

Original Research Article

Effect of melittin against the pilocarpine model of epilepsy in newborn mice

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Sent for review: 9 May 2025

Revised accepted: 20 October 2025

Abstract

Purpose: To evaluate the effect of melittin against pilocarpine-induced epilepsy in neonatal mice.

Methods: Twenty pups were divided into four groups; G1 (control) received normal saline, G2 was intraperitoneally injected with pilocarpine hydrochloride (400 mg/kg body weight) postnatally at day 1 and 7 after birth (P1 and P7), G3 was injected subcutaneously with melittin (0.5 µg/1g) twice a week, and G4 was first injected with pilocarpine at P1 and P7, and then with melittin (0.5 µg/1g). After two weeks of treatment, the neonates were anesthetized, anatomized, and the brains were prepared for general histology and immunohistochemical stains of glial fibrillary acidic protein (GFAP), alpha-synuclein and beta-amyloid.

Results: Histological studies of brains showed vacuolation, pale, faint cytoplasm and shrinking of pyramidal cells in the pilocarpine-treated group (G2) in comparison with control group (G1). The cerebral cortex region showed nuclear pyknosis and acidophilic cytoplasm. Dark-stained nuclei of the Purkinje cell layer and eosinophilic cytoplasm were detected in the pilocarpine-treated group in the cerebellum region, in contrast to control group. Furthermore, expression of GFAP and alpha-synuclein in the hippocampus region was inhibited in the pilocarpine-treated group (G2) in comparison with control group. Also, a significant increase in beta-amyloid expression in the cerebellum region was noted in the pilocarpine-treated group (G2) compared with control group (G1). In addition, melittin-treated group (G3) showed similarity with control group. A significant improvement in the previous histopathological markers in pilocarpine-treated group (G2) was noted after treatment with melittin in G4 group.

Conclusion: Melittin may offer a novel therapeutic approach for treating pilocarpine hydrochloride-induced status epilepticus.

Keywords: Alpha-synuclein, Beta-amyloid, Epilepsy, Glial fibrillary acidic protein, Melittin, Pilocarpine

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INTRODUCTION

Epilepsy is an unbalanced state between inhibitory and excitatory neurotransmission of the brain, which includes hyperexcitability, continuous disability, deficits in cognitive functions and memory deficits [1]. Status

epilepticus (SE) is defined by neuropathological features such as abnormal electrical activity [2]. The mice pilocarpine model resembles human temporal lobe epilepsy (TLE) in neuropathological damage [3]. Also, TLE is linked with neuronal cell loss and inhibitory pyramidal cells in cornu ammonis of hippocampal

regions with alterations of glial cells [4]. Induction of TLE in research in experimental setting occurs by administering pilocarpine and this model is important for evaluating the effectiveness of novel antiepileptic agents [5]. Animals (mice and rats) with pilocarpine-induced status epilepticus display severe cell damage, mossy fiber sprouting, pyramidal cell loss, and astrogliosis in the hippocampus, amygdala and thalamus during epileptogenesis, where the locations and timing of neuronal loss in pilocarpine model mice are distinguished by determination of the sites of brain lesions in hippocampal regions [6]. Different mouse strains and different doses have been used to induce status epilepticus by intraperitoneal injection of pilocarpine [7].

Pilocarpine-induced seizures cause significant alterations in the concentration levels of aspartate, glycine, glutamate, taurine, gamma-aminobutyric acid, and glycine in the homogenized brain tissues, while the lithium-pilocarpine model showed changes in the extracellular levels of cholinergic amino acid neurotransmitters during epileptogenesis. In addition, status epilepticus revealed an increase in glucose utilization and cerebral metabolic rate during epileptic activity [8]. Nowadays, searches for new natural compounds for epilepsy treatments are necessary.

Melittin, the main ingredient of honeybee (*Apis mellifera*) venom, comprising 26-amino acid peptide, is a cationic, hemolytic peptide, and contributes to reducing fibrosis in some animal models [9]. It participates in combating neurodegeneration and reduces the inflammatory protein expression in neurological disorders [9]. Melittin has diverse biological activities such as antiviral, cell cycle arrest, anti-apoptotic effect, anti-bacterial, wound-healing potential, antifungal, anti-inflammatory, hemolytic, anticancer, anti-mutagenic, antitumor, radioprotective, anti-nociceptive, aids in immune modulatory activity, and pain-relief effects [10].

Protective roles of melittin have been reported in osteoarthritic chondrocytes, rheumatoid arthritis, acute pancreatitis, acute liver inflammation, renal injury, renal fibrosis and gastric inflammation [11]. Previous research on melittin concentrated on inflammatory cytokine production, amelioration of hepatic failure and myocarditis, as well as antitumor activity and antiapoptotic effects [11].

The aim of the current study is geared towards evaluating the possible impact of melittin against pilocarpine model of epilepsy in neonatal mice.

