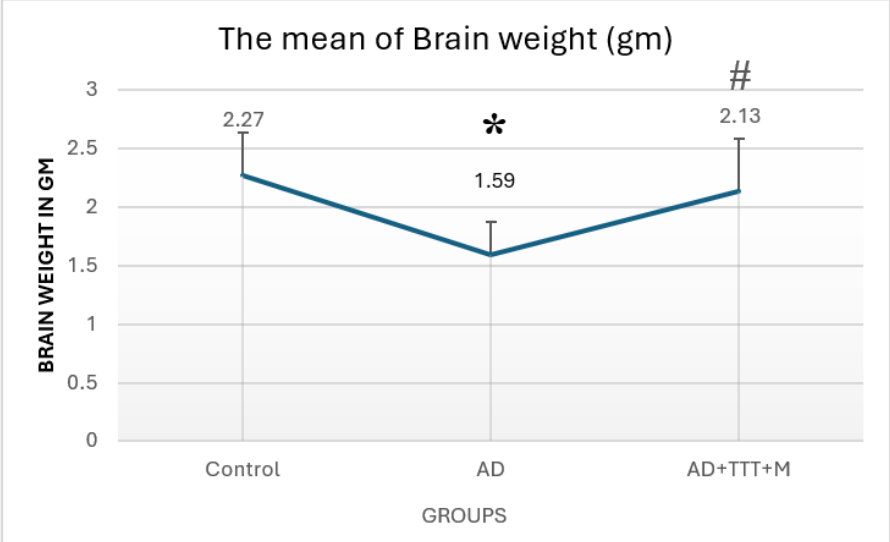
* Significant difference as compared with the control group.

# Significant difference as compared with AD group.

**Morphometric Results:**

**1. Brain Weight Of Different Groups:**

The Adrenalectomized (AD) group showed significant decrease in brain weight (1.59 gm±0.28) in comparison to control group (2.26 gm±0.37), but (AD+TTT+M) showed significant increase in brain weight (2.13 gm±0.45) in comparison to AD group (P < 0.001) (Histogram 2).




**Histogram 2:** Shows the mean of brain weight (gm) of different groups after 4 weeks.

* Significant difference as compared with the control group.

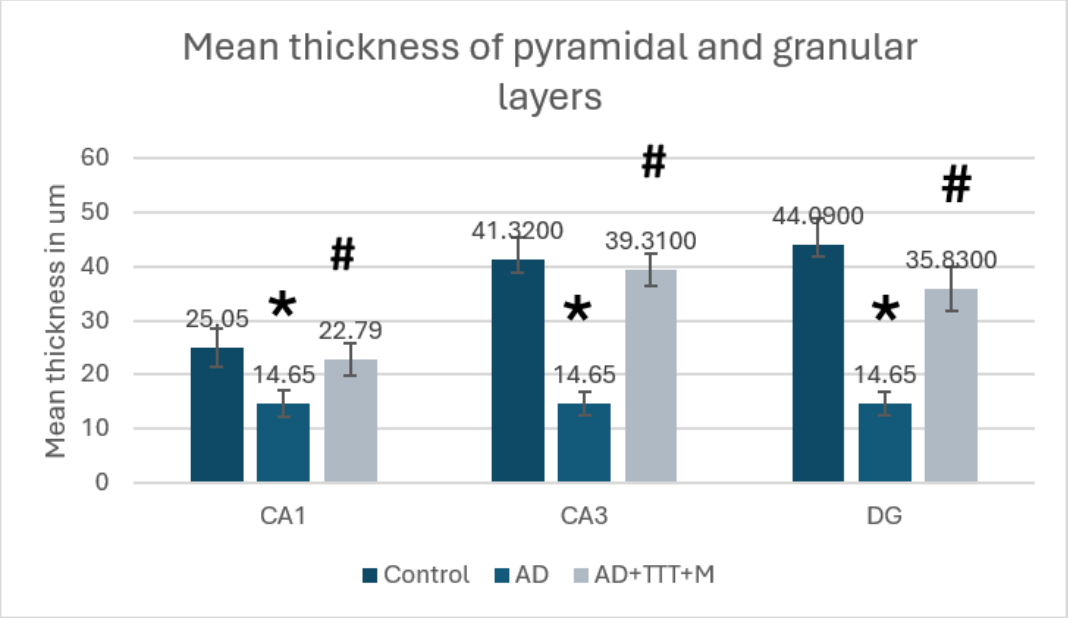
# Significant difference as compared with AD group.

