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Impact of Sleep Deprivation Combined with Cell Phone Radiation on Behavior, Cognitive Function, Neuroinflammation and Apoptosis in Rat Model: A Functional and Histological Study

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Abstract

Background: Sleep deprivation has become one of the most important problems affecting mood, learning, and memory function. On the other hand, the use of mobile phones has multiple hazards to neurological function, besides disturbing the physiological sleep pattern.

Aim of Study: This study assessed the effect of REM sleep deprivation combined with cell phone radiation on behavior and cognitive function and possible changes in markers of neuroinflammation and apoptosis in a rat model.

Material and Methods: Thirty-two adult female Wistar albino rats were assigned into 4 equal groups: (1) Control group, (2) REM sleep deprivation group, (3) Mobile EMR exposure group, and (4) the combined group. At the end of the study period, animals underwent behavior and cognitive function tests by open field and Y maze. Then rats were euthanized, the brain was excised, and prepared for histological assessment, ie.; H & E and measuring tissue levels of NFxB and CD45 by immunohistochemistry. REM sleep deprivation and mobile EMR exposure groups showed increased rearing and grooming in open field test, indicating higher anxiety.

Results: Ymaze test revealed a decreased percentage of correct alteration, indicating diminished cognitive function, where group 4 (combined group) was more affected. Significant increase in mean number of degenerated pyramidal cells and mean area % of NFxB and caspase-3 in the cerebral cortex in sleep deprivation, mobile EMR exposure, and combined groups. However, the combined group revealed a significant increase comparedto sleep deprivation, and mobile EMR exposure groups.

Conclusion: Both sleep deprivation and mobile exposure were able to induce cognitive dysfunction and hippocampal damage together with the increased number of degenerated cortical cells. Meanwhile, the most deleterious effect was observed in the combined group thus it may highlight the impaired neuronal assemblies (the basic units needed for the brain processing during wakefulness).

Key Words: REM sleep deprivation — EMR exposure — Neuroinflammation — Caspase-3 — NFxB.

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Introduction

SLEEP is a universal need of all higher life forms including humans, and humans spend about a third of their time asleep. Sleep deprivation refers to a sleep-time depression that cannot meet physiological needs due to several factors that cause various functional disorders such as endocrine, metabolic, cardiovascular, and nervous system diseases [1]. With the development of modem society and the quickening of the pace of life, sleep deprivation has become one of the most important problem affecting the work and life of human beings [2]. Non-rapid eye movement (non-REM) sleep is followed by a significantly shorter period of rapid eye movement (REM). Sleep deprivation, in particular, REM sleep deprivation, can affect mood, learning, and memory function and can even lead to the development of neuropsychiatric disorders such as Alzheimer's disease [3,4,5], Parkinson's disease **[6],** depression m.

Disturbance of sleep cycles and sleep deprivation alone are considered a type of stress having harmful physiological consequences.

Excessive use of mobile phones being a source of Electromagnetic radiation (EMR) poses multiple hazards to neurological function, besides disturbing the physiological sleep pattern. Exposure to EMR could disturb the redox environment of cells with excess production of reactive oxygen species (ROS) that can affect the different body systems in both animals and humans **[8].** The induced oxidative stress might affect postnatal development in the form of decreased weekly body weight gain and delay in anatomical and physiological development.

Material and Methods

Ethical approval:

Thirty-two adult female Wistar albino rats (130-150g) were involved in the study. The animals were bred and raised at the animal care unit of the faculty of medicine, Cairo University. This study period from June - Agust 2023. The animals were housed under ordinary living conditions (e.g., humidity, temperature, and light/dark cycles); with free access to rat chow and water throughout the study period. All animal procedures were in accordance with the Guide to the Care and Use of Experimental Animals available from the Canadian Council on Animal Care and approved by the Research Ethics Committee of 6th of October University (06U REC), number (PRE-Me-2306001).

Animal groups and the study protocol:

Rats were housed for 2 days before starting the study for acclimatization. The included rats were numbered and randomly assigned into 4 groups: (n=8 rats each). (1) The control group (control), (2) REM sleep deprivation group subjected to REM sleep deprivation for 72 hours, (3) Mobile EMR exposure group, the rats in this group were exposed to EMR of mobile phone for 2 hours a day, 6 days/week for four weeks and (4) combined group where animals were exposed to EMR of the mobile phone 6 days/week for four weeks duration, then 72 hours of REM sleep deprivation. All animal groups were maintained throughout the days of the experimental study without recorded death.

1- *Sleep deprivation:*

REM sleep deprivation was applied in groups 2 and 4 using the inverted pot technique **[9]** (Fig. 1), which was found to decrease REM sleep by about 90-99% in rats **[10].** Each cage was filled with water, and two small, inverted pots (6cm diameter) were fixed, so that the pot surface is 1cm above the water level. One rat was put in each cage. Pots allow the rat to sit and enter non-REM sleep. The principle of the model is that when muscle tone is lost in REM sleep, animals can't maintain their extended posture on such a small platform, so they fall off in the water and wake up, so REM sleep is prevented **[11].** No drowning was recorded as rats immediately wake up when their nose is immersed in water. Food and water were freely accessible.

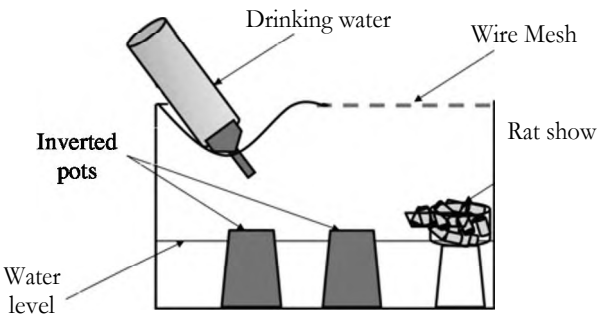


Fig. (1): Diagrammatic representation of a REM sleep depravation cage.

2- *Mobile EMR exposure:*

Rats of groups 3 and 4 were exposed to EMR from a cell phone, 2 hours a day, 6 days/week for 28 days **[12].** The used phone model (Huawei P9 smartphone) was of frequency 900-1800mHz at GSM (global system for mobile) mode; the value of specific absorption rate (SAR) was 0.620W/kg. The phone was put in the middle on top of the cage to ensure equal electromagnetic radiation to the body of all animals in the cage and was kept on continuously (autoredial) for 1 hour and 40 minutes daily and then in calling mode (to another phone number) for other 20min **[13].** Control group rats were put in a separate place to make sure that it is not exposed to any radiation.