EXPERIMENTAL

Animal grouping and experimental design

Twenty adult virgin females and ten male mice (*Mus musculus*; 25 – 30 g) were acquired from Animal Housing Facility, Faculty of Science, Sohag University, Egypt. The mice were housed in isolated polypropylene cages with available food and water, temperature (28 °C) and lighting (12 light/12 dark). The mice were distributed in ten cages (two females with one male per cage) and left overnight. The ten pregnant females were left to have their pups. After birth, newborns were divided into four groups (5 each group); the first group (G1) was control (injected intraperitoneally with 0.9 % NaCl), the second group (G2) was injected intraperitoneally with pilocarpine hydrochloride (400 mg/kg in 0.9 % NaCl) postnatally at days 1 and 7 after birth (P1 and P7) [6]. Atropine sulfate at dose (5 mg/kg (i.p.) was injected intraperitoneal (30 min before administration of pilocarpine) to minimize pilocarpine's peripheral side effects [12]. Also, melittin (0.5 µg/1 g) was injected subcutaneously twice a week to the third group (G3) for two weeks. The fourth group (G4) was injected first (30 mins earlier) with Atropine sulfate, then with pilocarpine hydrochloride (at P1 and P7), then after 30 min, injected with subcutaneous melittin (twice a week) for two weeks. Newborns at two weeks old from the different groups were anesthetized with xylazine and anatomized following treatment.

Tissue processing

General histology

Newborns' brains in each group were fixed in 10 % formalin alcohol for 4 h at room temperature. The dehydration process was performed with ascending series of ethyl alcohol (70 %, 90 %, 100 %, and 100 %) and the clearing step was carried out by three changes in xylene (2-hourly intervals) followed by three changes in toluene (every 1 h). Infiltration in Paraffin wax was achieved in an oven at 60 °C for 6 h (three changes; 2-hourly intervals). Furthermore, samples were orientated in the Paraffin blocks to be ready for cutting at 5 µm by a microtome (Leica Biosystems, Shanghai, China). Hematoxylin and Eosin (H & E) dye was used to stain the sections for conventional histology [13].

Immunohistochemistry study

Immunohistochemical staining was done using the protocol of the Millipore Company (Det-HP1000; Burlington, MA). Positive slides were

used during the steps of the immunohistochemical technique to prevent losing the sections. Sections were rinsed in xylene for deparaffinization, then rehydrated by a descending series of ethyl alcohol to water (100 %, 90 %, 70 %, 50 %, water).

Thereafter, the sections were treated with citrate buffer (pH = 6) to retrieve the antigen. Following each step, phosphate buffer (pH = 7.4) was used for washing. Endogenous peroxidase blocking was carried out by rinsing the slides in 3 % hydrogen peroxide. Brain sections of different groups were immersed for 10 min in the blocking reagent (Det-HP1000, 20773; Millipore).

The primary antibody step was done in different tissues of the brain; e.g., Hippocampus regions were incubated with two primary antibodies in separate sections of slides; GFAP (Rabbit polyclonal; ab227761; Abcam; 1:100) and alpha-synuclein (Rabbit polyclonal, E2681, Spring Bioscience, Pleasanton, CA) (Ready to use). Cerebral cortex regions were incubated with the primary antibody, alpha-synuclein, while the cerebellum region was incubated with primary anti-beta Amyloid antibody (polyclonal produced in Rabbit; ab74396; Abcam; 1:100).

Secondary antibody step was done by incubating with goat anti-rabbit immunoglobulin-G (IgG; 20775; Det-HP1000; Millipore Co.) for 30 min. The labeling enzyme step was carried out by incubating with streptavidin horseradish peroxidase (20774; Det- HP1000; Millipore Co.) for 30 min.

Freshly prepared 3,3'-diaminobenzidine tetrahydrochloride (DAB) with the substrate (D3939; Sigma, St. Louis, MO) was added to sections, which were subsequently dehydrated, cleared and then mounted with Distyrene Plasticizer Xylene (DPX). Photomicrographs were captured using an AxioCam ERc5s camera attached to a light microscope (Axio Lab. A1, Carl Zeiss, Berlin, Germany).

Analysis of immunohistochemical stained sections

The percentage area intensity of immunohistochemical stained sections was determined by ImageJ software (<https://fiji.sc/>) for quantifying color [14].

Two-tailed *t*-test analysis of the data was performed using the Microsoft Excel program. The histogram displayed the acquired data as mean $\pm$ standard error (SE).

RESULTS

Histological and immunohistochemical investigation of the hippocampus region

Sections of control group's hippocampus region stained with H & E (Figure 1 A) revealed four regions of *Cornu ammonis* (CA1, CA2, CA3, CA4) and dentate gyrus (DG). Control group (G1) in CA1 region showed astrocytes (star-shaped) with positive glial fibrillary acidic protein (GFAP) immunoreactive cells that were distributed among the hippocampus's several cell layers (red rectangle in Figure 1 B and C). Pilocarpine-treated group (G2) revealed a significant decrease in GFAP expression in astrocyte cells (Figure 1 D and G). Conversely, melittin-treated group didn't reveal any abnormality compared to control (Figure 1 E and G). Pilocarpine-induced mice treated with melittin (G4) exhibited improvement in GFAP signals in astrocyte cells (Figure 1 F and G) compared with G2.

Perivascular astrocytes (the end-feet of astrocytes that send cell expansions toward the blood vessels in the hippocampus area showed strong immunoreactivity of GFAP in control group (white rectangle in Figure 1 B and Figure 2 A). In the pilocarpine-treated group, a significant decrease in GFAP localization was noted in long processes of astrocytes (Figure 2 B and E). However, melittin-treated group (G3) did not show any change in GFAP immunoreactivity in the astrocytes of surrounding blood vessels (Figure 2 C and E). Pilocarpine plus melittin-treated group (G4) exhibited a significant improvement in the GFAP signals as compared to G2 group (Figure 2 D and E).

