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Research Article

Impact of Sleep Deprivation on the Central Nervous System Neurotransmitters and Immune Function in Male Albino Rats

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Abstract

Background: Sleep deprivation adversely affects both the central nervous system and immune function, leading to potential alteration in behavioral and physiological responses. ***Objectives***: To evaluate the impact of varying durations of sleep deprivation on brain neurotransmitters and immune responses in male albino rats. ***Methods***: A total of 32 rats were allocated into four groups: control, 18-hour, 24-hour, and 72-hour sleep deprivation. Sleep deprivation was induced using gentle handling and environmental noise. Brain levels of serotonin, GABA, and glutamate were measured, as well as serum markers including TNF-α, interleukin-6 (IL-6), and total antioxidant capacity (TAC). Inflammatory responses were also assessed through complete blood counts. Brain tissues were processed using standard histological techniques and stained with H&E, followed by semi-quantitative lesion scoring using image analysis software based on neuroglial pyknosis, vascular congestion, and perivascular edema, graded on a 0–100% scale. ***Results***: *S*erotonin levels initially decreased after 18 hours of sleep deprivation but normalized by 24 and 72 hours. Glutamate levels rose progressively with longer deprivation, while GABA, TAC, and IL-6 remained stable across all durations. Sleep deprivation triggered time-dependent immune changes, beginning with lymphocyte alterations and progressing to increased monocytes and inflammatory ratios, along with elevated TNF-α levels. Histopathology showed time-dependent brain damage from sleep deprivation, with severe vascular and glial changes at 72 hours, aligning with inflammatory marker elevations. ***Conclusions***: Sleep deprivation induces specific, duration-dependent alterations in neurotransmitter and immune profiles. Prolonged deprivation, especially at 72 hours, may provoke neuroinflammatory and anxiety-related behavioral changes.

Keywords: Albino Rats, Immune response, Neurotransmitters, Neuroinflammation, Sleep deprivation.

تأثير الحرمان من النوم على النواقل العصبية للجهاز العصبي المركزي والوظيفة المناعية لدى ذكور الجرذان البيضاء

الخلاصة

الخلفية: يؤثر الحرمان من النوم سلبا على كل من الجهاز العصبي المركزي ووظيفة المناعة ، مما يؤدي إلى تغيير محتمل في الاستجابات السلوكية والفسيولوجية. **الأهداف**: تقييم تأثير فترات متفاوتة من الحرمان من النوم على النواقل العصبية في الدماغ والاستجابات المناعية لدى ذكور الفئران المهقية.**الطرائق**: تم توزيع ما مجموعه 32 فأرا إلى أربع مجموعات: مجموعة التحكم، ومجموعة الحرمان من النوم لمدة 18 ساعة، و 24 ساعة، و 72 ساعة. تم تحفيز الحرمان من النوم باستخدام التعامل اللطيف والضوضاء البيئية. تم قياس مستويات السيروتونين وGABA والغلوتامات في الدماغ، بالإضافة إلى علامات المصل بما في ذلك TNF-α و interleukin-6 (IL-6) والقدرة الإجمالية المضادة للأكسدة (TAC). كما تم تقييم الاستجابات الالتهابية من خلال تعداد الدم الكامل. تمت معالجة أنسجة المخ باستخدام تقنيات نسيجية قياسية وصبغها ب H & E، متبوعا بتسجيل الآفات شبه الكمية باستخدام برنامج تحليل الصور القائم على البكنوس العصبي، واحتقان الأوعية الدموية، والوذمة حول الأوعية الدموية، متدرجة على مقياس 0-100٪. **النتائج**: انخفضت مستويات السيروتونين في البداية بعد 18 ساعة من الحرمان من النوم ولكنها عادت إلى طبيعتها بمقدار 24 و 72 ساعة. ارتفعت مستويات الغلوتامات تدريجيا مع الحرمان لفترة أطول، بينما ظلت GABA و TAC و IL-6 مستقرة طوال جميع الفترات. أدى الحرمان من النوم إلى تغيرات مناعية تعتمد على الوقت، بدءا من تغيرات الخلايا الليمفاوية والتقدم إلى زيادة الخلايا الوحيدة والنسب الالتهابية، إلى جانب ارتفاع مستويات α عامل نخر الورم. أظهر تحليل الأنسجة المرضي تلفا دماغيا يعتمد على الوقت من الحرمان من النوم، مع تغيرات شديدة في الأوعية الدموية والدبقية عند 72 ساعة، تتماشى مع ارتفاعات علامات الالتهاب. **الاستنتاجات**: يؤدي الحرمان من النوم إلى تغييرات محددة تعتمد على المدة في الناقل العصبي والملامح المناعية. قد يؤدي الحرمان المطول، خاصة لمدة 72 ساعة، إلى تغيرات سلوكية مرتبطة بالالتهابات العصبية والقلق.