3- *Assessment of cognitive function:*

Behavioral tests were conducted separately during a specific time range (9 a.m.-1 p.m.).

a- Open Field Test:

Open field is used for the assessment of anxiety levels, locomotion, and exploration eagerness.

The open field consists of a white square arena (50 x 40cm) with walls of 40cm height **[14].** On the day of the test, rats were transported to the testing room and left in their cages for 2h before the test. At the start of each session, the rat was placed in the center of the arena and allowed to explore for 5min **[15].** The rat's behavior was recorded for 5min using a camera fixed above the arena.

The apparatus was cleaned with 70% ethanol before placing each animal. The recorded videos were then analyzed, counting the number of lines crossed by the rat, the duration taken by each rat to leave the central square, and the number of grooming and rearing.

b- V-Maze Continuous Procedure:

For this test, the apparatus used consists of the Y-maze where the rat is placed in the center and the behavior was observed for 5min **[16].** The sequence of arm choices was recorded using a video camera. An arm entry was defined as the entry of four paws into the defined arm. Alternation was defined as multiple entries into the three arms (A, B, or C) on three overlapping sets.

4- *Sample collection and Histological preparation and Immunohistochemical evaluation:*

At the end of the experimental period, Brain tissue was obtained, fixed in 4% buffered paraformaldehyde, and processed into paraffin blocks. Five pm sections were cut and subjected to hematoxylin and eosin (H&E) staining and immunohistochemical staining with the avidin-biotin-peroxidase complex technique using: Anti-caspase-3 antibody (ab184787; a rabbit monoclonalantibody, Abcam, UK) and anti-NFxB antibody (ab16502; a rabbit monoclonal antibody; Abcam, UK). Sections were

placed in 10mM citrate buffer (pH 6, 25°C) for 10 minutes to perform antigen retrieval before being incubated with the primary antibodies overnight at 4°C. After that, slides were incubated for 20 minutes at 25°C (room temperature) with a secondary antibody (1:1000, Goat Anti-Rat IgG H&L, ab150165, Abcam). Immunohistochemical staining was identified using Mayer's hematoxylin as a counterstain and diaminobenzidine as a chromogen.

5- *Statistical analysis:*

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while non-parametric Kruskal-Wallis test and Mann-Whitney test were used for non-normally distributed quantitative variables **[17].** p-values less than 0 05 were considered statistically significant.

Results

Cognitive function assessment by open field and Y-maze tests:

Observing parameters of the open field revealed a significant increase in the number of line crossing in sleep deprivation and combined groups, and a significantly increased frequency of rearing and grooming in the sleep deprivation group, mobile EMR exposure group, and combined group in comparison to the control group ($p<0.05$) (Table 1). Also, there was a significant decrease in the percentage of spontaneous alternation (obtained from the Y-maze test) in rats of the tested groups compared to the control group ($p<0.05$) (Fig. 2). Indeed, number of rearing and grooming times in the combined group was significantly higher compared to sleep deprivation and mobile EMR exposure groups, and the percentage of spontaneous alternation in the mentioned group was significantly lower compared to the sleep deprivation and mobile EMR exposure groups ($p<0.05$).

Table (1): Assessments of cognitive functions in all studied groups using the open-field test.

	Control	Sleep deprivation	Mobile EMR exposure	Combined
No of line crossing	91.33±7.34	89±9 .27	58.67±6.38* #	56±5 .18*#
No of rearing	4.83±1.17	18±3.69*	19.5±2.74*	24.67±2.16*#$
No of grooming	2.83±0.75	6.83±1.17*	5.17±1.17*	9±1.41*#$

Values are presented as mean ± SD.
*: Statistically significant compared to the corresponding value in the control group ($p<0.05$).
#: Statistically significant compared to the corresponding value in the sleep deprivation group ($p<0.05$).
$: Statistically significant compared to the corresponding value in the Mobile EMR exposure group ($p<0.05$).

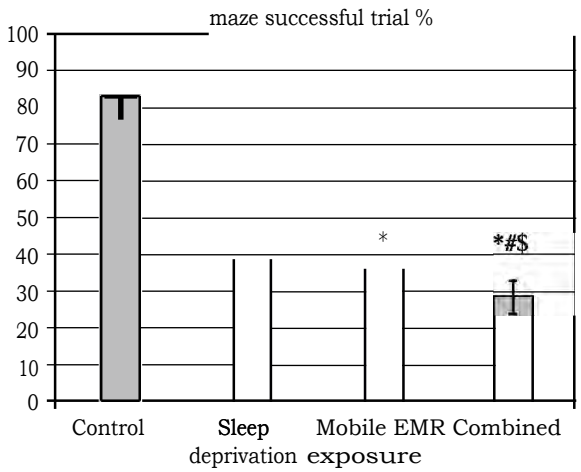


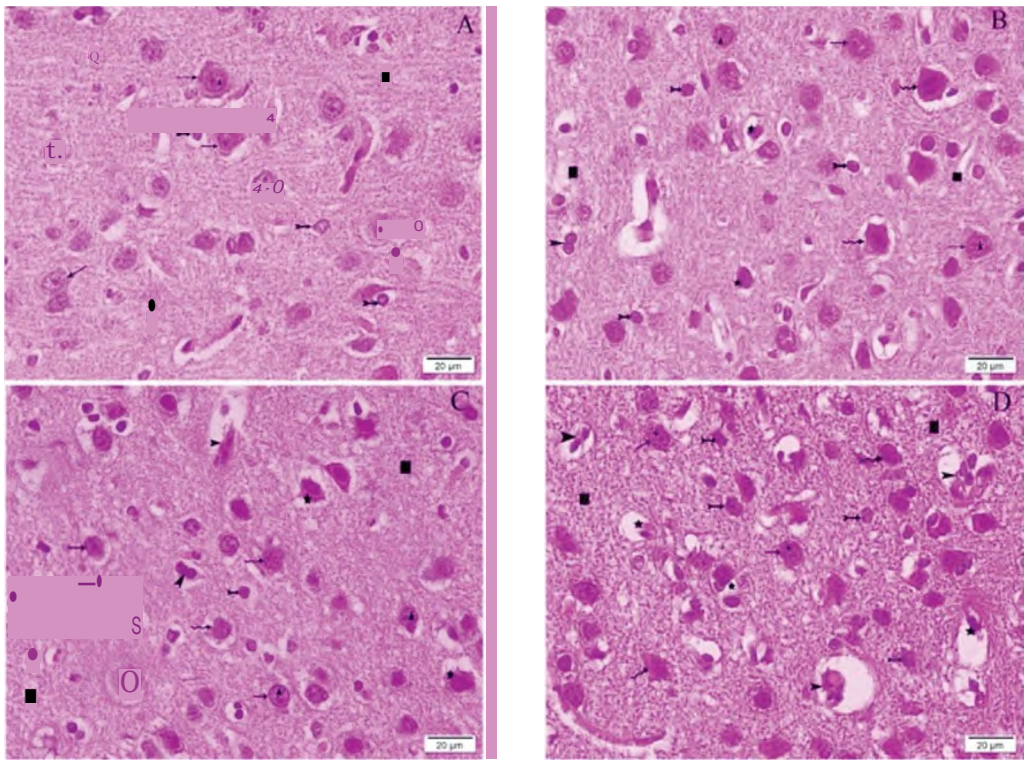
Fig. (2): Assessments of cognitive functions in all studied groups using the Y-maze test.

