Original Article

Histological and Immunohistochemical Study of the Effect of Early Maternal Deprivation on the Hippocampus and Dentate Gyrus of Male Albino Rat Offspring at Adulthood: The Role of Vitamin B_{12} Supplementation After Weaning

Walaa M. Elwan, Noha R. M. Elswaidy and Marwa A. A. Ibrahim

Department of Histology and Cell Biology, Faculty of Medicine, Tanta University, Egypt

ABSTRACT

Introduction: Early life stress (ELS) plays a role in determining normal physiological functions and predisposing to pathologic processes later in life. Maternal deprivation (MD) is an example of early childhood stress that has been related to long-term behavioral issues due to disturbance in normal brain development. Vitamin B_{12} is required for the normal functioning and development of the central nervous system.

Aim of the Work: Examine the effect of early MD as a model for ELS on the structure of hippocampus and dentate gyrus of male albino rat offspring at adulthood and evaluate the role of vitamin B_{12} supplementation after weaning.

Material and Methods: Thirty male albino pups with their respective mothers were assigned into three equal groups; Control group (I); pups were kept with their mothers till weaning, then were separated until adulthood. MD group (II): pups were deprived from their mothers for 24h on postnatal day 9 then kept like the control group until adulthood. MD group supplemented with vitamin B_{12} (III); The pups underwent MD as described in group II but were kept on 50 µg of vitamin B_{12}/kg of diet. Brain specimens were obtained for different biochemical, histological, and immunohistochemical techniques.

Results: MD group revealed a significant surge in the tissue malonaldehyde with evident histological alterations in the hippocampus and dentate gyrus. A significant reduction in Bcl2 immunoexpression was concomitant with a significant rise in GFAP immunoexpression. MD group supplemented with vitamin B_{12} depicted evident amelioration of the studied parameters.

Conclusion: Early MD altered the structure of hippocampus and dentate gyrus of male rat offspring at adulthood. Healthy infant-mother relationship is recommended to maintain the offspring's mental health. Adjuvant therapy with vitamin B_{12} could be helpful in ameliorating such negative effects possibly through its antiapoptotic, glial stabilizing effects, and neuroprotective properties.

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Key Words: Dentate gyrus; hippocampus; maternal deprivation; vitamin B_{12}.

Corresponding Author: Marwa A. A. Ibrahim, MD, Department of Histology and Cell Biology, Faculty of Medicine, Tanta University, Egypt, **Tel.**: +20 10 9918 6399, **E-mail:** maleox68@yahoo.com, marwa.ibrahim@med.tanta.edu.eg

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INTRODUCTION

For most species, mothers stimulate the natural development of their offspring through nurturing and providing affection and safety for them. Most newborn animals have a strong reliance on maternal care during their initial days or weeks of life, making this period of high vulnerability to alterations in mother-infant relations[1,2]. This vital period impacts the development of cognitive, motor, and socio-emotional skills during adolescence and adulthood as the brain develops rapidly throughout the prenatal period and the early period of postnatal life[3,4].

Early life stress (ELS) plays a role in determining normal physiological functions and predisposing to pathologic processes later in life. Furthermore, ELS generates neuroendocrine changes which are linked to social and emotional behavior changes that can last a lifetime[5,6]. Maternal deprivation (MD) is an example of early childhood stress that has already been related to long-term behavioral issues like anxiety and psychopathologies like schizophrenia due to disturbance in normal brain development[7,8]. Additionally, the long-term effects of MD appear to be age dependent, for example, MD at the third postnatal day was reported to reduce hippocampal neurogenesis in prepubertal rats. Furthermore, depression, that affects millions of people of all ages, is predisposed by the collaboration of genetic and environmental liabilities through critical developmental phases such as infancy and adolescence[9].

The hippocampus is particularly vulnerable to stress. The survival, differentiation, plasticity, and preservation of function in the developing brain are all aided by brain-derived neurotrophic factor (BDNF), which is broadly distributed in the hippocampus. Furthermore, recent clinical and laboratory studies have found that MD during important stages of brain development might damage hippocampus cytoarchitecture, resulting in learning disabilities and behavioral disorders[10,11].

The B vitamins are a category of water-soluble vitamins that are essential for brain growth and cognitive function maintenance[12]. Vitamin B_{12} is required for the normal

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functioning of the central nervous system as well as its development and initial myelination. The ability of vitamin B_{12} to promote neurite development, neuroregeneration, and antinociception has been investigated in various animal models of neuronal disorders[13]. Moreover, vitamin B_{12} is important for the functioning of hippocampus. Previous studies have identified a specific mechanism relating low vitamin B_{12} levels to the defect in cognition and memory functions as well as microstructural damage in hippocampal subfields[14,15].

Based on these data, this study was performed to examine the effect of early MD as a model for ELS on the structure of hippocampus and dentate gyrus of male albino rat offspring at adulthood and evaluate the role of vitamin B_{12} supplementation after weaning using different biochemical, histological, and immunohistochemical methods.

MATERIAL AND METHODS

Experimental design

The experiment was run at the animal house of the Histology department, under the permission of the Local Ethics Committee of Tanta Faculty of Medicine, Egypt (approval code 35511). Six adult female albino rats were habituated at the animal house for 2 weeks before starting the experiment. After mating, each female rat was placed in an individual clean properly ventilated cage until delivery. They were kept under similar standard housing conditions, allowed free access to standard laboratory diet and water. After delivery (considered to be postnatal day 0, PND0), the dams and their pups were left without disruption until PND9, thereafter, thirty of the male pups along with their respective mothers were randomly assigned into three equal groups (Table 1):

Group I (Control group): (n=10) Pups were kept with their respective mothers till weaning (PND21) then the offspring were separated to be kept under standard housing conditions with free access to standard laboratory diet until adulthood (PND90).

Group II (Maternally deprived group): (n=10) On PND9, pups of this group were totally deprived from their mothers for 24h by removing the mothers into a new cage in a different room, while their pups were kept in their home cages. On PND10, the mothers were put back with their corresponding pups and were kept together till weaning (PND21) then the offspring were separated to be kept under standard housing conditions with free access to standard laboratory diet until adulthood (PND90)[17].

Group III (Maternally deprived & supplemented with vitamin B_{12} group): (n=10) The pups underwent MD as described in group II. They were then separated upon weaning (PND21) to be kept under standard housing conditions until adulthood (PND90) while being supplemented with 50 µg vitamin B_{12} per kg of chow[16]. Vitamin B_{12} (Cyanocobalamin, 98%) was purchased from Merck (Cat# V2876, Darmstadt, Germany).

Table 1: Experimental design of the study groups

Groups	PND_0-PND_9	PND_9-PND_{10}	PND_{10}-PND_{21}	PND_{21}-PND_{90}
Control group	Pups with mothers	Pups with mothers		Pups separated from mothers
MD group		Pups deprived from mothers	Pups with mothers	
MD & vit B_{12} group				Pups separated from mothers + 50µg vit B_{12}

The offspring were euthanized on the PND90 using intraperitoneal pentobarbital (40mg/kg)[18]. The temporal lobe was instantly taken out for histological and biochemical processing.