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INTRODUCTION

Sleep is essential for maintaining brain function, immune health, metabolism, and emotional regulation. However, sleep deprivation (SD) has become increasingly common due to lifestyle factors and is linked to various health issues, including cognitive decline, mood disorders, and weakened immunity [1]. Key neurotransmitters (serotonin, GABA, and glutamate) play crucial roles in sleep regulation and brain balance. SD disrupts these neurotransmitters, often decreasing serotonin and GABA while increasing glutamate, which can lead to behavioral and cognitive disturbances [2]. Additionally, SD negatively affects immune function

by promoting inflammation through elevated cytokines like TNF-α, contributing to increased disease risk. Sleep deprivation is a significant public health issue, with 30–35% of adults sleeping less than 6 hours per night and 10–15% experiencing chronic insomnia, while 25–35% report occasional insomnia [3]. Studies also indicate a reduction in average sleep duration by up to 18 minutes per night over the past three decades [4]. Sleep deprivation disrupts serotonin (5-HT) synthesis and release by reducing the availability of tryptophan—due to elevated cortisol—and by decreasing the activity of tryptophan hydroxylase (TPH), a key enzyme in serotonin production. It also impairs the function of serotonergic neurons in the raphe nuclei, lowering extracellular serotonin levels in regions like the prefrontal cortex and hippocampus [5]. Additionally, sleep deprivation increases the expression of the serotonin transporter (SERT), which enhances serotonin reuptake and depletes synaptic levels [6]. GABA, the brain's main inhibitory neurotransmitter, is crucial for regulating sleep. It is synthesized from glutamate via glutamic acid decarboxylase (GAD), particularly GAD65 and GAD67, which play roles in synaptic and metabolic GABA production, respectively. GABA acts through ionotropic and metabotropic receptors, with GABA being central to sleep regulation. Sleep deprivation is associated with reduced GABAergic tone, which contributes to cortical hyperexcitability and impaired sleep [7]. Glutamate serves as the principal excitatory neurotransmitter and is crucial to learning and memory processes [8]. Sleep deprivation elevates extracellular glutamate levels by impairing its clearance, especially via astrocytic excitatory amino acid transporters (EAAT1 and EAAT2), leading to excitotoxicity and neuronal stress [9]. This imbalance contributes to disrupted neural plasticity and cognitive deficits associated with sleep loss. Sleep deprivation (SD) induces oxidative stress mainly through increased ROS production and impaired clearance [10,11]. Contributing factors include elevated metabolism, stress hormone activation, mitochondrial dysfunction, endoplasmic reticulum impairment, and gut dysbiosis via Nox enzyme activation [10]. Normally, sleep reduces glucose metabolism and ROS generation, but SD reverses this, leading to greater oxidative damage and higher sensitivity to acute oxidative stress [10,12]. Sleep deprivation (SD) causes significant brain damage, particularly in regions like the hippocampus, prefrontal cortex, and amygdala. Key histopathological changes include neuronal loss (via apoptosis and necrosis), oxidative stress (marked by increased ROS and MDA and reduced antioxidants like SOD and GSH) [13], and neuroinflammation, with elevated pro-inflammatory cytokines and microglial activation. Synaptic dysfunction is also observed, with reduced synaptic proteins (synaptophysin, PSD-95) and dendritic spine loss [14,15]. SD disrupts blood-brain barrier integrity, allowing harmful substances to enter the brain [16], and causes mitochondrial damage, impairing synaptic energy metabolism [17]. These effects collectively increase vulnerability to neurodegenerative diseases like Alzheimer's, linked to impaired clearance of β-amyloid and tau proteins [15,18]. Several key factors contribute to sleep deprivation. Chronic health conditions such as sleep apnea, asthma, diabetes, and chronic pain can disrupt sleep. Disruptions in circadian rhythms, especially in conditions such as Alzheimer's and Parkinson's disease, also play a role. Shift work and long working hours disrupt circadian rhythms, especially among healthcare workers. Adolescents are especially vulnerable due to social jetlag and early school start times, compounded by excessive screen exposure before bed. Additionally, the use of substances like alcohol and caffeine can interfere with sleep patterns [19]. Sleep deprivation compromises both innate and adaptive immunity, increasing vulnerability to infections and chronic inflammation. It elevates pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interleukin-1β(IL-1β) through activation of the NF-κB pathway, a key mediator of stress-induced inflammation [20]. The objectives of this study are to investigate how sleep deprivation affects central nervous system (CNS) function by measuring brain levels of serotonin, GABA, and glutamate, and to examine its impact on immune function by quantifying pro-inflammatory biomarkers such as TNF-α and IL-6, along with other blood-derived inflammatory markers.