Values are presented as mean ± SD.
*: Statistically significant compared to the corresponding value in the control group ($p<0.05$).
#: Statistically significant compared to the corresponding value in the sleep deprivation group ($p<0.05$).
$: Statistically significant compared to the corresponding value in the Mobile EMR exposure group ($p<0.05$) (n=8).

Histological results:

Examination of the cerebral cortex in the control group revealed homogenous acidophilic neuropil with neuroglial cells and large pyramidal cells having basophilic cytoplasm and pale vesicular nucleus with prominent nucleolus. Sleep deprivation and Mobile EMR exposure groups exhibited pale vacuolated neuropil with a large number of neuroglial cells. Some pyramidal cells are shrunken and surrounded with a halo, a few degenerated with dark nuclei, few cells are basophilicwith pale vesicular nuclei. Multinucleated cells could be detected. Both groups exhibited a significant increase inthemean number of affected cells in the cerebral cortex when compared to the control group. In addition, the combined group showed pale vacuolated neuropil with a large number of neuroglial cells. Many pyramidal cells are either degenerated with dark nuclei or shrunken surrounded with a halo associated with a significant increase inthe mean number of abnormal cells when compared to control, Sleep deprivation, and Mobile EMR exposure groups. Only a few pyramidal cells are basophilic with pale vesicular nuclei. Many multinucleated cells could be detected (Figs. 3 ,5A).

Fig. (3): Photomicrograph for Hematoxylin and Eosin sections in the cerebral cortex of (x400):



A: Control group demonstrating homogenous acidophilic neuropil (■) with neuroglial cells (bifid arrows) and large pyramidal cells (arrows) having basophilic cytoplasm and pale vesicular nucleus with prominent nucleolus (A).

B: Sleep deprivation group revealing acidophilic neuropil (•) with large number of neuroglial cells (bifid arrows). Some pyramidal cells are degenerated with dark nucleus (wavy arrows), few cells are shrunken and surrounded with a halo (astrex) and some cells are basophilic (arrows) with pale vesicular nuclei (A). Multinucleated cells could be detected (arrowhead).

C: Mobile EMR exposure group exhibiting pale vacuolated neuropil (■) with large number of neuroglial cells (bifid arrows). Many pyramidal cells are shrunken surrounded with a halo (astrex), few degenerated with dark nucleus (wavy arrows), few cells are basophilic (arrows) with pale vesicular nuclei (A). Note the presence of multinucleated cells (arrowheads).

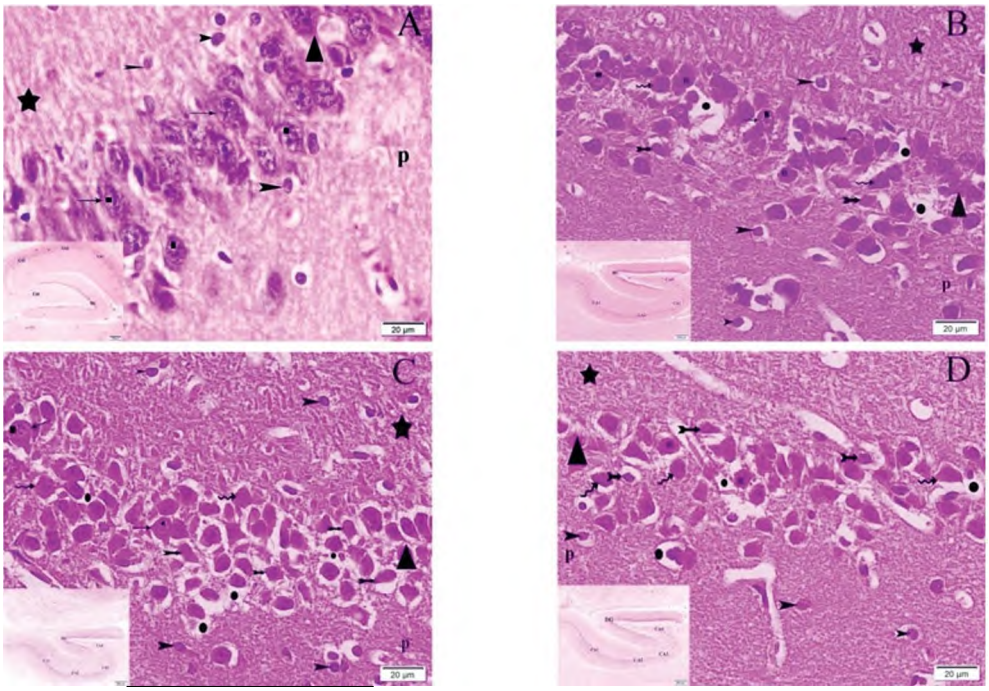
D: Combined group showing pale vacuolated neuropil (•) with large number of neuroglial cells (bifid arrows). Many pyramidal cells are either degenerated with dark nucleus (wavy arrows) or shrunken surrounded with a halo (astrex). Only few pyramidal cells are basophilic (arrows) with pale vesicular nuclei (A). Many multinucleated cells could be detected (arrowheads).