Normal architecture of pyramidal cell layer, i.e., rounded vesicular nuclei and basophilic cytoplasm, was seen in control group at CA1 (Figure 3 A). The pyramidal cells in the pilocarpine-treated group (G2) appeared vacuolated, disorganized, detached, with pale, altered-stained nuclei and faint cytoplasm (Figure 3 B). The pyramidal cells in melittin-treated group appeared in normal architecture (Figure 3 C) while those in pilocarpine plus melittin-treated group (G4) revealed improvement in previous alterations (Figure 3 D). In control and melittin-treated groups, alpha-synuclein immunohistochemical staining showed normal distribution around the cell membranes of pyramidal cells (Figure 3 E and G), while the pilocarpine-treated group revealed complete inhibition and a significant decrease of alpha-synuclein localization (Figure 3 F and I). Slight improvement of alpha-synuclein signals in some

pyramidal cells was observed in pilocarpine plus melittin-treated group (Figure 3 H and I).

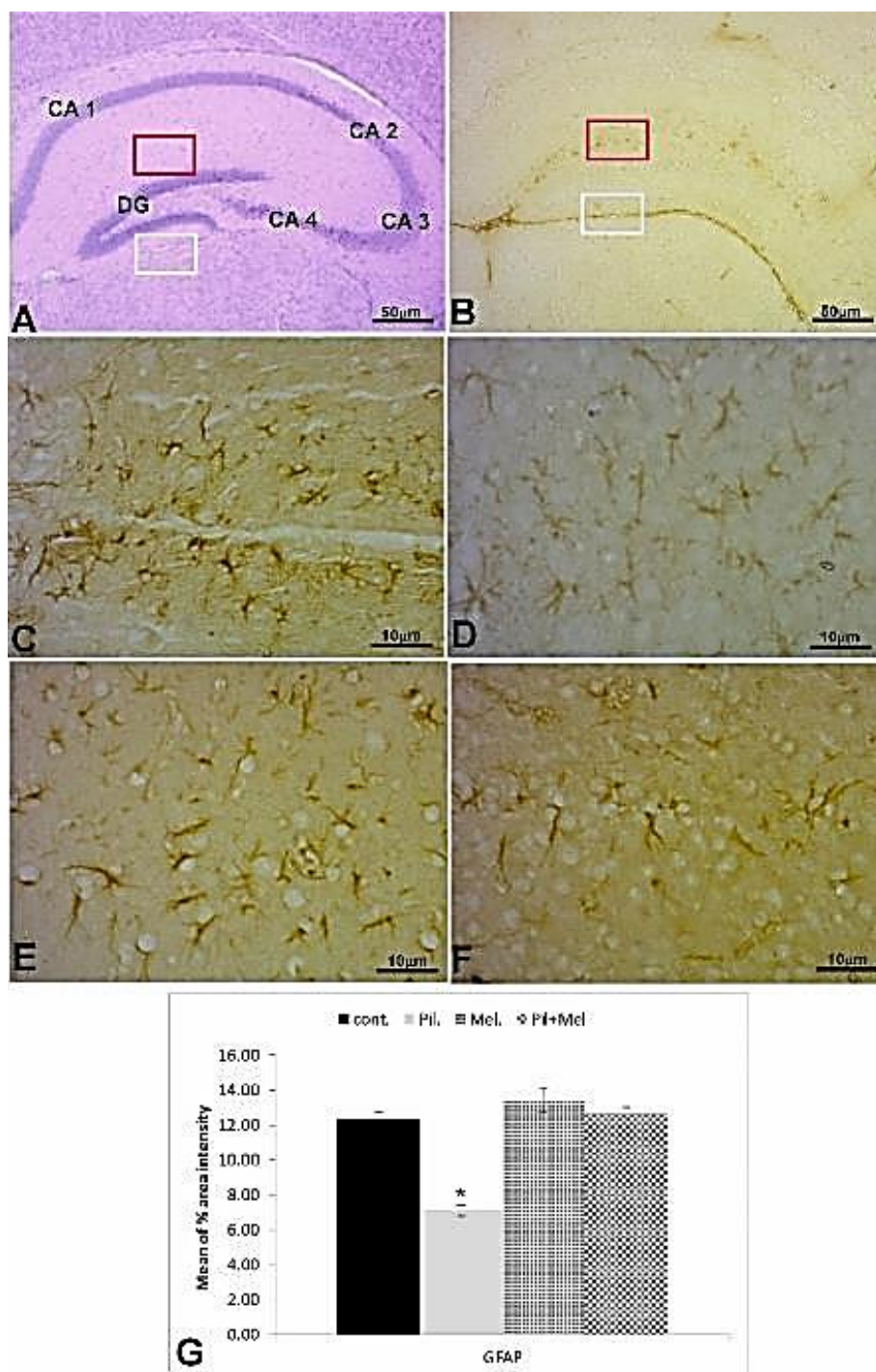


Figure 1: Photomicrographs of H&E-stained sections show histological structure of hippocampus (A), which consists of four areas of cornu ammonis (CA1, CA2, CA3, CA4) and dentate gyrus (DG). (B) The GFAP immunostaining sections in the hippocampus region show normal GFAP expression in the hippocampus fissure region (red rectangle) and blood vessels (white rectangle) of control group. (C) High magnification of control group (G1) revealed positively-stained astrocytes for GFAP expression in CA1 region. (D) Inhibition of GFAP localization was detected in pilocarpine-treated group (G2). (E) Melittin-treated groups (G3) didn't exhibit any change in the GFAP expression. (F) Improvement in GFAP signals of astrocyte cells was detected in pilocarpine plus melittin-treated group (G4). Scale bar in A-B is 50 μ m; C-F is 10 μ m. The histogram represents mean % area intensity of GFAP expression in different groups. (*) indicates a significant decrease in G2 (pil) compared to G1 (cont.), while G3 (mel) and G4 (pil+mel) are not significantly different