Biochemical study

Spectrophotometry was used to detect the level of tissue malondialdehyde (MDA) as a marker for oxidative stress[19].

Histological staining

Sagittal temporal lobe sections were fixed in 10% formalin and processed to obtain paraffin sections for staining with hematoxylin & eosin (H&E)[20].

Immunohistochemical staining

The primary antibodies, rabbit polyclonal antibodies against GFAP and Bcl2 (ab7260 and ab59348 respectively, Abcam, USA), both at a dilution of 1:500, were used as markers for astrocytes and apoptosis respectively. Immunohistochemical staining proceeded using the standard Labeled StreptAvidin Biotin (LSAB) method as previously described[21], using 3,3′-diaminobenzidine (DAB) hydrogen peroxide as the chromogen followed by Mayer's hematoxylin counterstaining

Morphometric analysis

Microphotography was done using a light microscope (Leica, Switzerland) equipped with a digital camera (Leica, Switzerland). Image analysis was performed using the software "ImageJ" (National Institute of Health, USA), where ten non-overlapping high power (x400) fields from each slide were quantified for:

1. The mean thickness of the pyramidal cell layer (µm) and mean number of the pyramidal cells/HPF of the hippocampus.
2. The mean thickness of the granular cell layer (µm) and the mean number of the granular cells/HPF of the dentate gyrus.

3. The mean color intensity of Bcl2-positive immunohistochemical expression
4. The mean area percentage (%) of GFAP-positive astrocytes.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's test (IBM SPSS Statistics for Windows, USA) were used for data analysis. Differences were considered significant if probability value $p<0.05$.

RESULTS

Biochemical assay

Tissue MDA of MD group II was significantly higher ($p<0.05$) compared to the control group I, while MD&vit B_{12} group III expressed a significant drop ($p<0.05$) compared to group II, yet it was non-significantly different ($p>0.05$) from the control group (Table 1).

Histological findings

H&E-stained sections of the temporal lobe of the brain from the control group I showed two locking C-shaped arcs: Cornu Ammonis and the dentate gyrus. The Cornu Ammonis of the hippocampus showed three zones: CA1, CA2, and CA3 (Figure 1). Each zone appeared in triple layers: polymorphic, pyramidal, and molecular. The pyramidal nerve cells were large cells with big vesicular nuclei and protuberant cytoplasmic processes directed toward the molecular layer. Neuroglial cells and blood vessels were detected (Figure 2).

The dentate gyrus (DG) also showed triple layers: the molecular, granular, and polymorphic layers. The granular layer was composed of closely packed granular cells. The granular cells had rounded pale vesicular nuclei overlying some immature neurons in the subgranular zone. Neuroglial cells and blood vessels were observed in the molecular and polymorphic layers (Figure 2).

Examination of different areas of hippocampus from the MD group II showed an apparently thinner pyramidal cell layer with relatively fewer pyramidal cells compared to the control group. Most pyramidal cells appeared dispersed, widely separated, and shrunken with dark condensed nuclei and dark cytoplasm, whereas some cells had vesicular nuclei. Numerous glial cells and dilated capillaries were observed in the polymorphic and molecular layers (Figure 3).

Examination of H&E-stained sections from the dentate gyrus area depicted an apparently shrunken granular layer with relatively fewer granular cells compared to the control group. The granular cell layer showed many dark shrunken granular cells and numerous immature neurons. Some granular cells had vesicular nuclei. The cells of the polymorphic layer appeared disturbed with some dark-stained pyramidal cells. Numerous glial cells and dilated capillaries were observed in the polymorphic and molecular layers (Figure 3).

The different areas of hippocampus from the MD&vit B_{12} group III showed an apparently normal histoarchitecture, yet some pyramidal cells appeared shrunken with dark condensed nuclei and dark cytoplasm (Figure 4). Moreover, examination of H&E-stained sections from the dentate gyrus area revealed an apparently normal histology, but some dark shrunken granular cells were detected (Figure 4).

Both mean thickness of the pyramidal cell layer and mean number of the pyramidal cells of the hippocampus in group II revealed a significant decrease ($p<0.05$) of both parameters in all three zones compared to the control group, whereas group III recorded a significant increase ($p<0.05$) of both parameters in all three zones with regard to group II, yet they were non-significantly different ($p>0.05$) from the control group (Table 2, Histogram 1).

Similarly, both mean thickness of the granular cell layer and the mean number of the granular cells of DG in group II depicted a significant decrease ($p<0.05$) of both parameters compared to the control group, whereas group III expressed a significant increase ($p<0.05$) of both parameters compared to group II, but they had a non-significant difference ($p>0.05$) compared to the control group (Table 2, Histogram 1).

Bcl2 immunohistochemical findings

Immunohistochemically stained sections for Bcl2 from the different areas of hippocampus and DG of the control group I showed a strong cytoplasmic Bcl2 immunoreaction detected in the nerve cells throughout the different layers (Figure 5). Whereas section from the MD group II revealed a weak Bcl2 immunoreaction in the nerve cells throughout the different layers (Figure 6). Meanwhile, sections from the MD&vit B_{12} group III depicted a moderately strong Bcl2 immunoreaction in the nerve cells throughout the different layers (Figure 7).

The mean color intensity of Bcl2 immunoreaction in group II showed a significant decrease ($p<0.05$) compared to the control group, whereas group III expressed a significant increase ($p<0.05$) compared to group II, yet it was non-significantly different ($p>0.05$) from the control group (Table 2, Histogram 1).

GFAP immunohistochemical findings

Immunohistochemically stained sections for GFAP from the different areas of hippocampus and DG of the control group I showed some GFAP-positive astrocytes mainly in the molecular and polymorphic layers and to a lesser extent in the pyramidal and the granular cell layers (Figure 8). Whereas sections from the MD group II showed numerous GFAP-positive large astrocytes with extensive branching outspreading into the pyramidal and the granular cell layers (Figure 9). On the other hand, sections from the MD&vit B_{12} group III depicted many GFAP-positive astrocytes mainly in the molecular and polymorphic layers and to a lesser extent in the pyramidal and the granular cell layers (Figure 10).

The mean area percentage of GFAP immunoreaction in group II depicted a significant increase ($p<0.05$) in comparison to the control group, whereas group III expressed a significant decrease ($p<0.05$) with regard to group II, yet it was non-significantly different ($p>0.05$) from the control group (Table 2, Histogram 1).