Hippocampal examination ofthe control group exhibited Comu Ammonis (CA) divided into CA1, CA2, CA3, and CA4. The Dentate gyrus (DG), which surrounds CA4, could be seen by its upper and lower extremities. Inside the concavity of the CA and DG, the Molecular Layer could be seen. The CA1 region was seen formed of Pyramidal, Molecular, and Polymorphic layers. The neuropil in the polymorphic and molecular layers had neuroglial cells with pale vesicular nuclei. Pyramidal cells were present in the pyramidal layerhaving pale vesicular nuclei and prominent nucleoli. However, the sleep deprivation and the mobile EMR exposure groups showed a shrunken hippocampus withmany neuroglial cells with pale vesicular nuclei in the neuropil of polymorphic and molecular layers. Many pyramidal cells in the pyramidal layer had pale vesicular nuclei and prominent nucleoli. Some pyramidal cells are either degenerated with dark nuclei or shrunken with pericellular halo associated with a significant increase in the mean number of degenerated cells in comparison with the control group. In addition, the hippocampus of the combined group was shrunken with many pyramidal cells in the pyramidal layer were either degenerated with a dark nucleus or shrunken with a pericellular halo. Few pyramidal cells had pale vesicular nuclei and prominent nucleoli. The mean number of abnormal cells in the hippocampus of the combined group revealed a significant increase when compared to the control and sleep deprivation groups (Figs. 4,5B).

Immunohistochemical staining:

Examination of the cerebral cortex and CA1 area of the hippocampus in the control group revealed negative Caspase-3 and NFxB immunostaining. Sleep deprivation and mobile EMR groups exhibited a significant increase in mean area % of caspase-3 and NFxB immunostaining when compared to the control group. In addition, the combined group revealed marked cytoplasmic and nuclear caspase-3 and NFxB immunostaining associated with a significant increase of mean area % in comparison with control, sleep deprivation, and mobile EMR groups (Figs. 6, 8C; caspase-3 immunostaining, Figs. 7, 8D; NFxB immunostaining).

Fig. (4): Photomicrograph for Hematoxylin and Eosin sections in the Hippocampus of (x400 & inset x40):



A: Control group: Cornu Ammonis (CA) can be seen as CAl, CA2, CA3, and CA4. The Dentate gyrus (DG), which surrounds CA4, can be seen by its upper and lower extremities. Inside the concavity of the CA and DG, the Molecular Layer (astrex) is seen. Pyramidal layer (A), Molecular layer (astrex), and Polymorphic layer (P) are the three layers that make up the CAl region. The neuropil in the polymorphic (P) and molecular (astrex) layers contains neuroglial cells (arrowheads) with pale vesicular nuclei. Pyramidal cells (arrows) are present in the pyramidal layer *(A)* having pale vesicular nuclei (■) and prominent nucleoli.

B: Sleep deprivation group: Cornu Ammonis (CA) of the shrunken hippocampus is divided into CAl, CA2, CA3, and CA4. Outside the CA4's upper and lower extremities is the dentate gyrus (DG). Many neuroglial cells (arrowheads) with pale vesicular nuclei are seen in the neuropil of polymorphic (P) and molecular (astrex) layers. Many pyramidal cells in the pyramidal layer *(A)* have pale vesicular nuclei (■) and prominent nucleoli. Some pyramidal cells are either degenerated (wavy arrows) with dark nuclei (n) or shrunken (bifid arrows) with pericellular halo (0).

C: Mobile EMR exposure group: The hippocampus is shrunken and divided into CAl, CA2, CA3, and CA4. Outside the CA4's upper and lower extremities is the dentate gyrus (DG). The neuropil in the polymorphic (P) and molecular (astrex) layers contains many neuroglial cells (arrowheads) with pale vesicular nuclei. Many pyramidal cells in the pyramidal layer *(A)* are either degenerated with dark nucleus (wavy arrows) or shrunken (bifid arrows) with pericellular halo (•). Few pyramidal cells (arrows) have pale vesicular nuclei (■) and prominent nucleoli.

D: Combined group: The hippocampus's Cornu Ammonis (CA) is made up of CAl, CA2, CA3, and CA4. The dentate gyrus (DG) is located outside the CA4's upper and lower extremities. The polymorphic (P) and molecular (astrex) layers exhibits many neuroglial cells (arrowheads) with pale vesicular nuclei. The pyramidal layer *(A)* reveals either degenerated pyramidal cells (wavy arrows) with dark nuclei (n) or shrunken cells (bifid arrows) with pericellular halo (•).

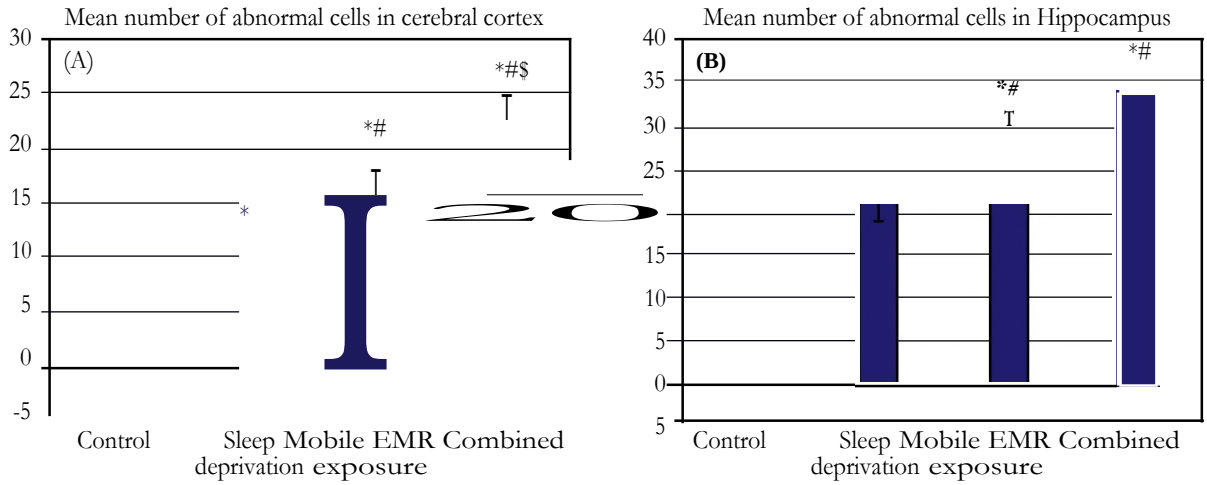


Fig. (5): (A) Histogram: Mean number of degenerated pyramidal cells in the cerebral cortex. (B) Histogram: Mean number of degenerated pyramidal cells in Hippocampus.

Values are presented as mean ± SD.

*: Statistically significant compared to the corresponding value in the control group ($p<0.05$).

#: Statistically significant compared to the corresponding value in the sleep deprivation group ($p<0.05$).