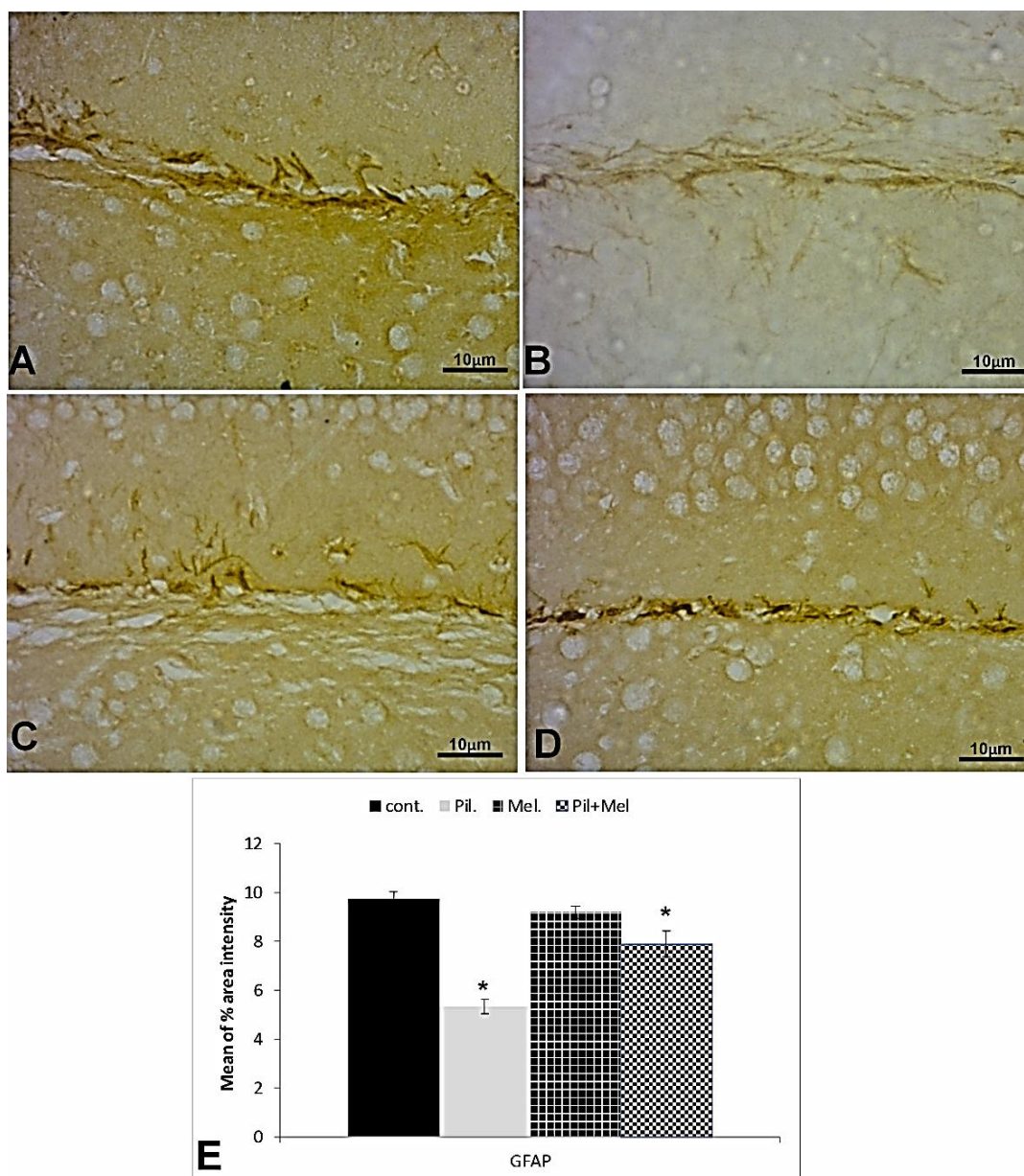


Figure 2: Photomicrographs show GFAP immunostaining of peri-vascular astrocytes in the blood vessel of hippocampus region. (A) Control group (G1) shows normal GFAP astrocytes. (B) Pilocarpine-treated group (G2) exhibited decrease in GFAP astrocytes. (C) Melittin-treated group (G3) didn't show any abnormalities in GFAP expression. (D) Pilocarpine plus melittin-treated group (G4) revealed improvement in the GFAP astrocytes. Scale bar in A-D is 10µm. (E) Histogram shows mean % area intensity of GFAP expression. (*) indicates a significant decrease in G2 (pil.) compared to control

Histological and immunohistochemical investigation of the cerebral cortex region

Normal neurons with rounded nuclei, soma and dendrites were noted in the cerebral cortex of control group (Figure 4 A). The pilocarpine-treated group exhibited abnormal nuclei and pyknosis of nuclei (Figure 4 B) in comparison with control group. However, no histological alterations were noted in the neurons in melittin-treated group (Figure 4 C). Improvement in the neuron's morphology was observed in

pilocarpine plus melittin-treated group (Figure 4 D) as compared with G2 and alpha-synuclein was normally expressed in the cerebral cortex neurons of control and melittin-treated groups (Figure 4 E and G). Furthermore, a significant decrease in the alpha-synuclein localization was detected in the pilocarpine-treated group (Figure 4 F and I). The pilocarpine plus melittin-treated group (G4) exhibited a significant improvement in the expression of alpha-synuclein (Figure 4 H and I).