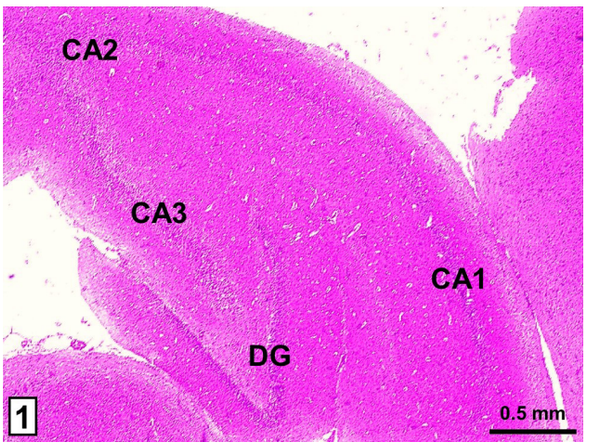


Fig. 1: A photomicrograph of the temporal lobe of the brain of the control group shows two locking C-shaped arcs; Cornu Ammonis with its three zones (CA1), (CA2), and (CA3) and the dentate gyrus (DG). (H&Ex40, scale bar=0.5mm)

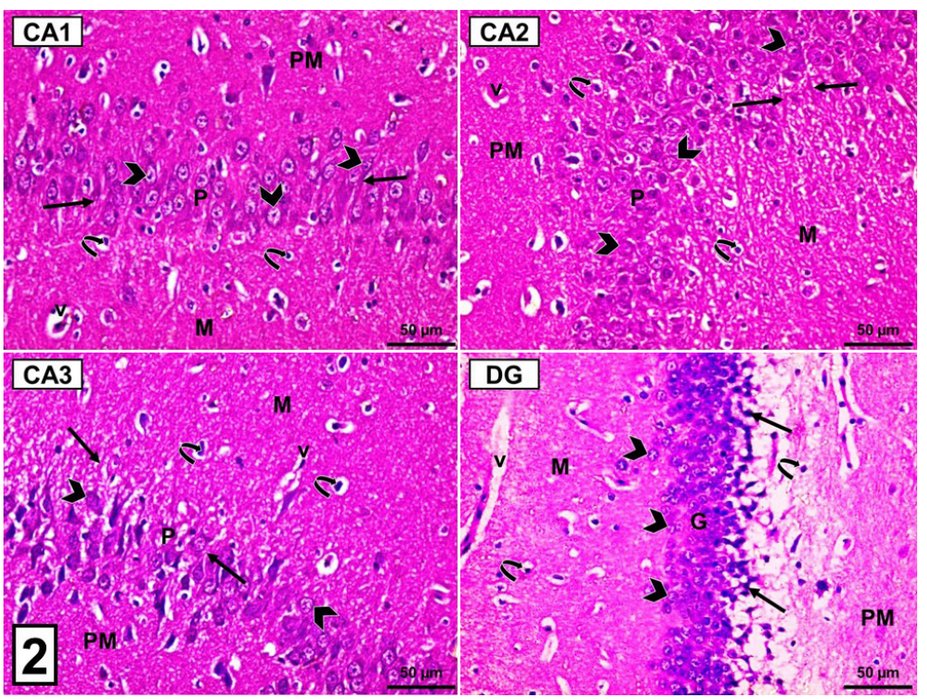


Fig. 2: Photomicrographs from the CA1, CA2, and CA3 regions of the hippocampus of the control group show their triple layers: polymorphic (PM), pyramidal (P), and molecular (M) layers. The pyramidal nerve cells are large cells with big vesicular nuclei (arrowheads) and protuberant cytoplasmic processes (arrows) directed toward the molecular layer. Neuroglial cells (curved arrows) and blood vessels (v) are observed. The DG region of the control group shows its triple layers: the molecular (M), granular (G), and polymorphic (P) layers. The granular cells have rounded pale vesicular nuclei (arrowheads) overlying some immature neurons (thin arrows) in the subgranular zone. Neuroglial cells (curved arrows), and blood vessels (v) are observed in the molecular and polymorphic layers. (H&E x400, scale bar=50 µm)

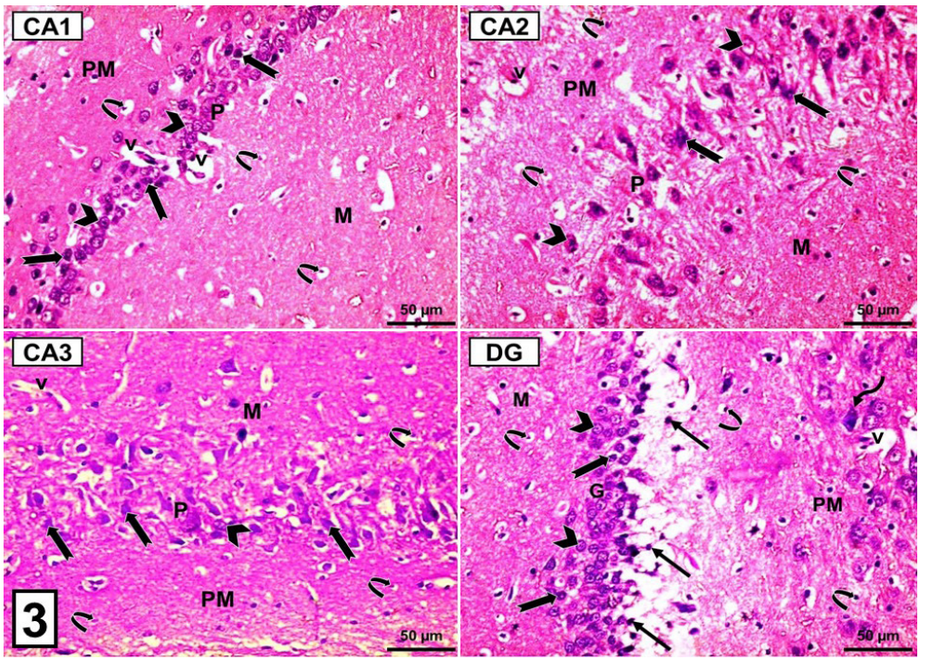


Fig. 3: Photomicrographs from the CA1, CA2, and CA3 regions of the hippocampus of group II show an apparently thinner pyramidal cell layer (P) with relatively fewer pyramidal cells compared to the control group. Most pyramidal cells (notched arrows) appear shrunken with dark condensed nuclei and dark cytoplasm, whereas some cells have vesicular nuclei (arrowheads). Numerous glial cells (curved arrows) and dilated capillaries (v) are observed in the polymorphic (PM) and molecular (M) layers. The DG region of group II shows an apparently shrunken granular layer (G) with relatively fewer granular cells compared to the control group. The granular cell layer shows many dark shrunken granular cells (notched arrows) and numerous immature neurons (thin arrows). Some granular cells have vesicular nuclei (arrowheads). The cells of the polymorphic layer appear disturbed with some dark-stained pyramidal cells (wavy arrow). Numerous glial cells (curved arrows) and dilated capillaries (v) are observed in the polymorphic (PM) and molecular (M) layers. (H&E x400, scale bar=50 µm)