$: Statistically significant compared to the corresponding value in the Mobile EMR exposure group ($p<0.05$) (n=8).

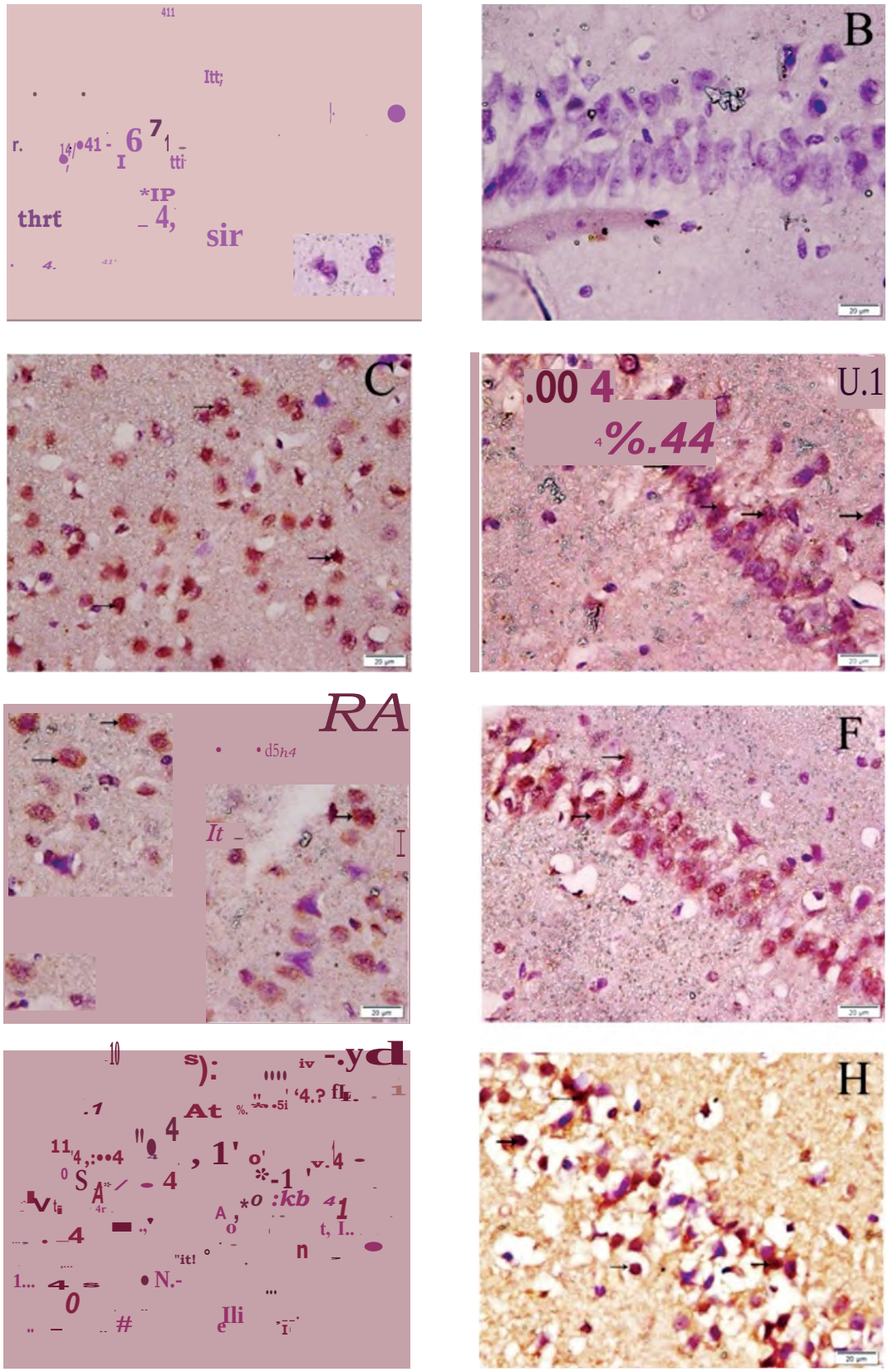


Fig. (6): A photomicrograph in cerebral cortex and CAl area of the Hippocampus immunostained by Caspase-3 (x400). A,B: Control group reveals negative caspase-3 immunostaining C,D: Sleep deprivation group shows some caspase-3 immunopositive cells (arrows). E,F: Mobile EMR exposure group demonstrates few caspase-3 immunopositive cells (arrows). G,H: Combined group: demonstrates many caspase-3 immunopositive cells (arrows).