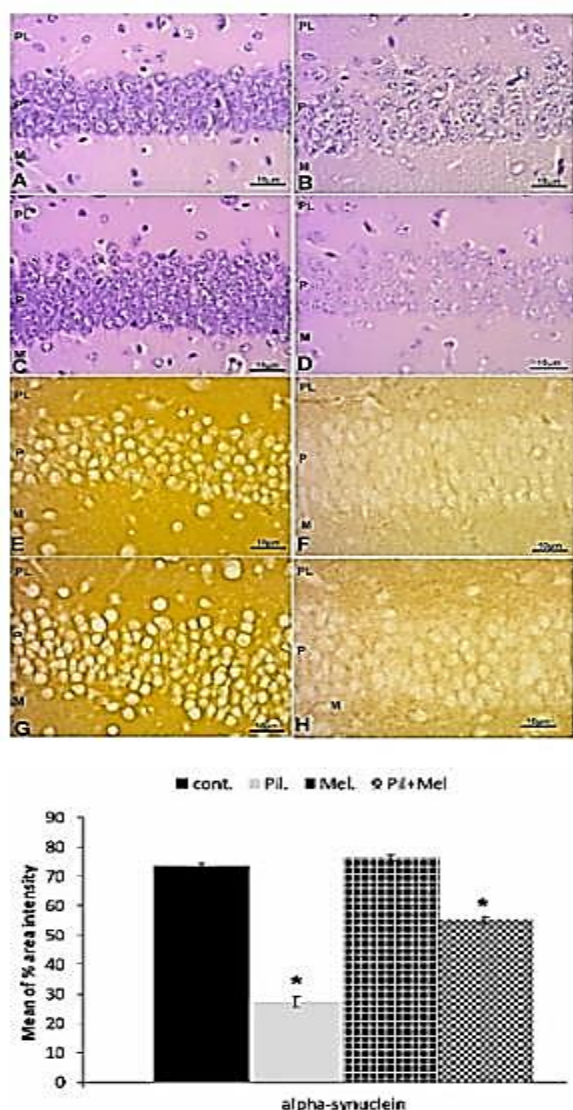


Figure 3: Photomicrographs of H&E-stained sections of hippocampus (CA1) that consist of three layers: polymorphic (PL), pyramidal (P), and molecular (M). (A) Normal pyramidal cell layer was detected in control group (G1). (B) Detachment, disorganization, and shrinkage of cells were observed among pyramidal cells of pilocarpine-treated group (G2). (C) The pyramidal cells in melittin-treated group appeared similar to control group. (D) Pyramidal cells in pilocarpine plus melittin-treated group revealed improvement. (E) Alpha-synuclein immunostained sections in the hippocampus region (CA1) of control show the normal distribution of alpha-synuclein around the cell membranes of pyramidal cells. (F) Inhibition of alpha-synuclein localization was noted in the pilocarpine-treated group (G2). (G) Similar alpha-synuclein expression was noted in melittin-treated group (G3). (H) Improvement in alpha-synuclein expression was detected in pyramidal cells in pilocarpine plus melittin-treated group (G4). Scale bar in A-H is 10µm. (I) Histogram shows mean % area intensity of alpha-synuclein expression in the hippocampus region. (*) indicates a significant decrease in G2 (pil.) compared to control