METHODS

Study design and ethical approval

This is an experimental study design. Male Wistar Albino rats, weighing between 230 and 350 grams, were procured from the animal facility of the University of Tikrit and accommodated in the College of Pharmacy at the University of Sulaimani. The subjects were housed in well-ventilated plastic enclosures under standardized laboratory settings, maintaining a temperature of 25±2°C and a consistent light-dark cycle. The animals were given a regular pellet meal and had unrestricted access to water. A two-week acclimatization phase preceded the commencement of the experiment. All procedures were conducted in compliance with the Guidelines for Animal Experimentation and received approval from the Ethical Committee of the College of Pharmacy, University of Sulaimani (Certificate no. PH151-25, dated 22/01/2025). The study adhered to both institutional and Canadian Council on Animal Care (CCAC) guidelines. The study comprised 32 male Wistar rats, randomly assigned to four groups: control, 18-hour sleep deprivation, 24-hour sleep deprivation, and 72-hour sleep deprivation. Each group contained eight rats (Figure 1).

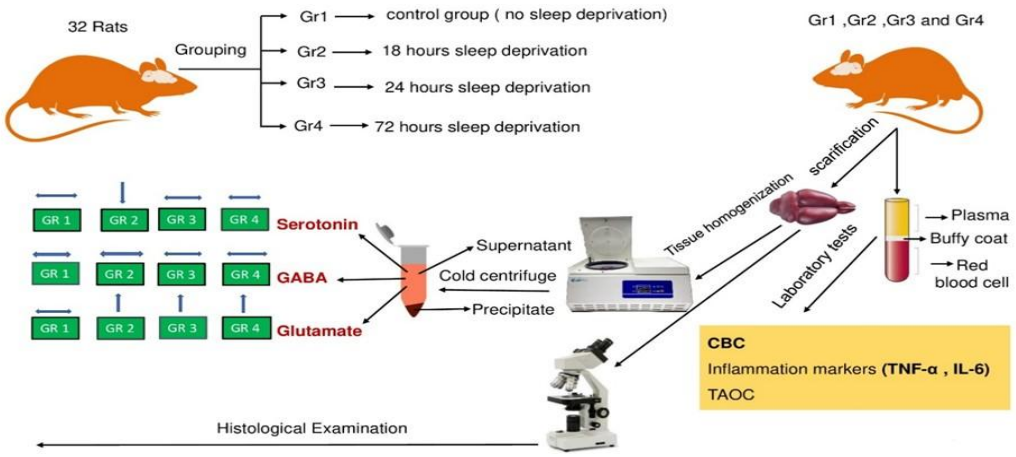

Figure 1: Study design flowchart illustrating animal enrolment, group randomization, and duration of the study.

Sleep deprivation model

The rats faced complete sleep deprivation (SD) during the daytime, starting at 10:00 AM. The SD was implemented by using gentle handling techniques, which included disturbing the bedding, mildly shaking the cage, and softly touching the animals with a brush when they attempted to sleep [21]. Additionally, environmental noise was employed to keep the rats awake. A speaker was installed inside the sound-attenuating box housing the animals, connected to an amplifier with a digital audio player (Model: GY-802 5.0 + EDR(A2DP)). The 15-minute noise sequence, featuring random street sounds such as vehicle horns, ambulance sirens, hammering, sudden braking, bells, alarms, and airplane noises, was played repeatedly over the 12-hour SD period. To avert habituation, the auditory stimuli exhibited random variations in duration, frequency (800–20,000 Hz), amplitude (85–100 dB, with an average intensity of 85 dB), and intervals between noises. This approach has been shown to avoid causing cochlear damage in rodents [22,23]. Also, light exposure was integrated into the sleep deprivation procedure as an additional arousing stimulus. During the SD period, rats were continuously monitored, while control rats remained undisturbed in their home cages, allowed to sleep normally during the same timeframe [22–24].