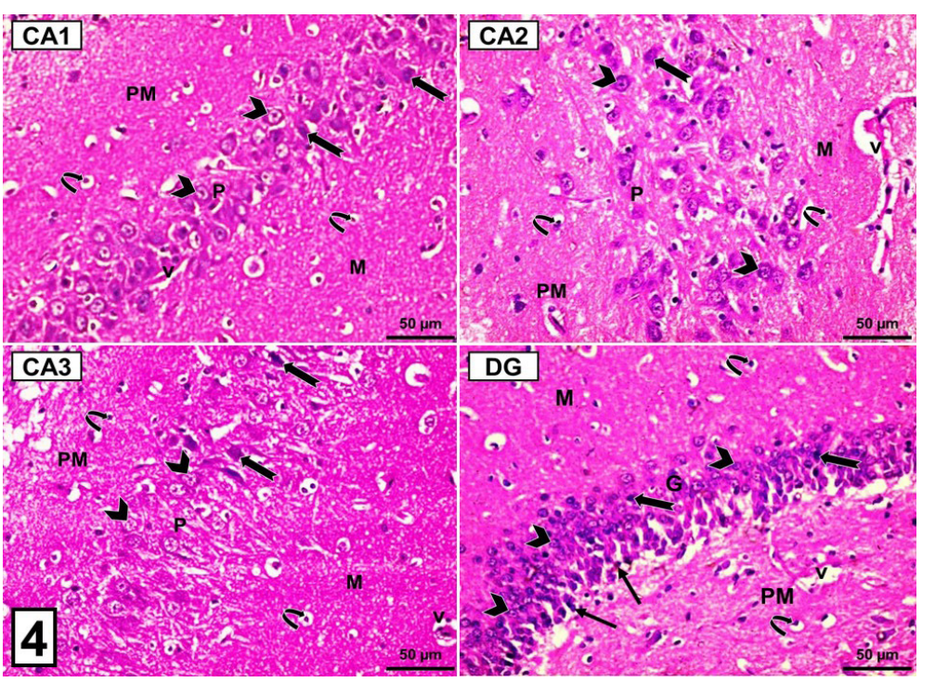


Fig. 4: Photomicrographs from the CA1, CA2, and CA3 regions of the hippocampus of group III show an apparently normal histoarchitecture with the triple layers: polymorphic (PM), pyramidal (P), and molecular (M) layers. Most pyramidal nerve cells appear as large cells with big vesicular nuclei (arrowheads). Some pyramidal cells (notched arrows) appear shrunken with dark condensed nuclei and dark cytoplasm. Neuroglial cells (curved arrows) and blood vessels (v) are observed. The DG region of group III shows an apparently normal histology with its triple layers: the molecular (M), granular (G), and polymorphic (P) layers. The granular cells of the granular layer appear with pale vesicular nuclei (arrowheads) overlying some immature neurons (thin arrows) in the subgranular zone. Some dark shrunken granular cells (notched arrows) are noticed. Some neuroglial cells (curved arrows) and blood vessels (v) are observed. (H&E x400, scale bar=50 µm)

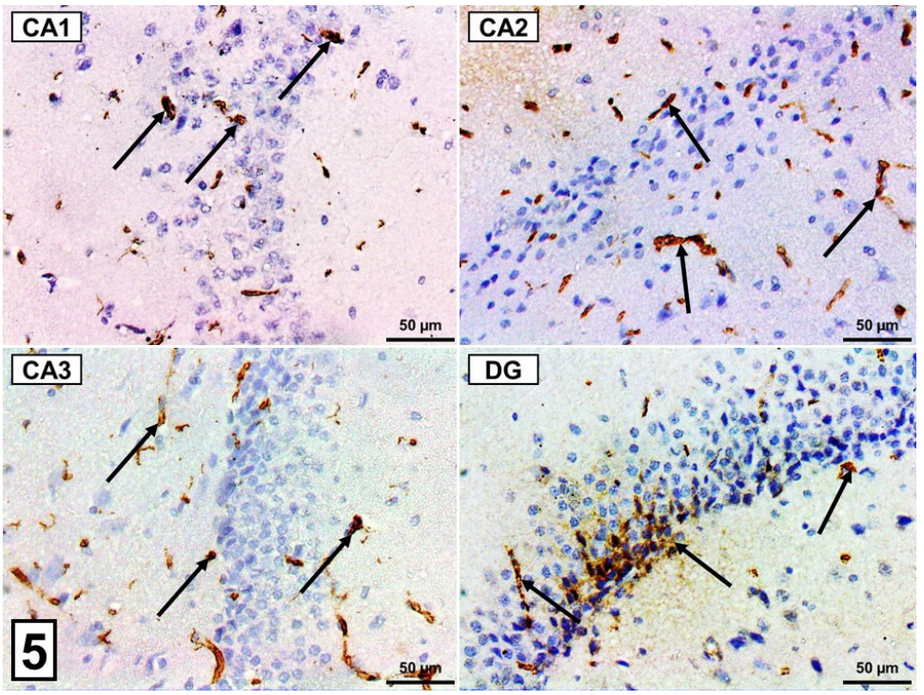


Fig. 5: Photomicrographs from the CA1, CA2, and CA3 regions of the hippocampus and the DG region of the control group show a strong cytoplasmic Bcl2 immunoreaction in the nerve cells (arrows) detected throughout the different layers. (Bcl2x400, scale bar=50 µm)

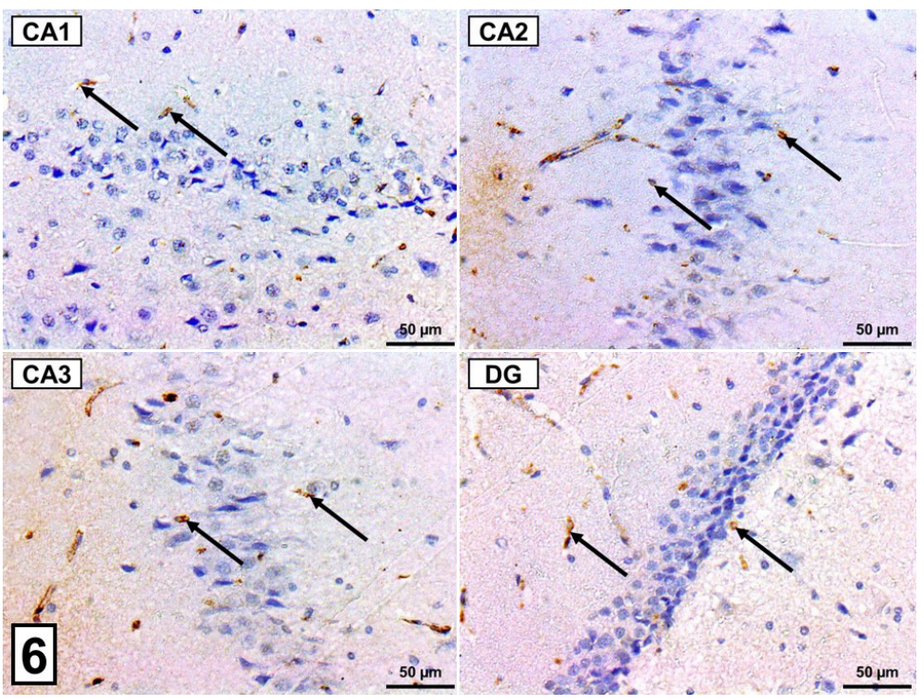


Fig. 6: Photomicrographs from the CA1, CA2, and CA3 regions of the hippocampus and the DG region of group II show a weak cytoplasmic Bcl2 immunoreaction in the nerve cells (arrows). (Bcl2x400, scale bar=50 µm)