**2. The Thickness of the Pyramidal Layer (CA1) and (CA3) of Hippocampus Proper:**

There was a significant reduction in thickness of the pyramidal layer CA1 (14.654 µm ± 2.466) and CA3(22.305 µm ± 2.237) of the AD group in comparison to control group CA1(25.053 µm ± 3.516) and CA3 (41.323 µm ± 4.172). There was an elevation in thickness of CA1(22.797 µm ± 2.993) and CA3(39.311 µm ± 2.983) of (AD+TTT+M) group in comparison to AD group (P < 0.001) (Histogram 3).

**3. The thickness of the Granule Layer of Dentate Gyrus:**

There was a reduction in the granule cells layer thickness of the adrenalectomized group (19.146 µm ± 2.113) in comparison to control group (44.096 µm ± 4.721). There was an elevation in thickness of granule cells layer of (AD+TTT+M) group (35.834 µm ± 4.153) in comparison to AD group (p<0.001) (Histogram 3).



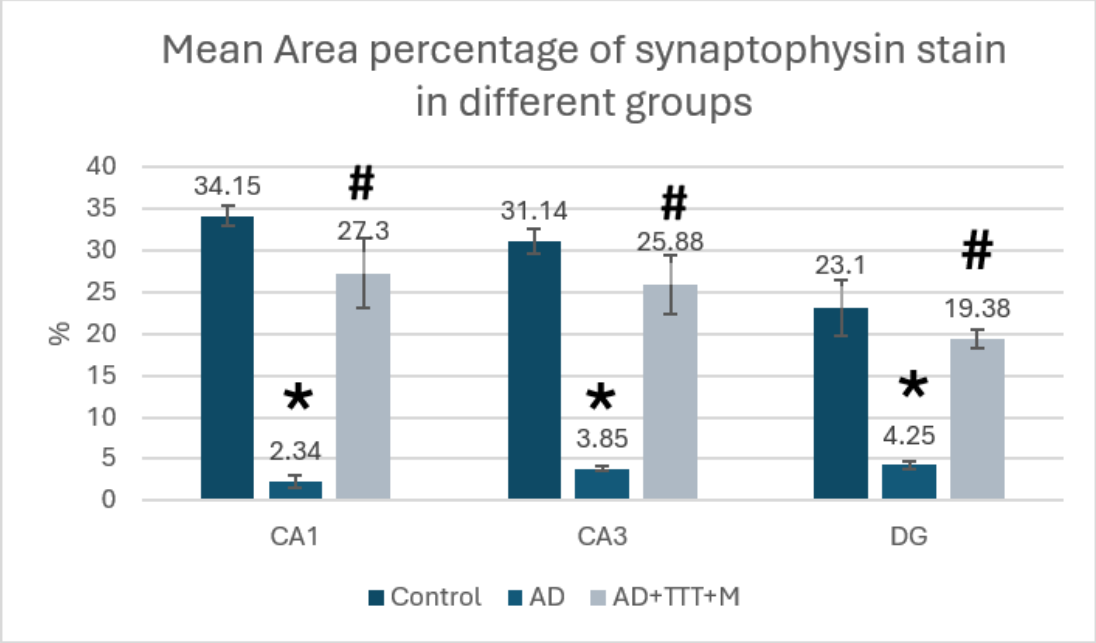

**Histogram 3:** Shows the mean thickness of pyramidal and granular layers in different groups.

* Significant difference as compared with the control group.