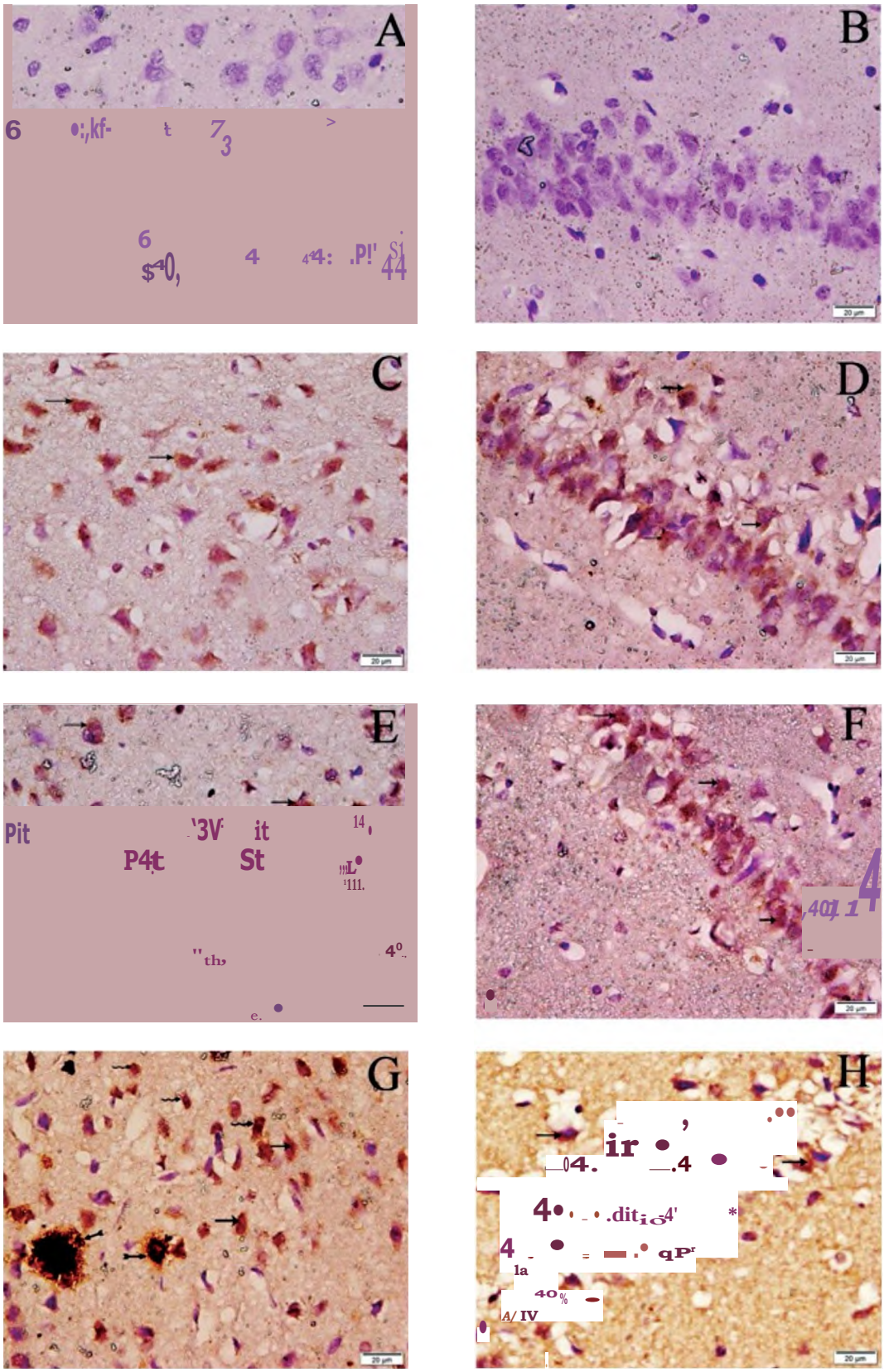


Fig. (7): A photomicrograph in cerebral cortex and CAl area of the Hippocampus immunostained by NFxB (x400). A,B: Control group exhibits negative cytoplasmic NFxB immunostaining. C,D: Sleep deprivation group reveals moderate cytoplasmic NFxB immunostaining (arrows). E,F: Mobile EMR exposure group shows minimal cytoplasmic NFxB immunostaining (arrows) G,H: Combined group: Demonstrates marked cytoplasmic (arrows) and nuclear (wavy arrows) NFxB immunostaining Note the presence of giant cells with marked cytoplasmic NFxB immunostaining (bifid arrows).

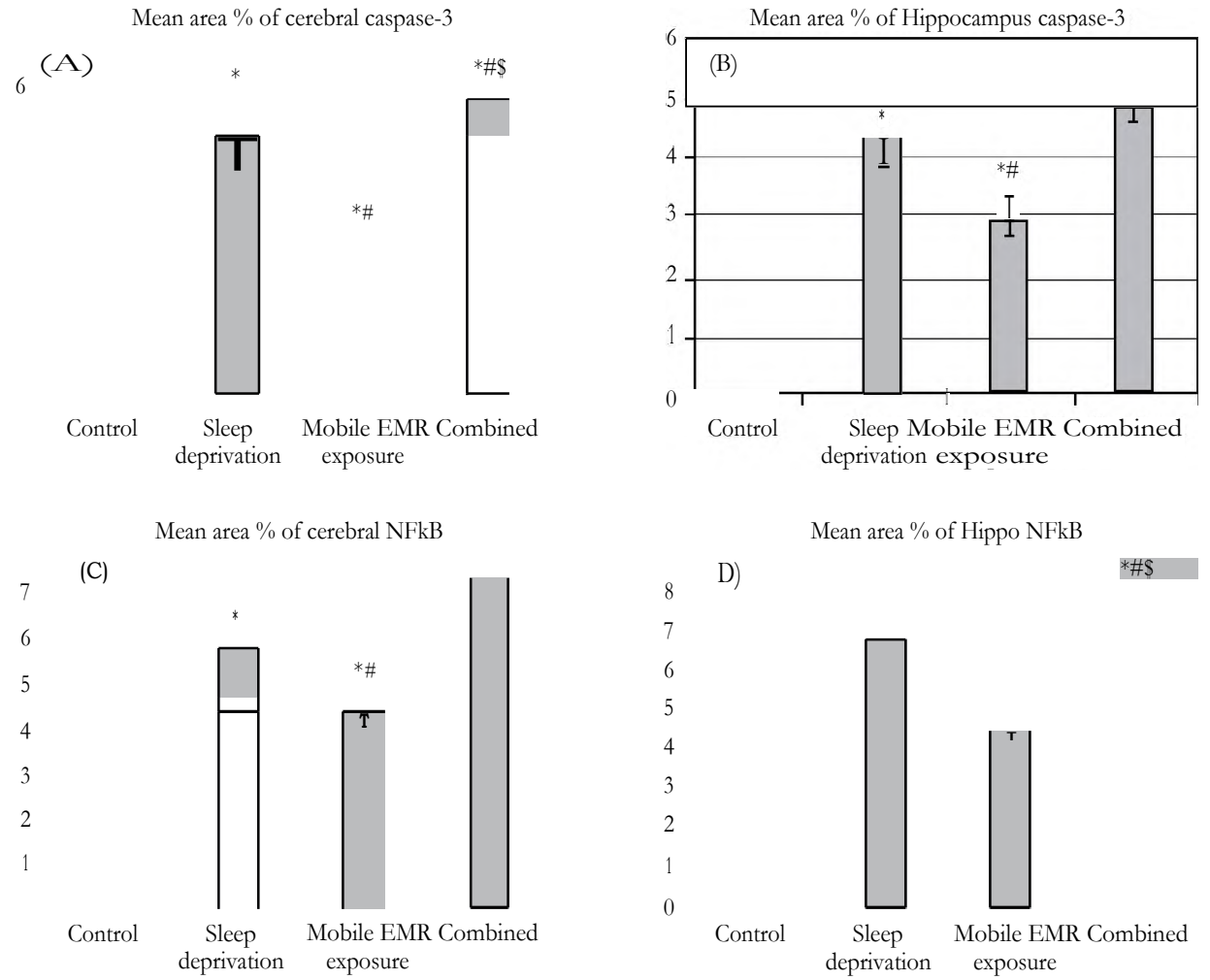


Fig. (8): (A,B) Histogram: Mean Area % of caspase-3 immunoreactivity in the cerebral cortex and Hippocampus. (C,D) Histogram: Mean Area % of NFxB immunoreactivity in the cerebral cortex and Hippocampus.