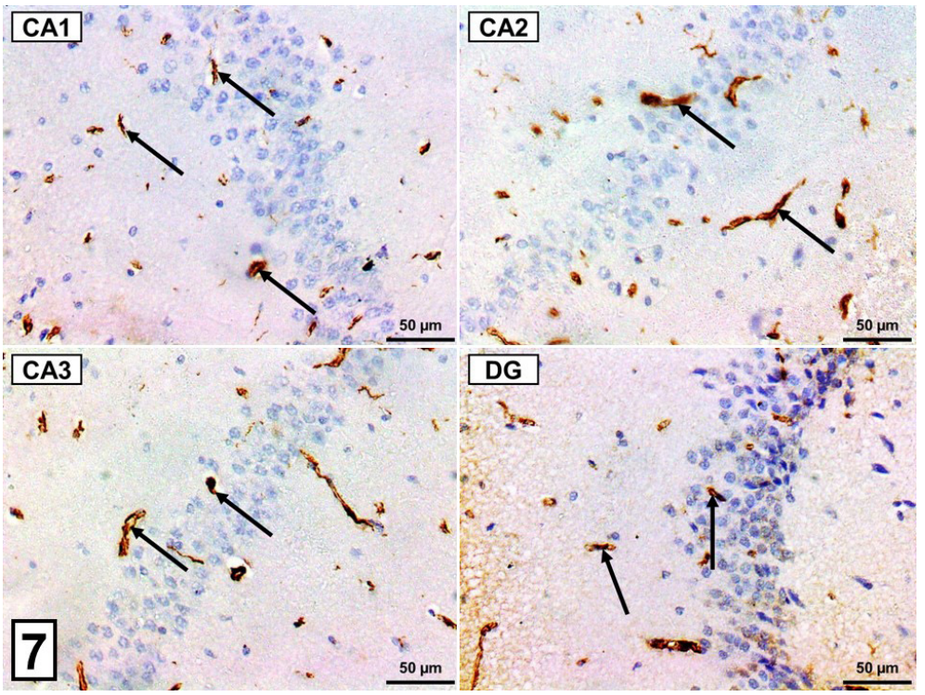


Fig. 7: Photomicrographs from the CA1, CA2, and CA3 regions of the hippocampus and the DG region of group III show a moderately strong cytoplasmic Bcl2 immunoreaction in the nerve cells (arrows). (Bcl2x400, scale bar=50 µm)

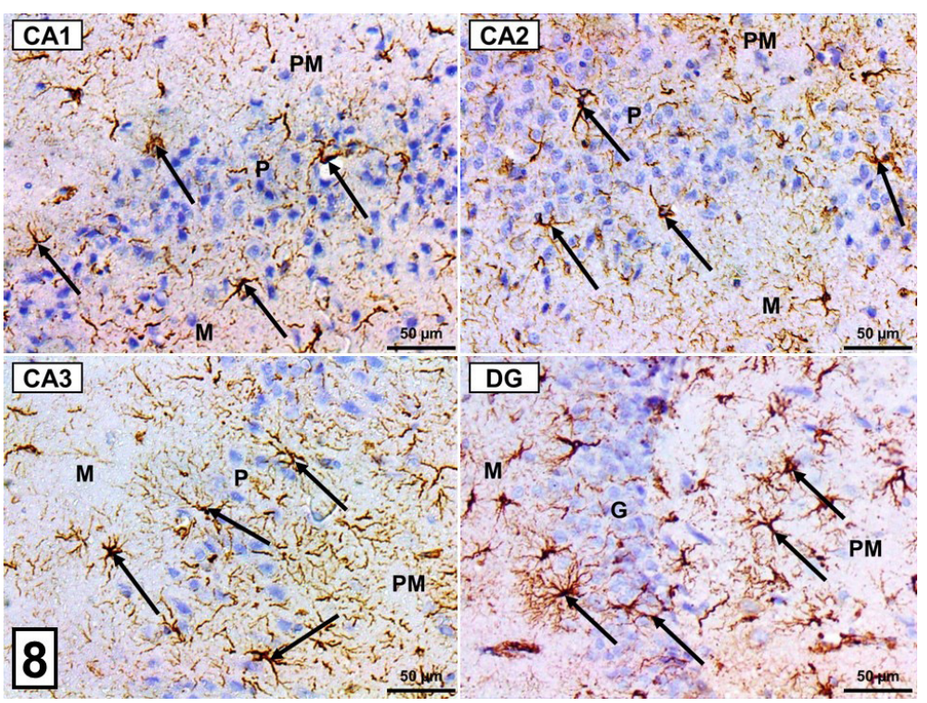


Fig. 8: Photomicrographs from the CA1, CA2, and CA3 regions of the hippocampus of the control group show some GFAP-positive astrocytes (arrows) mainly in the molecular (M) and polymorphic layers (PM) and to a lesser extent in the pyramidal cell layer (P). The DG region of the control group shows some GFAP-positive astrocytes (arrows) mainly in the molecular (M) and polymorphic layers (PM) and to a lesser extent in the granular cell layer (G). (GFAPx400, scale bar=50 µm)