# Significant difference as compared with AD group.

**4. Area Percentage of Synaptophysin Immunohistochemical Staining:**

There was highly significant reduction in the synaptophysin immune reaction in CA1 (2.34 % ± 0.714), CA3 (3.86 % ± 0.282) and DG (4.26 % ± 0.383) in adrenalectomized group in comparison to control group CA1 (34.16 % ± 1.212), CA3 (31.14% ± 1.526) and DG (23.10 % ± 3.35). There was highly significant elevation in the synaptophysin immune reaction in all layers of (AD+TTT+M) group: CA1 (27.31 % ± 4.155), CA3 (25.89 % ± 3.517) and DG (19.38 % ± 1.062) in comparison to AD group (p<0.001) (Histogram 4).




**Histogram 4:** Showing the mean area percentage of synaptophysin immune reaction in different groups.

* Significant difference as compared with the control group.

# Significant difference as compared with AD group.

## DISCUSSION

Primary Adrenal insufficiency leads to neuronal cells apoptosis, especially throughout the hippocampus and dentate gyrus**.** Neurodegeneration that occurs in the hippocampus leads to an increase in oxidative stress, mitochondrial dysfunction and metabolic changes (Knezevic *et al.,* 2023).

Melatonin has anti-inflammatory, immunomodulatory, anti- apoptotic functions, as well as neuroprotective effects (Shin, 2023).

In the present study, the levels of malondialdehyde (MDA) in hippocampus tissue of adrenalectomized group were elevated in 4 weeks. Abi Issa (2017) correlated the elevated MDA levels in case of adrenalectomy for 2 weeks due to peroxidation of polyunsaturated fatty acids.

Levels of MDA were back to approximate normal levels Adrenalectomized with hormone replacement plus melatonin (AD+TTT+M) group. Monteiro *et al.*, (2024) reported that melatonin decreases oxidative damage by increasing antioxidant enzymes and controlling lipid peroxidation.

In the current study, the cresyl-stained coronal sections of AD groups hippocampi showed that CA1, CA3 and dorsal blade of dentate gyrus are the first to be affected. Abi Issa (2017) also reported disarrangement, shrinking and apoptosis of granule cells in dorsal blade of dentate gyrus in adrenalectomy model for 2 weeks. Higher magnification of CA1, CA3 and dorsal blade of DG in AD group revealed that most of the pyramidal cell and granule cell bodies were loosely packed, disarranged, appeared dark, shrunken and having pyknotic nuclei. These findings came in agreement with other studies like Mandour *et al.* (2021) who noticed same changes in CA1 and CA3 in Alzheimer disease in albino rats in 37 days, which is a neurodegenerative condition as primary adrenal insufficiency. He explained these changes due to elevation of acetylcholinesterase enzyme levels in Alzheimer disease which led to central acetylcholine transmitter break down and form a highly neurotoxin that causes more neurodegeneration.

In the current study, adrenalectomized with hormone replacement plus melatonin (AD+TTT+M) group showed mild histopathological changes. Rat hippocampi had preserved architecture and normal cells of CA1, CA3 and dorsal blade of DG regions. These findings were explained by Shin. (2023) who reported that melatonin is a powerful antioxidant in Alzheimer and other neurodegenerative diseases. Melatonin reduced reactive oxygen species production and regulated the level of mRNA encoding in some antioxidant enzymes. It improved hippocampal synaptic growth and preserved the structure of neuronal and glial cells.

In the current study, CA1, CA3 and DG regions of hippocampus in the control group with synaptophysin staining revealed widespread dense positive immune reaction of presynaptic terminals which was seen in the form of coarse beaded granules scattered through the pyramidal cells, granule cells and their apical dendrites. These findings were in agreement with Mandour *et al.* (2021) who described positive synaptophysin coarse granules seen through the pyramidal cells in different layers of hippocampus and prefrontal cortex in the control group of rat model of Alzheimer like-disease.

Pyramidal cell of CA1, CA3 and granule cell bodies of the dorsal blade of DG in AD group showed negative immune reaction with absence of synaptophysin positive granules reflecting synaptic loss or damage. This comes in agreement with the results of Chen *et al.*, (2017), who described the same findings in Alzheimer hippocampus of rat model which resulted from inhibited protein biosynthesis. He confirmed that reduction of synaptophysin expression in the hippocampus is a major finding in systemic neurodegenerative disease.