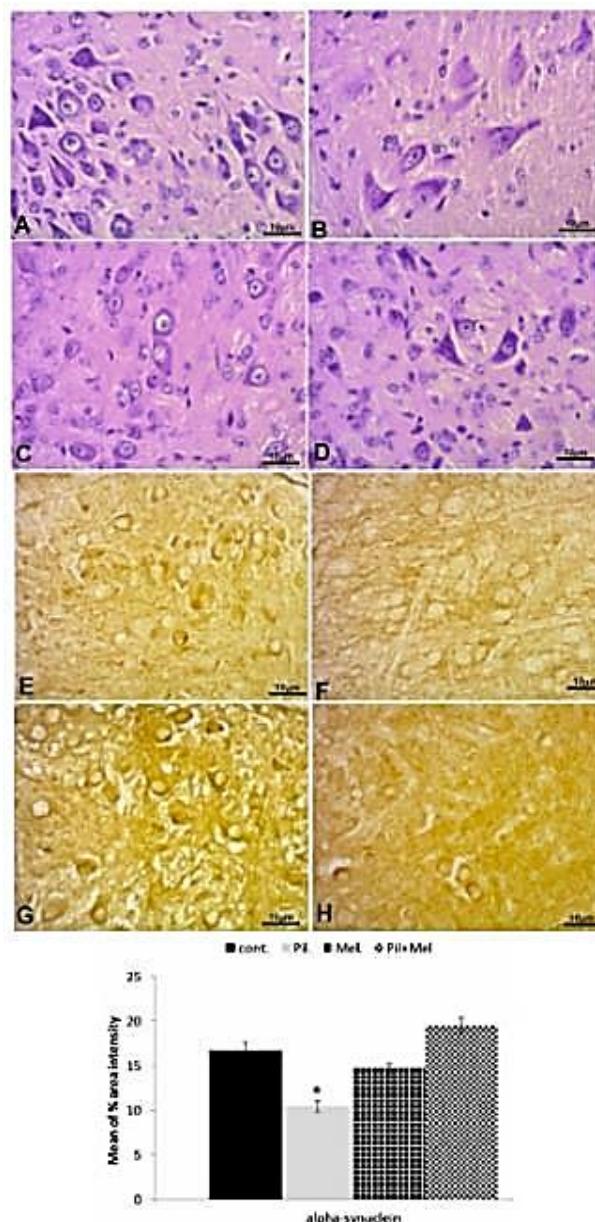


Figure 4: Photomicrographs showing H&E staining in the cerebral cortex; control group (A) and melittin-treated group (C) with normal neurons. (B) Pilocarpine-treated group exhibited abnormality and pyknosis of neuronal nuclei. (D) Recovery in the morphological appearance of neurons was observed in pilocarpine plus melittin-treated group. Alpha-synuclein immunostaining in the cerebral cortex; control group (E) and Melittin-treated group (G) showed the normal localization of alpha-synuclein expression in the neurons (G). Pilocarpine-treated group displayed a significant decrease in the alpha-synuclein localization (F, I). Pilocarpine plus melittin-treated group exhibited a significant improvement in the expression of alpha-synuclein (H, I). Scale bar in A-H is 10 µm. (I) Histogram shows mean % area intensity of alpha-synuclein expression in the cerebral cortex. (*) indicates a significant decrease in G2 (pil.) compared to control

Histological and immunohistochemical investigation of the cerebellum region

Histological staining of the cerebellum gray matter in control group (Figure 5A) revealed three layers: molecular layer, Purkinje cell layer, and granular cell layer. Pyriform Purkinje neuronal cells appeared in the middle of the Purkinje cell layer with clear vesicular nuclei and a basophilic cytoplasm. Increase in size and eosinophilic cytoplasm in some cells of Purkinje cell layer was observed in pilocarpine-treated group (Figure 5 B) in comparison with control group. Also, no histological changes were noted in melittin-treated group (Figure 5 C). Alterations in the Purkinje cell layer were absent in the pilocarpine plus melittin-treated group (Figure 5 D), while the pilocarpine-treated group (G2) showed a marked and significant increase in the beta-amyloid expression (Figure 5 F and I) compared with the normal expression in control group (Figure 5 E and I) and melittin-treated group (Figure 5 G). A significant improvement in the accumulation of beta-amyloid was noted in the pilocarpine plus melittin-treated group compared with pilocarpine-treated group (Figure 5 H and I).

DISCUSSION

The present immunohistochemical study of GFAP expression, alpha-synuclein, and beta-amyloid in pilocarpine-treated brain tissues provides evidence of neuropathological alterations in the pilocarpine-induced epilepsy model. Glial fibrillary acidic protein (GFAP) is the principal intermediate filament protein and is considered a classical hallmark of reactive astrogliosis and also determines the functions of astrocytes in the CNS, where astrocytes play a significant role in the spread and generation of epileptic action [15]. Astrocytes play important functions such as generation of growth factors and cytokines, glutamate and potassium absorption, protein synthesis in the extracellular matrix, and are also crucial in the normal functioning of the central nervous system [16].

The current investigation revealed a decline in GFAP expression in the pilocarpinized hippocampus region that is a result of astrocyte alterations. Astrocyte degeneration by pilocarpine is specific to different mouse strains, i.e., it is absent in mice that had either kainate-induced or pilocarpine-induced status epilepticus [16]. Overexpression of GFAP in astrocytes (gliosis) has been described in some models that were accompanied by proliferation and swelling of astrocytes [17].