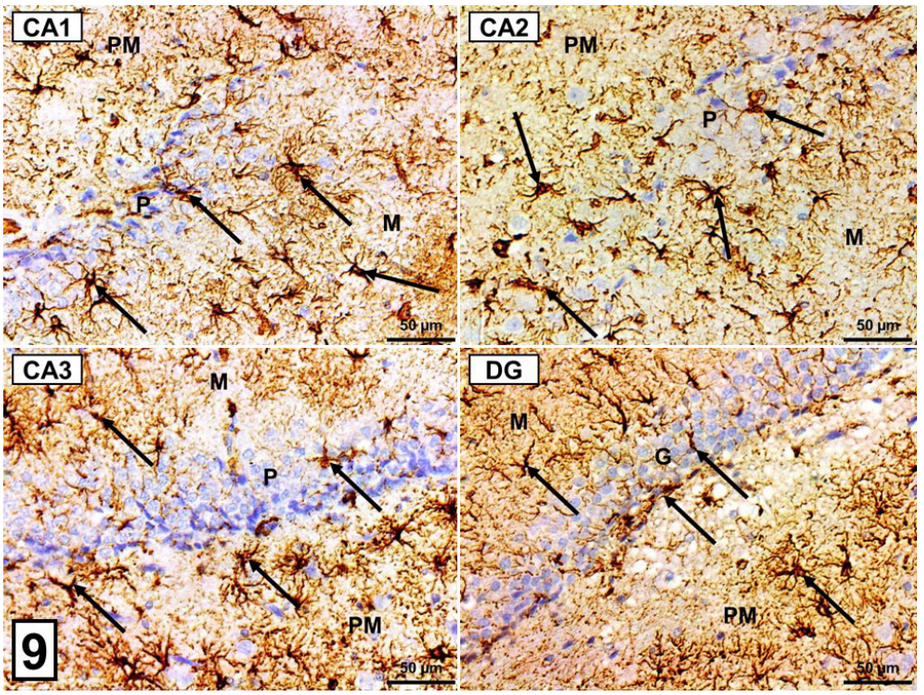


Fig. 9: Photomicrographs from the CA1, CA2, and CA3 regions of the hippocampus of group II show numerous GFAP-positive large astrocytes (arrows) in the molecular (M) and polymorphic layers (PM) with extensive branching outspreading into the pyramidal cell layer (P). The DG region of group II shows numerous GFAP-positive large astrocytes (arrows) in the molecular (M) and polymorphic layers (PM) with extensive branching outspreading into the granular cell layer (G). (GFAPx400, scale bar=50 µm)

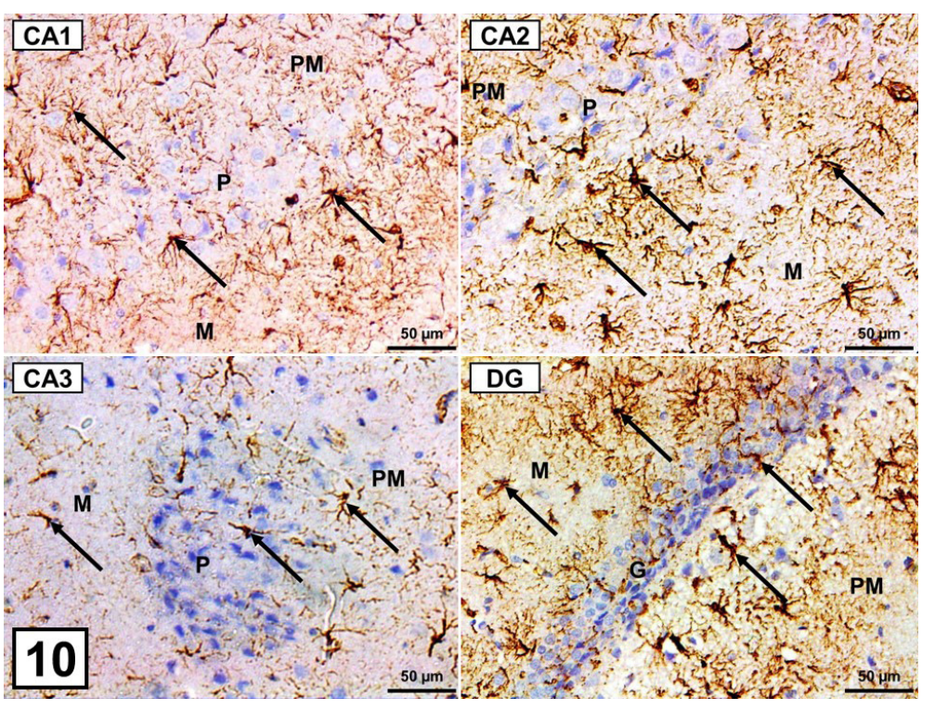
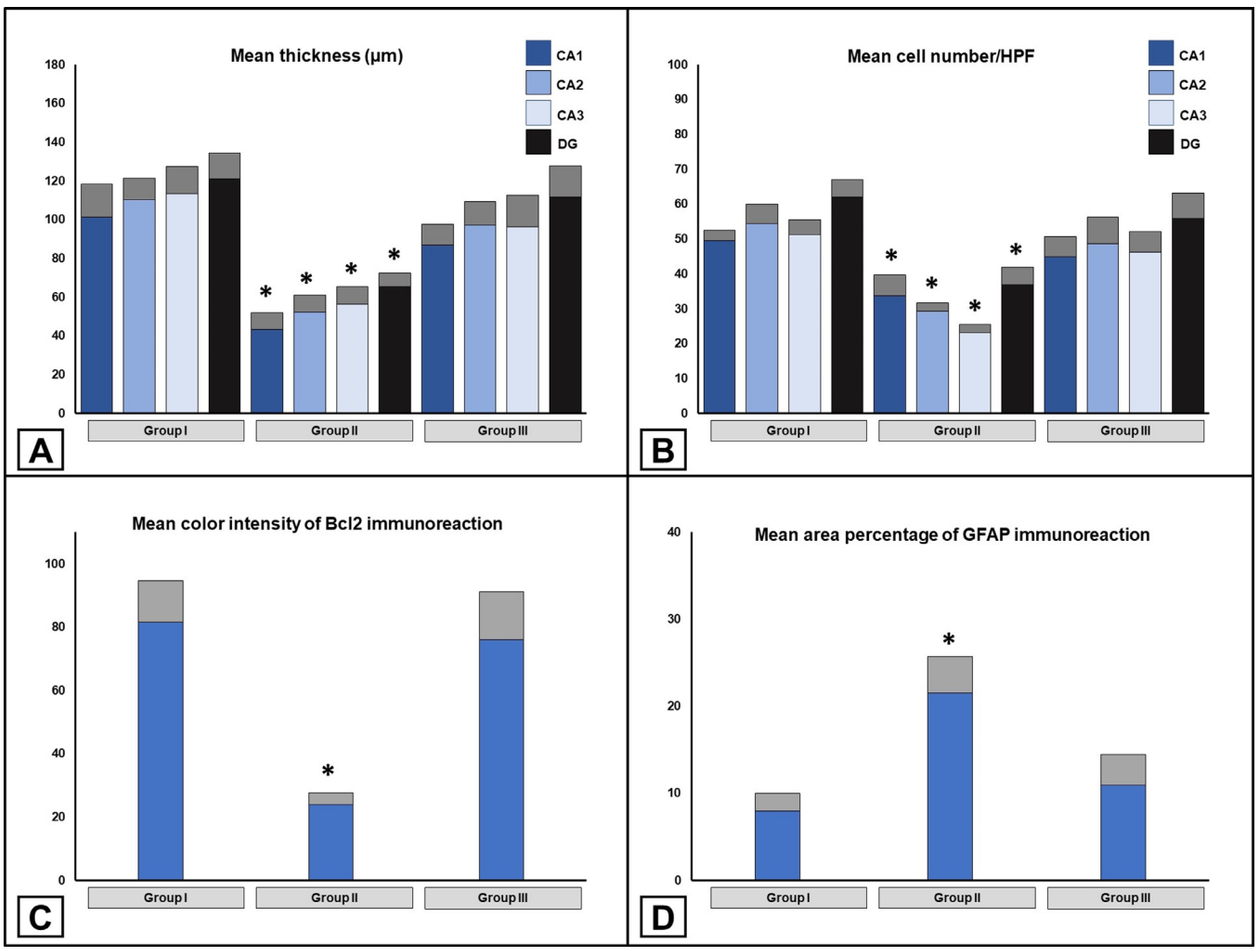