In Adrenalectomized with hormone replacement plus melatonin (AD+TTT+M) group, CA1, CA3 and dorsal blade of DG presented with a widespread reappearance of synaptophysin positive granules. This distribution indicates a widespread restoration of presynaptic terminals. Sayed *et al.,* (2025) explained melatonin's neuroprotective effect on the cerebellar cortex of adult male albino rats following neurodegenerative effect of monosodium glutamate. He reported that melatonin reduces oxidative stress especially MDA levels which affect synaptophysin expression in tissue.

In our current study, Adrenalectomized (AD) group showed significant decrease in brain weight, but adrenalectomized with hormone replacement plus melatonin (AD+TTT+M) group showed significant increase in brain weight as compared to AD group. This came in agreement with Feng *et al.* (2019) who observed reduction in brain weight in neurodegenerative disease that happens with lead exposure and referred it to high oxidative stress factors that lead to apoptosis and decrease neuronal densities. Tabatabaei-Jafari *et al.* (2020) also illustrated brain weight reduction in Alzheimer model, which is a neurodegenerative condition, due to central neuronal degeneration, cell death and shrinkage of several brain areas including hippocampus, cerebral and entorhinal cortices.

In the current study, we observed a highly significant reduction in thickness of the CA1, CA3 and dorsal blade of DG of the adrenalectomized group and significant increase in thickness of CA1, CA3 and dorsal blade of DG of the adrenalectomized with hormone replacement plus melatonin (AD+TTT+M) group. Mandour ***et al.*** (2021) observed the same findings in hippocampus and prefrontal cortex in a rat model of Alzheimer like-disease. He referred the reasons of hippocampal atrophy in Alzheimer disease to the activated microglia that release pro-inflammatory cytokines. They induce a central inflammatory process which in sequence promotes neurodegeneration of the neurons.

According to our statical analysis, there was a highly significant reduction in area percentage of Synaptophysin in all layers (CA1/CA3 and DG) in adrenalectomized groups. There was a highly significant elevation in the area percentage of Synaptophysin immunohistochemical staining in all layers (CA1/CA3 and DG) of Adrenalectomized with hormone replacement plus melatonin (AD+TTT+M) group. These findings come in agreement with El-Adli *et al*, (2023) who noticed same changes in area percentage of synaptophysin in diabetes model for 8 weeks.

**Conclusion**

The current study concluded that the primary adrenal insufficiency had a harmful effect on the cellular structure of the hippocampus of the adult albino rats. Also, reduction of synaptophysin expression in CA1, CA3 and dorsal blade of DG which might be correlated to memory loss and cognitive decline. Melatonin hormone had an ameliorating effect on the harmful effects of adrenal hormone reduction. Melatonin protected the hippocampal cells from the effects of the primary adrenal insufficiency to a great extent so that the hippocampus returned near to normal cellular structure.

**Declarations:**

**Ethics Approval:** Institutional Research Board (IRB) approval code is (code number: MED.MS.23.06.15). Faculty of Medicine, Mansoura University and followed the NIH guidelines for the Care and Use of Laboratory Animals.

**Conflict of Interest:** The authors declare no conflict of interest.

**Author contribution:** Dina Badawi contributed to data interpretation and preparation of the manuscript. Hagar Hashis contributed to study design, execution and data interpretation. Reham Ali and Salwa Mustafa contributed to the revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

**Data Availability Statement:** Data is contained within the article.

**Funding Information:** This research received no external funding.

**Acknowledgment:** We acknowledge the support and the help rendered by Mansoura faculty of medicine where the study was carried out. We also thank vet surgeons who helped in surgery and collecting samples.

**REFERENCE**

Abi Issa, A. S. (2017). Short-Term Bilateral Adrenalectomy: Biochemical and Morphological Alterations in the Rat Hippocampus. Theses. https://scholarworks.uaeu.ac.ae/all_theses/610

Abdelzaher, L. A., Hussein, O. A., & Ashry, I. E. M. (2021). The Novel Potential Therapeutic Utility of Montelukast in Alleviating Autistic Behavior Induced by Early Postnatal Administration of Thimerosal in Mice. *Cellular and Molecular Neurobiology*, 41(1), 129–150. https://doi.org/10.1007/s10571-020-00841-2

Canonico, B., Carloni, S., Montanari, M., Ambrogini, P., Papa, S., Alonso-Alconada, D., & Balduini, W. (2024). Melatonin Modulates Cell Cycle Dynamics and Promotes Hippocampal Cell Proliferation After Ischemic Injury in Neonatal Rats. *Molecular Neurobiology*, 61(9), 6910–6919.