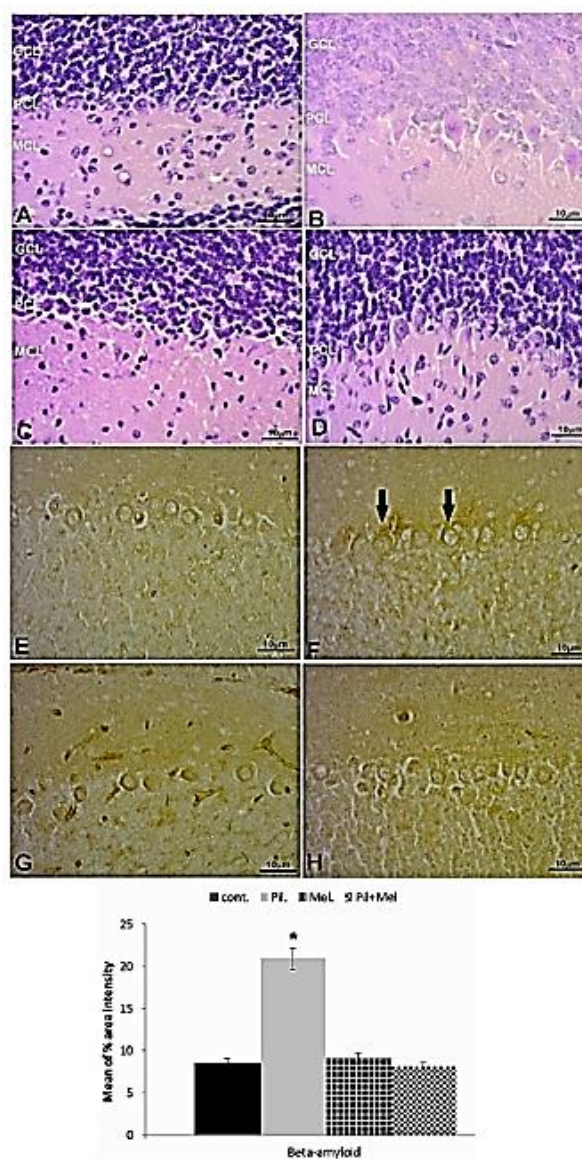


Figure 5: Photomicrographs of H&E-stained cells showing the layers of cerebellar region: granular cell layer (GC), Purkinje cell layer (PCL), and molecular layer (MCL). (A) PCL of control group has pyriform neuronal cells with clear vesicular nuclei and a basophilic cytoplasm arranged in a single row. (B) PCL of Pilocarpine-treated group (G2) displayed increase in size and eosinophilic cytoplasm in some cells. (C) No histological changes were noted in melittin-treated group (G3), and (D) improvement was noted in pilocarpine plus melittin-treated group (G4). (E) Beta-amyloid immunostaining in the cerebellar region; control group (G1) showed the normal localization of beta-amyloid. Pilocarpine-treated group (G2) showed a marked increase in beta amyloid expression (F, arrows, I). Melittin-treated group (G3) did not express any histopathological alteration (G). (H) Improvement of beta amyloid expression was exhibited in the pilocarpine plus melittin-treated group (G4). Scale bar in A-H is 10 μ m. (I) Histogram shows the mean % area intensity of beta-amyloid expression in the cerebellar region. (*) indicates a significant increase in G2 (pil.) compared to control

Spontaneous seizures induced by pilocarpine or lithium/pilocarpine were associated with significant astroglial and neuronal degeneration in the piriform cortex [16].

The present study showed inhibition of alpha-synuclein expression in the areas of the cerebral cortex and hippocampus of pilocarpine-treated group. Alpha-synuclein deficiency or absence in mice causes neuronal dysfunction, changes in synaptic structure, axonal structure, and synaptic protein composition, as well as functional deficiencies in the nigrostriatal dopamine system [18]. The results of the present study revealed a deposition of β -amyloid in the cerebellar region of pilocarpine-treated group. Previous studies have shown that β -amyloid protein deposition is a histopathological hallmark of neurodegenerative diseases such as Alzheimer, Down syndrome, dementia, neurotoxicity, epilepsy and brain injuries [19]. Accumulation of soluble β -amyloid monomers leads to deposition of insoluble fibrillar polymers. Irregular β -amyloid deposition would have spread to the Purkinje or granule cell layers. Uncommon patterns of amyloid deposition might be due to an excessive or unusual astrocyte activity or as a result of variations in molecules such as apolipoproteins, proteoglycans, and acute phase proteins. A possible therapeutic approach to neurodegenerative disease therapy is the clearance or removal of the β -amyloid deposits [20].

The ameliorating results obtained by melittin were in harmony with the antiepileptic effects of *Nigella sativa*, Thymoquinone, *Rosa damascena*, *Wuling mycelia*, erythravine, 11 α -hydroxy-erythravine, Scopolamine, neural Per-Arnt-Sim protein 4 (Npas4), Annexin A1-derived peptide and peptides obtained from wasp and scorpion venoms [21,22]. Bee venom has been shown to possess radioprotective and neuroprotective effects against acrylamide. Melittin, the active ingredient of bee venom, has been documented to prevent seizures in the bicuculline-induced epileptic model [23]. Melittin stimulates the glutamatergic and GABAergic neurotransmission by activation of Ca^{2+} and phospholipase A2. Melittin does not damage cell membranes at low concentrations, but it exerts lytic effects at high concentrations. The improvement might be due to the antioxidant power of melittin against the oxidant damage [11]. The antioxidant property of melittin might be due to suppression of nitric oxide (NO), inducible nitric oxide synthase (iNOS), cyclooxygenase-2/prostaglandins expression (COX-2/PGE2), Reactive Oxygen Species (ROS), and malondialdehyde (MDA) levels [9].

Antioxidant defense of melittin might be associated with TrkB/CREB/BDNF pathway, where it stimulates p-TrkB activation, increases the amount of p-CREB transcription factor, and then upregulates the expression of brain-derived neurotrophic factor (BDNF) or through regulating the activation of Nrf2/HO-1 pathway [13]. Melittin inhibits aortic vascular smooth muscle cell proliferation by inhibiting NF- κ B, Akt activation, mitogen-activated protein kinase pathway, and regulating the expression of extracellular matrix (ECM). It also suppresses liver fibrosis by a decline of NF- κ B activation, inhibition of the activation of the NF- κ B pathway, and suppression of IL-6 expression.

Furthermore, melittin suppresses cell death by hydrogen peroxide (H_2O_2)-induced apoptosis, hence used in the treatment of cholangitis and biliary fibrosis, with analgesic and anti-inflammatory effects. Thus, the effect of melittin might be related to the modification of the NF- κ B signaling pathway or the probability of melittin blocking the integral interaction between astrocytes and microglia that stimulates continuous inflammation [24]. It has been suggested that the mechanisms of action of melittin might be attributed to interaction with diverse molecules, or it may be due to direct pilocarpine inactivation or receptor inactivation at the surface of cells. Thus, more investigation is required for future uses of melittin in the treatment of epilepsy and to ascertain melittin's mode of action, delivery, effectiveness and safety in an epilepsy model.