Fig. 10: Photomicrographs from the CA1, CA2, and CA3 regions of the hippocampus of group III show many GFAP-positive astrocytes (arrows) mainly in the molecular (M) and polymorphic layers (PM) and to a lesser extent in the pyramidal cell layer (P). The DG region of group III shows many GFAP-positive astrocytes (arrows) mainly in the molecular (M) and polymorphic layers (PM) and to a lesser extent in the granular cell layer (G). (GFAPx400, scale bar=50 µm)

Table 2: Biochemical and morphometrical analysis of the different study groups

Parameters			Group I	Group II	Group III
Mean MDA nmol/g-tissue protein			59.16±5.58	77.03±7.21[a]	62.11±7.44[b]
Mean thickness (µm)	Pyramidal cell layer	CA1	101.14±17.25	43.40±8.47[a]	86.91±10.51[b]
		CA2	110.43±11.08	52.35±8.81[a]	97.07±12.20[b]
		CA3	113.26±14.12	56.24±9.09[a]	96.33±16.33[b]
	Granular cell layer	DG	121.09±13.36	65.38±7.19[a]	111.80±15.90[b]
Mean cell number /HPF	Pyramidal cells	CA1	49.51±3.01	33.71±5.94[a]	44.82±5.79[b]
		CA2	54.39±5.64	29.18±2.55[a]	48.62±7.57[b]
		CA3	51.22±4.22	23.11±2.48[a]	46.16±6.06[b]
	Granular cells	DG	62.01±4.98	36.80±5.10[a]	55.84±7.33[b]
Mean color intensity of Bcl2 immunoreaction			81.56±13.09	23.88±3.80[a]	76.07±15.02[b]
Mean area percentage of GFAP immunoreaction			7.98±1.97	21.55±4.12[a]	10.95±3.51[b]

The superscript letters [a,b] denote significant difference compared to groups I and II respectively



Histogram 1: Morphometrical analysis of A] Mean thickness (µm) and B] Mean cell number/HPF of different zones of hippocampus and DG. C] Mean color intensity of Bcl2 immunoreaction. D] Mean area percentage of GFAP immunoreaction. * denotes significance versus the control group.

DISCUSSION

Early life stress (ELS), such as postnatal maternal deprivation (MD), can expose the neonates for various complications, as it alters the hypothalamic-pituitary-adrenal (HPA) axis and inversely affects the neurobehavior and neuroimmune systems causing many psychopathologies later in life[22,23].

The present study revealed that early MD caused numerous degenerative changes in the hippocampus and dentate gyrus. Early MD group showed a significant decrease in the thickness of the pyramidal cell layer of the different hippocampal areas, associating with a significant decrease in the pyramidal cell number. Additionally, the hippocampal nerve cells were shrunken with pyknotic nuclei and darkly stained cytoplasm. Similar histological results were recorded by other studies using Alzheimer's disease models[24]. Hippocampus, particularly CA1 and CA3 areas, was previously reported to play important roles in initial learning and memory in rats[25]. This goes in agreement with previous clinical studies that reported that early postnatal life stress disrupts the hippocampus-dependent spatial learning and memory in adult rats, accompanied with many hippocampal morphological, physiological and molecular abnormalities[26].

Moreover, the DG of MD group depicted reduced thickness of the granular cell layer coupling with a significant decrease in the number of granular cells. Similar results were recorded by another study[27]. Additionally, the nerve cells of the DG were shrunken and contained many immature neurons. This coincides with other experimental animal studies that declared that ELS enhances directly the proliferation and differentiation of DG immature cells in the migration stream, but this is followed by a reduction on the long-term survival of these cells as well as DG size in adult animals, suggesting that ELS firstly enhance the neurogenesis in the immature DG, with a subsequent slow depletion of the stem cell pool in the adult DG[28,29].

Moreover, it was reported that common early stress models in rats are based on temporary MD when the DG is still forming during the first postnatal weeks, and the stressful events during this period are more able to cause persistent injuries when compared to stress during adulthood[30]. During the postnatal period, the hippocampus undergoes essential processes including neurogenesis, synaptogenesis, glial growth and dendritic remodeling, making it a potential target for stress-induced structural changes. These processes continue till adulthood, such as adult neurogenesis as well as the addition of new granular cells of dentate gyrus, that become incorporated into organized neuronal circuits[31].

Furthermore, the present work revealed that MD induced neuronal apoptosis as most of neurons of hippocampus as well as DG had pyknotic nuclei, this was further confirmed by the current immunohistochemical results that revealed that MD induced apoptosis through a significant down-regulation of expression of the anti-apoptotic marker Bcl2. This goes in line with other studies using MD-induced depression models, they revealed that MD induces apoptosis in hippocampus and DG by augmenting the Bax and caspase-3 expression and decreasing the Bcl2 expression[32-34]. Similarly, previous experimental studies showed that depressed animals depicted a greater number of apoptotic cells in the hippocampus compared with normal ones[35]. Nevertheless, it was reported that adult neurogenesis in DG is accompanied by apoptosis of the immature, excessive and constantly generated precursor cells[36].

In the present study, the dilated blood capillaries observed in the hippocampus and DG of MD group could be a sign of inflammation, as some authors declared that MD increased the inflammatory cytokine interleukin-1 (IL-1) receptor in the hippocampus of adult rat offspring[37]. Moreover, other clinical evidence revealed that people with history of ELS have higher pro-inflammatory signaling in general and higher inflammatory response to acute stress[38].

Furthermore, the current study recorded a significant increase of tissue MDA in the MD group. It is widely accepted that oxidative stress has been implicated in the stress-induced neuronal degeneration[39]. The hippocampal nerve cells are reported to be rich in glucocorticoid receptor (GR)[40]. Therefore, they could be negatively affected by stress induced-glucocorticoids high levels. In response to stress, the glucocorticoids released from the adrenal gland activates the HPA axis that may trigger the cellular reduction–oxidation (redox) system leading to oxidative stress and neuronal damage[41].