Chen, Y.-Y., Zhang, L., Shi, D.-L., Song, X.-H., Shen, Y.-L., Zheng, M.-Z., & Wang, L.-L. (2017). Resveratrol Attenuates Subacute Systemic Inflammation-Induced Spatial Memory Impairment via Inhibition of Astrocyte Activation and Enhancement of Synaptophysin Expression in the Hippocampus. *Annals of Clinical & Laboratory Science*, 47(1), 17–24.

de Kloet, E. R. (2024). Coping with the multifaceted and

multifunctional role of cortisol in the brain. Neuroscience Applied, 3, 104047. https://doi.org/10.1016/j.nsa.2024.104047

El-Adli, D., Gawish, S. A., AbdElFattah, A. A. M., & Soliman, M. Fm. (2023). The effect of experimentally- induced diabetes on rat hippocampus and the potential neuroprotective effect of Cerebrolysin combined with insulin. A histological and immunohistochemical study. *Egyptian Journal of Basic and Applied Sciences*, 10(1), 255–273.

Feng, C., Liu, S., Zhou, F., Gao, Y., Li, Y., Du, G., Chen, Y., Jiao, H., Feng, J., Zhang, Y., Bo, D., Li, Z., & Fan, G. (2019). Oxidative stress in the neurodegenerative brain following lifetime exposure to lead in rats: Changes in lifespan profiles. *Toxicology*, *411*, 101–109. doi.org/10.1016/j.tox.2018.11.003

Feng, Y., Jiang, X., Liu, W., & Lu, H. (2023). The location, physiology, pathology of hippocampus Melatonin MT2 receptor and MT2-selective modulators. European Journal of Medicinal Chemistry, 262, 115888. https://doi.org/10.1016/j.ejmech.2023.115888

Hamadi, N., Deniz, Ö. G., Issa, A. S. A., Islam, A. U. S., Amir, N., Minhas, S. T., Madjid, N., Khelifi-Touhami, F., Kaplan, S., & Adem, A. (2023). Stereological Evidence of Non-Selective Hippocampal Neurodegeneration, IGF-1 Depletion, and Behavioral Deficit following Short Term Bilateral Adrenalectomy in Wistar Rats. Biomolecules, 13(1), Article 1. doi.org/10.3390/biom13010022

Ibrahim, I. Y., Abdel-Hakim, S. M., Nazmy, W. H., Saad, A. H., & Ali, F. F. (2015). Effect of unilateral adrenalectomy on acute immobilization stress response in rats. Endocr. Regul, 49, 206-216.

Knezevic, E., Nenic, K., Milanovic, V., & Knezevic, N. N. (2023). The Role of Cortisol in Chronic Stress, Neurodegenerative Diseases, and Psychological Disorders. *Cells*, *12*(23), 2726.

Lathan P, Thompson AL. (2018) Management of hypoadrenocorticism (Addison's disease) in dogs. ***Veterinary Medicine*** (Auckl). 9;9:1-10. doi: 10.2147/VMRR.S125617. PMID: 30050862; PMCID: PMC 6055912.

Lee, S. *et al.* (2008). The antioxidant effects of melatonin in surgical brain injury in rats. In: Steiger, H.J. (eds) Acta Neurochirurgica Supplements. *Acta Neurochirurgica Supplementum*, vol 102. Springer, Vienna.

Liu, Y. T., Chen, H. W., Lii, C. K., Jhuang, J. H., Huang, C. S., Li, M. L., & Yao, H. T. (2020). A Diterpenoid, 14-Deoxy-11, 12-Didehydroandrographolide, in Andrographis paniculata Reduces Steatohepatitis and Liver Injury in Mice Fed a High-Fat and High-Cholesterol Diet. *Nutrients*, 12(2), 523.