Values are presented as mean ± SD.
*: Statistically significant compared to the corresponding value in the control group ($p<0.05$).
#: Statistically significant compared to the corresponding value in the sleep deprivation group ($p<0.05$).
$: Statistically significant compared to the corresponding value in the Mobile EMR exposure group ($p<0.05$) (n=8).

Discussion

In the present study, we investigated the effects of sleep deprivation combined with cell phone radiation on the behavior and cognitive function of rats, in addition to the effects on brain histology and immunohistochemical markers of neuroinflammation (NFxB and Caspase-3).

Our results showed an increase in the rats' locomotor activity tested by an open field, evidenced by increased line crossing in rats exposed to REM sleep deprivation, alone or together with EMR exposure, while EMR exposure alone hasn't significantly affected the locomotor activity. The open field also revealed increased grooming and rearing (supported against the wall of the maze) in rats exposed to REM sleep deprivation and/or EMR exposure, denoting increased anxiety.

In concordance with our findings, Daniels et al., **[18]** and Sultangaliyeva et al., **[19]** found that rats-exposed to mobile phone EMR showed increased grooming, in the open field. According to these findings, Daniels et al., **[18]** suggested that EMR exposure may lead to brain function abnormalities. However, in these studies, contrary to our results, locomotor activity was suppressed in rats exposed to EMR. This may be related to different strains, mobile model, and/or longer duration of exposure.

In studies of Kim et al., **[20]** and Leem et al. **[21],** REM sleep deprivation increased locomotion in the open-field test, which together with other findings was referred to as "manic-like behaviors". Furthermore, Tartar et al., **[22]** reported increased anxiety in open field testing of rats exposed to sleep deprivation. REM sleep deprivation significantly increased the mitochondrial DNA copy number, and the protein expression of COX4I1 in the hippocampus **[20].**

The Y-maze test is a tool for measuring the willingness of rodents to explore new environments. Rodents usually prefer to investigate a new arm in the maze rather than returning to the previously visited one **[23].** It accentuates visuospatial tasks as well as hippocampus-dependent tasks **[24].** It's also used to assess short-term spatial working memory **[25].**

In agreement with our results of REM sleep deprivation, Soto-Rodriguez et al. [26] stated that 72 hours of REM sleep deprivation induces spatial memory impairment, reduction in the number of hippocampal BrdU + cells, and persistent apoptosis rate. These effects persisted for at least 3 weeks after sleep deprivation. Also in mice sleep-deprived for 48 and 72 hours, showed worse performance in working memory as evaluated by the Y-maze test, compared to control mice **[27].**

REM sleep deprivation impairs spatial memory performance in rats **[28],** and 72 hours REM sleep deprivation induces an impairment effect on spatial learning and memory of rats in the Morris water maze apparatus **[29].** Even brief periods of sleep deprivation can disrupt consolidation of hippocampus-dependent associative and spatial learning in rodents **[30,31].**

It has been declared that sleep deprivation suppresses neurogenesis induced by hippocampus-dependent learning in rats **[32].**

On the contrary, some reports showed an improvement / or no impairment effect of sleep deprivation on learning and memory in splenectomized rats **[33],** normal rats **[34]** and it even stimulated hippocampal neurogenesis in rats after cerebral ischemia/reperfusion **[35].** However, these studies used acute short-term sleep deprivation for 24 hours or less, which is different from our protocol.

Histological examination of the cerebral cortex and hippocampus in the sleep deprivation group revealed some shrunken pyramidal cells surrounded with a halo, and few degenerated with a dark nucleus, associated with a significant increase of the mean number of degenerated pyramidal cells when compared to the control group. A previous study by Sexton et al., 2014 stated that groups who live in communities exhibited a longitudinal association between cortical atrophy and sleep quality **[36].** It has been reported that sleep deprivation induces hippocampal neuronal apoptosis, neurodegeneration, and microglial activation. Through the noradrenaline-mediated activation of Na+-K+ ATPase activity, REM sleep deprivation has been observed to increase brain excitability. Lack of sleep can damage the brain's metabolite clearance system and repair system, leading to oxidative stress and neuronal impairment **[37,38].**

Lack of sleep leads to neuronal death, which is with the Bcl-2/Bax ratio and the mitochondria-mediated apoptosis pathway. New evidence established that the destruction of the permeability of mitochondrial membranes required the production of the Box monomer. In addition, the activation of caspase-3 and caspase-9 led to a significant decrease in the flexibility of neuronal function and neurodegenerative lesions, which in turn led to several cognitive dysfunctions. Cyclic AMP, a crucial physiological signaling molecule, participates in numerous pathways that regulate apoptosis. PKA and CREB are phosphorylated by intracellular cAMP, which activates them. Activated p-CREB can then drive the transcription of several downstream genes, increasing the levels of Bcl-2 family proteins and speeding up the progression of apoptosis **[39,40].** This was detected in our study where degenerated pyramidal cells showed a significant increase in the sleep deprivation group when compared to the control group associated with a significant increase in mean area% of caspase-3.

It has been found that sleep deprivation increased the mean area % of NFxB immunostaining when compared to the control group. This could be explained by inducing inflammatory response through inducing cytokine release as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF), and interleukin-6 (IL-6) through activating NFxB which induces neuronal apoptosis, cerebral and hippocampal thinning In addition, melatonin disruption could lead to immunological responses that increase brain inflammation **[41-43].**

In the current work, the groups exposed to either sleep deprivation or mobile EMR showed a significantly decreased percentage of spontaneous alteration in the Y-maze test in comparison to control groups.

Mobile phone exposure negatively impacted the acquisition of learned responses in Wistar rats, tested by the Morris water maze **[44].** This indicated poor spatial navigation in the phone-exposed animals. In another study, adult rats exposed to 10 minutes of open call daily for 1 month, showed a significant decrease in the number of right arm entries in the eight-arm maze, and after two months there was a more significant decrease **[45].** The exposure of rats to EMR caused disturbances in monoamine neurotransmitters and this may underlie many adverse effects of EMR including memory, learning, and stress **[46].**

Gupta et al., **[47]** reported that EMR of 2450 MHz exposure causes mitochondrial dysfunction and activation of the intrinsic pathway of apoptosis in rats which results in cognition deficits However, EMR-900 and EMR-1800 exposure did not show any effect on cognitive function assessed by Y-maze in their study. They used a different study design where the emission of EMR was controlled by an

electromagnetic radiation exposure system instead of the mobile phone used in our study, and the daily exposure duration was only 1 hour, while we exposed the rats for 2 hours/ day.