In the present study, an immunohistochemical localization of GFAP, the main astroglial cytoskeletal protein[42], was performed to demonstrate the astrocytes and evaluate their response to neuronal injury, where GFAP upregulation is the most commonly hallmark of astrogliosis in various experimental models[43]. Our results revealed a significant increase in the mean area percentage of GFAP immunohistochemical positive astrocytes of MD group compared to the control group. Similar results were recorded by other investigators who reported that ELS exacerbates glial activation besides its negative neurogenesis effect[44]. Growing evidence indicates that astrogliosis, this defensive reaction of astrocytes, is involved in a wide range of neuropathologies, as it handles the acute stress and limits the tissue damage[45,46].

In the last decade, there is an increasing trend in research towards the dietary factors necessary for brain health[47]. Vitamin B_{12} has been indicated by many studies to possess neuroprotective effects[48,49]. Vitamin B_{12} supplementation after weaning was used in this study to assess its ability to attenuate MD-induced neuronal injury. The current results showed that vitamin B_{12} caused evident improvement in the different histological and immunohistochemical findings. This came in accordance with previous clinical studies that demonstrated the

closed relationship between vitamin B_{12} and proper brain development and function, as they revealed that low serum vitamin B_{12} level is strongly related to cognitive dysfunction[50]. Additionally, there is growing evidence indicating that vitamin B_{12} stimulates the synthesis of the neurotrophic factors needed for neuronal growth and survival[51]. In addition, vitamin B_{12} is a cofactor in folate metabolism and nucleotide biosynthesis, and this makes it crucial for normal nervous system function[52]. Previous in *vivo* studies have demonstrated the critical role of vitamin B_{12} in DNA methylation and epigenetic phenomenon in the CNS essential for maintenance of adult neurogenesis. These studies attributed Vitamin B_{12} neuroprotective effect to be through modulation of hippocampal DNA methylation on experimental pneumococcal meningitis rat model[53].

The immunohistochemical results of the present study demonstrated that vitamin B_{12} could ameliorate the degree of apoptosis of neuronal cells, this might be mediated through upregulation of the Bcl2 protein[54]. These results were in accordance with other studies that attributed anti-apoptotic effect of vitamin B_{12} against neuronal injury by reducing Bax protein and increasing Bcl2 protein, thus inhibiting neuronal death and allowing the recovery of neuronal functions[55].

In addition, in the present study, vitamin B_{12} supplementation has improved the reactive gliosis induced by MD as indicated by the immunohistochemical results. This finding might suggest that vitamin B_{12} exerts neuroprotective effects possibly by stabilizing glial activity against neuronal injury. Other in *vitro* study found that cultured astrocytes became reactive under conditions of hypovitaminosis B_{12}, suggesting the induction of astrogliosis to be one of the mechanisms of the hypovitaminosis B_{12} associated neurological symptoms[56].

Moreover, vitamin B_{12} supplementation evidently restored the level of tissue MDA in this study. Vitamin B_{12} was documented to possess antioxidant properties through superoxide scavenging effect directly in cytosol and mitochondria that contributes to neuronal growth, and indirectly by glutathione preservation[57,58]. Moreover, vitamin B_{12} was reported to alleviate the inflammation induced oxidative stress by modulating cytokine and growth factor production[59].

CONCLUSION

The results of the current study revealed that early MD altered the structure of hippocampus and DG of male rat offspring at adulthood. Accordingly, incorporation of healthy infant-mother relationship is extremely important for proper hippocampal and DG development, that improves the offspring mental health later in life. In addition, results from the present study demonstrate that adjuvant therapy with vitamin B_{12} could be helpful in ameliorating such negative effects possibly through its anti-apoptotic and glial stabilizing effects, in addition to its neuroprotective properties.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة هستولوجية و وهستوكيميائية مناعية لتأثير الحرمان المبكر من الأم على الحُصَين والتلفيف المسنن لذكور نسل الجرذ الأبيض في مرحلة البلوغ: دور مكملات فيتامين (ب١٢) بعد الفطام

ولاء محمد علوان، نهى رمضان محمد السويدى، مروة عوض عبد الحميد إبراهيم

قسم الهستولوجيا وبيولوجيا الخلية ـ كلية الطب ـ جامعة طنطا

مقدمة: تلعب ضغوط الحياة المبكرة دورًا في تحديد الوظائف الفسيولوجية الطبيعية والإستعداد للعمليات المرضية في وقت لاحق من الحياة. يُعد الحرمان من الأمهات مثالاً على ضغوط الطفولة المبكرة التي ارتبطت بقضايا سلوكية طويلة الأمد بسبب اضطراب النمو الطبيعي للدماغ. فيتامين (ب١٢) ضروري للعمل والتطور الطبيعي للجهاز العصبي المركزي.

الهدف من العمل: فحص تأثير الحرمان من الأمهات المبكر كنموذج لضغوط الحياة المبكرة على بنية الحُصين والتلفيف المسنن لنسل الجرذان البيضاء في مرحلة البلوغ وتقييم دور إعطاء فيتامين (ب١٢) بعد الفطام.

مواد و طرق البحث: تم تقسيم ثلاثين من ذكور جراء الجرذان البيضاء وأمهاتهم إلى ثلاث مجموعات متساوية. مجموعة التحكم (I) ؛ ظلت الجراء مع أمهاتها حتى الفطام، ثم تم فصلها حتى سن البلوغ. مجموعة الحرمان من الأمهات (II): تم حرمان الجراء من أمهاتهم لمدة ٢٤ ساعة في اليوم ٩ التالي للولادة ثم بقوا مثل المجموعة الضابطة حتى سن البلوغ. مجموعة الحرمان من الأمهات المكملة بفيتامين (ب١٢):خضعت الجراء إلى الحرمان من الأمهات كما هو موصوف في المجموعة الثانية ولكن تم الاحتفاظ بها على ٥٠ ميكروجرام من فيتامين (ب١٢) لكل كجم من الغذاء. تم الحصول على عينات المخ لمختلف التقنيات البيوكيميائية والنسيجية والهستوكيميائية المناعية.

النتائج: كشفت مجموعة الحرمان من الأمهات عن زيادة ذات دلالة إحصائية في مستوى مالونالديهيد النسيجي مع تغيرات نسيجية واضحة في الحُصين والتلفيف المسنن. ارتبط انخفاض كبير في التعبير المناعي لـ Bcl٢ بزيادة كبيرة في التعبير المناعي لـ GFAP. أظهرت مجموعة الحرمان من الأمهات المكملة بفيتامين (ب١٢) تحسن واضح للمعلمات المدروسة.

الاستنتاج: غيَّر الحرمان من الأمهات في وقت مبكر بنية الحُصين والتلفيف المسنن لذكور نسل الجرذ الأبيض في مرحلة البلوغ. لذلك يوصى بعلاقة صحية بين الرضيع والأم للحفاظ على الصحة العقلية للنسل. يمكن أن يكون العلاج المكمل بفيتامين (ب١٢) مفيدًا في تخفيف مثل هذه الآثار السلبية ربما من خلال تأثيره المضاد لموت الخلايا المبرمج، و وخصائص تثبيت الخلايا الدبقية و الحماية العصبية.