CONCLUSION

The present study provides a deeper understanding of how pilocarpine causes status epilepticus and the possible novel treatment of epilepsy by melittin. Therefore, melittin may offer a novel therapeutic approach for treating pilocarpine hydrochloride-induced status epilepticus.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

The design was approved by the Scientific Research Ethics Committee, Faculty of Science, Sohag University, Egypt (no. CSRE-1-25).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

- Koh HK, Kobau R, Whittemore VH, Mann MY, Johnson JG, Hutter JD, Jones WK. Toward an integrated public health approach for epilepsy in the 21st century. *Prev Chronic Dis* 2014; 28; 11: E146. doi: 10.5888/pcd11.140270. PMID: 25167091; PMCID: PMC4149320.
- Dichter MA. Emerging insights into mechanisms of epilepsy: implications for new antiepileptic drug development. *Epilepsia* 1994; 35: S51-S57.
- Mello LE, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, Finch DM. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia* 1993; 34(6): 985-995.
- Navidhamidi M, Ghasemi M, Mehranfard N. Epilepsy-associated alterations in hippocampal excitability. *Rev Neurosci* 2017; 28(3): 307-334.
- Hong N, Choi YS, Kim SY, Kim HJ. Neuroprotective effect of lithium after pilocarpine-induced status epilepticus in mice. *Korean J Physiol Pharmacol* 2017; 21(1): 125-131.
- Borges K, Gearing M, McDermott DL, Smith AB, Almonte AG, Wainer BH, Dingledine R. Neuronal and glial pathological changes during epileptogenesis in the mouse pilocarpine model. *Exp Neurol* 2003; 182(1): 21-34.
- Berkeley JL, Decker MJ, Levey AI. The role of muscarinic acetylcholine receptor-mediated activation of extracellular signal-regulated kinase 1/2 in pilocarpine-induced seizures. *J Neurochem* 2002; 82(1): 192-201.
- Meller S, Brandt C, Theilmann W, Klein J, Löscher W. Commonalities and differences in extracellular levels of hippocampal acetylcholine and amino acid neurotransmitters during status epilepticus and subsequent epileptogenesis in two rat models of temporal lobe epilepsy. *Brain Res* 2019; 1712: 109-123.
- Nguyen CD, Lee G. Neuroprotective activity of melittin—the main component of bee venom—against oxidative stress induced by A β 25–35 in in vitro and in vivo models. *Antioxidants* 2021; 10(11): 1654.
- Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (*Apis mellifera*) against 915-MHz microwave radiation-induced DNA damage in Wistar rat lymphocytes: in vitro study. *Int J Toxicol* 2009; 28: 88–98.
- Rady I, Siddiqui IA, Rady M, Mukhtar H. Melittin, a major peptide component of bee venom, and its conjugates in cancer therapy. *Cancer Lett* 2017; 402: 16-31.
- Williams MB, Jope RS. Protein synthesis inhibitors attenuate seizures induced in rats by lithium plus pilocarpine. *Exp Neurol* 1994; 129: 169–173.
- Suvarna KS, Layton C, Bancroft JD, editors. *Bancroft's theory and practice of histological techniques E-Book*. Elsevier Health Sciences 2018.
- Nguyen DH, Zhou T, Shu J, Mao J. Quantifying chromogen intensity in immunohistochemistry via reciprocal intensity. *Cancer InCites* 2013; 2(1): 1–4.
- Kodam A, Ourdev D, Maulik M, Hariharakrishnan J, Banerjee M, Wang Y, Kar S. A role for astrocyte-derived amyloid β peptides in the degeneration of neurons in an animal model of temporal lobe epilepsy. *Brain Pathol* 2019; 29(1): 28-44.
- Borges K, McDermott D, Irier H, Smith Y, Dingledine R. Degeneration and proliferation of astrocytes in the mouse dentate gyrus after pilocarpine-induced status epilepticus. *Exp Neurol* 2006; 201(2): 416-427.
- Brenner M, Messing A. Regulation of GFAP expression. *ASN Neuro* 2021; 13: 1759091420981206. doi: 10.1177/1759091420981206. PMID: 33601918; PMCID: PMC7897836.
- Greten-Harrison B, Polydoro M, Morimoto-Tomita M, Diao L, Williams AM, Nie EH, Chandra SS. $\alpha\beta\gamma$ -Synuclein triple knockout mice reveal age-dependent neuronal dysfunction. *Proc Natl Acad Sci USA* 2010; 107(45): 19573-19578.
- Mann DM, Iwatsubo T, Snowden JS. Atypical amyloid (A β) deposition in the cerebellum in Alzheimer's disease: an immunohistochemical study using end-specific A β monoclonal antibodies. *Acta Neuropathol* 1996; 91(6): 647-653.
- Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Roses A. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci USA* 1993; 90(20): 9649-9653.
- Aboul Ezz HS, Khadrawy YA, Noor NA. The neuroprotective effect of curcumin and *Nigella sativa* oil against oxidative stress in the pilocarpine model of epilepsy: a comparison with valproate. *Neurochem Res* 2011; 36(11): 2195-2204.
- Wei L, Cao B, Jia C, Zhang R, Liu Y, Qiao Y. Protective effect of *Wuling mycelia*, alone and in combination with

- valproic acid, on pentylenetetrazol induced epilepsy in rats. *Trop J Pharm Res* 2021; 20(1): 135-143.
23. Soares-Silva B, Beserra-Filho JIA, Morera PMA, Custódio-Silva AC, Maria-Macêdo A, Silva-Martins S, Ribeiro AM. The bee venom active compound melittin protects against bicuculline-induced seizures and hippocampal astrocyte activation in rats. *Neuropeptides* 2022; 91: 102209.
24. Verhoog QP, Holtman L, Aronica E, van Vliet EA. Astrocytes as guardians of neuronal excitability: mechanisms underlying epileptogenesis. *Front Neurol* 2020; 11: 591690.