Exposure to mobile EMR demonstrated some degenerated pyramidal cells with dark pyknotic nuclei and a few shrunken cells surrounded by a halo. Excess production of reactive oxygen species (ROS) during exposure to electromagnetic radiation (EMR) is closely associated with neuronal cell apoptosis. Increased ROS production has the potential to cause oxidative stress, inflammation, and alterations in antioxidant defense mechanisms Numerous studies have demonstrated that tissues experienced oxidative damage as a result of electromagnetic radiation. Malondialdehyde, catalase, and superoxide dismutase concentrations at higher levels may aid in assessing the relationship between apoptosis and radiation **[48,49].**

The effects of radiation in mobile phones include stress response, inflammation, proliferation, and apoptosis (P53, BAD, NF-kB, p-NF-kB, and p-STAT3). Apoptosis is a programmed cell death which is the mechanism used by mammalian cells to respond to any biological stress, aging, and cell damage. One of the important regulators of the intrinsic pathway of apoptosis is the tumor suppressor protein P53. P53 is activated by phosphorylation in response to DNA damage or any other genomic abnormality, which causes cell cycle arrest and DNA repair or apoptosis. P53's (p-P53's) phosphorylation occurs at Ser20 and Ser15 impairing the interaction between P53 and MDM2, a negative regulator of P53, which results in P53 overexpression. activation and accumulation. Following P53 activation, the apoptosis intrinsic pathway takes place **[50,51].**

According to evidence, caspase-3 is essential for a variety of events, including cell death, the breakdown of certain cells, and the production of apoptotic bodies **[52].** Additionally, caspase-3 participates in cellular remodeling procedures. It is widely known that proapoptotic chemicals such as cytochrome c produced from mitochondria can activate caspase-3 **[53,54].** A study by Xing et al., **[55].** Revealed that EMR-induced cytochrome c release and caspase-3 activation demonstrated in our study where the exposure to mobile EMR revealed a significant increase of mean area % of caspase-3 in the cerebral cortex and hippocampus in comparison with the control group.

The mean area % ofNFxB in the mobile EMR group was significantly increased when compared to the control group. Akefe et al., **[56].** Stated that exposure to EMR causes a significant increase in TNF-a levels TNF-a activation may lead to the activation of NFxB, which is well known for its role in the control of immune response and inflammation. IxB family members, which are NFxB inhibitory molecules, are polyubiquitinated and phosphorylated to limit NFxB activity. IxB is phosphorylated in response to an initiating signal, which causes its polyubiquitination and subsequent destruction. The released NFxB travels to the nucleus where it attaches to the precise DNA sequences of the genes that it controls, including those for immunoreceptors, cytokines, chemokines, and genes that control proliferation and death. The transcription of these target genes may be up- or down-regulated by the phosphorylation of NFxB subunits **[57].**

To our knowledge, this is the first work that studies the combined effect of REM sleep deprivation with mobile EMR exposure on rats' locomotor activity, anxiety, and spatial memory. Combining REM sleep deprivation and EMR exposure in our study, showed significantly increased grooming and supported rearing, indicating increased anxiety in comparison to either REM sleep deprivation or EMR exposure alone. In addition, in Y-maze, the combined group showed a significantly decreased percentage of spontaneous alteration in comparison to the REM sleep deprivation group and EMR exposure group, indicating a significant decrease in spatial short-term memory and exploration behavior in rats.

These findings were associated with a significant elevation of degenerated pyramidal cells and mean area % of caspase-3 and NFxB in the combined group in comparison with control, sleep deprivation, and mobile EMR groups. This could be explained by the synergistic effect of sleep deprivation and electromagnetic radiation on the cerebral cortex and hippocampus.

Conclusion:

The diminished cognitive functions together with the significant increase in the mean number of degenerated pyramidal cells and the area % of NFxB and caspase in the cortical and hippocampal were detected in both sleep deprivation and mobile EMR exposure groups. However, the combined SD, and mobile EMR exposure had a synergistic effect that was observed functionally and at cellular levels.

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دراسه التاثير المشترك للحرمان من النوم واشعاعات الهواتف الخلويه على الوظائف السلوكيه والمعرفيه والالتهابات العصبيه وموت الخلايا المبرمج فى نموذج الجرذان دراسه وظيفيه وخلويه

الخلفيه البحثيه: اصبح الحرمان من النوم احد اهم المشاكل التى تؤثر على الحاله المزاجيه والقدره على التعلم والذاكرة، كما اصبح لاستخدام الهواتف الخلويه العديد من التاثيرات على الوظائف العصبية بجانب التاثير على النمط الفسيولوجى للنوم

الهدف من البحث: دراسة التاثير المشترك للحرمان من النوم مع اشاعات الهواتف الخلويه على الوظائف السلوكيه والمعرفيه والتغييرات فى دلالات الالتهابات العصبيه وموت الخلايا المبرمج فى نموذج الجرذان

طرق البحث: تم تقسيم ٣٢ انثى جرذبالغه الى اربعه مجموعات متساويه وهم مجموعه الحكم ومجموعه الحرمان من النوم، مجموعه التعرض لاشعاعات الهواتف الخلويه للموجات الكهرومغناطيسيه والمجموعه التعرض المشترك. بعد ذلك تم ذبح الجرذان واستخراج المخ وتجهيزه للفحص النسيجى وقياس مستويات الانسجه من دلالات الالتهاب

النتائج: اظهر اختبار متاهه «Y» انخفاض نسبة التبادل الصحيح وهو ما يدل على انخفاض الوظائف المعرفيه خصوصاً فى المجموعه الرابعه (التاثير المشترك) كما كان هناك ارتفاعا ملحوظا في متوسط اعداد الخلايا الهرميه المضمحله وارتفاع نسبياً فى دلالات دلالة NFkB والكاسبيز ٣ فى القشره المخيه فى المجموعات الثلاث خلاف مجموعه الحكم.

الاستنتاج: كلا من الحرمان من النوم والتعرض لاشعاع الهواتف الخلويه قادر على الاخلال فى الوظائف المعرفيه واحداث ضرر فى منطقه الهيبوكامبس مع زياده اعداد خلايا القشرة المخية المضمحلة الا ان التاثير الاكبر لوحظ فى مجموعه التعرض المشترك.