Mandour, D. A., Bendary, M. A., & Alsemeh, A. E. (2021). Histological and imunohistochemical alterations of hippocampus and prefrontal cortex in a rat model of Alzheimer like-disease with a preferential role of the flavonoid “hesperidin”. *Journal of Molecular Histology*, *52*(5), 1043–1065.

Monteiro, K. K. A. C., Shiroma, M. E., Damous, L. L., Simões, M. de J., Simões, R. dos S., Cipolla-Neto, J., Baracat, E. C., & Soares-Jr, J. M. (2024). Antioxidant Actions of

Melatonin: A Systematic Review of Animal Studies. *Antioxidants*, *13*(4), 439.

Pradip Chauhan, M., Kinjal Jethwa, M., Ashish Rathawa, M., Girish Chauhan, B. D. S., & Simmi Mehra, M. (2021). The Anatomy of the Hippocampus. *Exon Publications*, 17–30.

Sayed, H., Mehdar, K., Alqahtani, S., Haredy, H., Elshafie, S., & Shediwah, A. (2025). Evaluating melatonin's neuroprotective effect on the cerebellar cortex of adult male albino rats following monosodium glutamate exposure: A comprehensive biochemical, histopathological and immunohistochemical study. *Tropical Journal of Pharmaceutical Research*, *23*(12), 2059–2071. doi.org/ 10. 4314/tjpr.v23i12.11

Shin, J.-W. (2023a). Neuroprotective effects of melatonin in neurodegenerative and autoimmune central nervous system diseases. *Encephalitis*, *3*(2), 44–53.

Tabatabaei-Jafari, H., Shaw, M. E., Walsh, E., & Cherbuin, N. (2020). Cognitive/Functional Measures Predict Alzheimer's Disease, Dependent on Hippocampal Volume. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, 75(7), 1393–1402.

White, S., Mauer, R., Lange, C., Klimecki, O., Huijbers, W., & Wirth, M. (2023). The effect of plasma cortisol on hippocampal atrophy and clinical progression in mild cognitive impairment. *Alzheimer's & Dementia : Diagnosis, Assessment & Disease Monitoring*, *15*(3), e12463.

## ARABIC SUMMARY

### تقييم دور الميلاتونين الوقائي في قرن امون الدماغي لذكور الجرذان البالغة البيضاء في نموذج قصور الغدة الكظرية الأولي: دراسة كيميائية، هستوباثولوجية وهستوكيميائية مناعية

**دينا عبد الله بدوي، ريهام إسماعيل علي، سلوى مصطفى الخياط وهاجر عطا الله حشيش**
**قسم التشريح والاجنة – كلية الطب – جامعة المنصورة**

أُجريت هذه الدراسة لتقييم تأثير قصور هرمونات الغدة الكظرية الأولي على قرن امون الدماغي وتقييم الدور الوقائي المحتمل للميلاتونين على الخلايا العصبية في قرن امون الدماغي في هذه الحالة.

تم إجراء هذه الدراسة على ثمان عشر فأرًا أبيضا من الذكور البالغين. تم تقسيمها إلى ثلاث مجموعات من ستة فئران كل منها عبارة عن مجموعة التحكم ومجموعة استئصال الغدة الكظرية، ومجموعة معالجة بالهرمونات بالإضافة إلى الميلاتونين. تم تحفيز القصور الكظري الأولي جراحياً عن طريق استئصال كلتا الغدتين الكظريتين لدى الفئران. تم إعطاء فلودروكورتيزون (أقراص كورتيلون 0.1 ملجم/كجم/يوم) بجرعة تكميلية (0.01-0.02 ملجم/كجم/يوم). تم إعطاء بريدنيزون (أقراص ديسبريلون-أو دي 5 ملجم) بجرعة تكميلية (0.5 ملجم/كجم/يوم). والميلاتونين (ناترول ميلاتونين 3 ملجم) بجرعة 15 ملجم/كجم. وأعطيت جميعها عن طريق الفم من خلال أنبوب المعدة من اليوم الأول حتى نهاية التجربة.

في نهاية التجربة، تم تخدير الفئران تخديراً عميقاً باستخدام الزيلازين (15 ملغم/كغم) والكيتامين (90 ملغم/كغم) للتضحية بها، ثم تم تشريح أدمغتها ووزنها. تم استخدام أحد نصفي المخ لعمل مقاطع على مستوى قرن امون الدماغي وتم تحضير مقاطع البارافين لصباغتها بالكريزيل البنفسجي والصبغات الكيميائية المناعية. تم تشريح قرن امون الدماغي من النصف الاخر من المخ واستخدامه للتحليل الكمي للعلامات المؤكسدة.

كان هناك اختلال في مستويات دلالات الأكسدة في قرن امون الدماغي المجموعة التي تم استئصال الغدة الكظرية منها مقارنة بمجوعة التحكم. أما المجموعة المعالجة بالميلاتونين مع الهرمون

فقد كانت هذه المستويات طبيعية تقريبًا. كان لدى المجموعة المعالجة بالميلاتونين مع الهرمون تغيرًا ملحوظًا في هذه العلامات مقارنة بالمجموعة التي تم استئصال الغدة الكظرية منها وفقًا للتحليل الإحصائي.

كانت طبقات الخلايا الهرمية مفككة وبدت داكنة ومنكمشة وذات نوى متخثرة في المقاطع المصبوغة بالكريسيل فى المجموعة التي تم استئصال الغدة منها، أظهرت المقاطع تلفاً عصبياً كبيراً خاصة في المنطقة الأولى والثالثة من قرن امون والشفرة الظهرية للتلفيف المسنن. أظهرت مجموعة الميلاتونين بنية محفوظة أكثر مع وجود خلايا شبه طبيعية في جميع الطبقات.

وفقًا للتحليل الإحصائي، كان لدى المجموعة التي تم استئصال الغدة الكظرية منها انخفاض في سمك المنطقة الأولى والثالثة من قرن امون والشفرة الظهرية للتلفيف المسنن. أظهرت المجموعة المعالجة بالميلاتونين بالإضافة إلى الهرمونات زيادة كبيرة في سمك الطبقات مقارنة بالمجموعة التي تم استئصال الغدة منها.

لم تظهر المقاطع المصبوغة بالسينابتوفيسين من قرن امون الدماغي للفئران ذات الغدة الكظرية المستأصلة أي نقاطاً أو مجموعات إيجابية من صبغة السينابتوفيسين في جميع الطبقات. أظهرت المقاطع المصبوغة بالسينابتوفيزين من الفئران المعالجة بالهرمونات والميلاتونين استعادة كبيرة في ظهور الحبيبات المصبوغة باللسنابتوفيزين.

وأظهر التحليل الإحصائي للنسبة المئوية لمساحة السينابتوفيسين أن المجموعة ذات الغدة الكظرية المستأصلة كان لديها انخفاض ملحوظ في نسبة مساحة السينابتوفيسين بالمقارنة مع مجموعة التحكم. أظهرت المجموعة المعالجة بالميلاتونين مع الهرمونات زيادة ملحوظة في النسبة المئوية لمساحة السينابتوفيسين في جميع الطبقات مقارنة بمجموعة الغدة الكظرية المستأصلة.

ووفقاً للتحليل الإحصائي، فإن نقص هرمونات الغدة الكظرية يقلل من وزن الدماغ. كان وزن الدماغ لدى مجموعة الميلاتونين بالإضافة إلى الهرمونات البديلة طبيعيًا تقريبًا.

**خاتمة**

من خلال الدراسة الحالية يمكن استنتاج أن قصور الغدة الكظرية الأولي كان له تأثير ضار على التركيب الخلوي لقرن آمون الدماغي في الجرذان البيضاء البالغة. وأن نقص تركيز صبغة السينابتوفزين في أجزاء قرن آمون الدماغي قد يؤدي الى التدهور المعرفي وفقدان الذاكرة. قصور الغدة الكظرية الأولي كان له تأثير أخف ضررا عند اضافة الميلاتونين الى العلاج الهرموني البديل. قام الميلاتونين بحماية خلايا قرن آمون الدماغي من تأثيرات قصور الغدة الكظرية الأولي إلى حد كبير بحيث عاد الحصين بالقرب من البنية الخلوية الطبيعية.