Tissue homogenization and blood sampling

After the respective sleep deprivation periods, the rats were anesthetized with chloroform as the anesthetic agent. Following the euthanasia of the rats, roughly 5 milliliters of blood was obtained via heart puncture. The blood was deposited in a tube and permitted to coagulate. Subsequently, it was centrifuged at 3000 rpm for 20 minutes, and the resultant serum was preserved at -20°C for later inflammatory and oxidative stress biomarker analysis. Brain tissue was harvested, and a portion of the brain tissue was rinsed with ice-cold normal saline and subsequently immersed in 25 ml of 10% formaldehyde to be used for subsequent histopathological analysis. The remaining portion, primarily the hypothalamus, was excised and rinsed with ice-cold phosphate-buffered saline (PBS) solution. Subsequently, it was homogenized with each gram of brain tissue, to which nine milliliters of PBS was added in compliance with the directives from the bioassay technology ELISA kits. Homogenization was conducted via a homogenizer apparatus. The homogenized samples were immediately centrifuged at 5000 g for 5 min in a chilled centrifuge, and the supernatant was collected and stored at -80°C for later neurotransmitter (serotonin, GABA, and glutamate) analysis.

Neurotransmitter biomarkers measurement

To measure neurotransmitter levels in brain tissue, an enzyme-linked immunosorbent assay (ELISA) was used for serotonin, GABA, and glutamate. Brain tissue homogenates were prepared, and the supernatant was analyzed using ELISA kits. The supernatant of brain tissue homogenate was analyzed using ELISA kits (Bioassay Technology Laboratory, Shanghai, China) to quantify serotonin (Cat. No. E0866Ra), GABA (Cat. No. E0102Ra), and glutamate (Cat. No. E1474Ra). For serotonin, the procedure involved adding the sample to pre-coated wells with rat 5-HT antibodies, followed by incubation with biotinylated 5-HT antibody and streptavidin-HRP. After washing away unbound substances, a substrate solution was added, and the optical density (OD) was measured at 450 nm. Similarly, for glutamate, the sample was added to pre-coated wells with rat GLM antibodies, followed by biotinylated GLM antibody and streptavidin-HRP. After incubation and washing, a substrate solution was added, and the OD was measured at 450 nm. For GABA, the sample was added to wells coated with rat GABA antibodies, followed by biotinylated GABA antibody and streptavidin-HRP. After washing, a substrate solution was added, and the OD was measured at 450 nm. The OD values for each neurotransmitter corresponded to their concentrations in the samples.

Measurement of inflammatory markers

Blood serum was used for measuring inflammatory markers, including Interleukin 6 (IL-6), Tumor Necrosis Factor Alpha (TNFA), and Total Antioxidant Capacity (T-AOC). These markers were analyzed

using ELISA kits from Bioassay Technology Laboratory (Shanghai, China). Each analysis involved binding the respective markers in the sample to a pre-coated antibody on an ELISA plate, followed by incubation with biotinylated antibodies and streptavidin-HRP. The reaction was terminated by adding a stop solution, and the absorbance was measured at 450 nm to quantify the concentration of each marker. Interleukin 6 (IL-6): The concentration of IL-6 was measured using a pre-coated rat IL-6 antibody, followed by the binding with biotinylated anti-IL-6 antibody and streptavidin-HRP. The absorbance was measured at 450 nm. Tumor Necrosis Factor Alpha (TNFA): TNFA was measured using a pre-coated rat TNFA antibody, biotinylated anti-TNFA antibody, and streptavidin-HRP, with absorbance also read at 450 nm. Total Antioxidant Capacity (T-AOC): T-AOC was quantified using a pre-coated rat T-AOC antibody, biotinylated anti-T-AOC antibody, and streptavidin-HRP. The absorbance was quantified at 450 nm.

Histological analysis

At the last stage of the experiment, rats were subjected to a fasting period of no less than 10 hours prior to being humanely euthanized with an overdose of inhaled chloroform. Following euthanasia, brain tissue specimens were obtained for histological analysis. Fixation and Dehydration: Brain specimens were positioned in tissue cassettes and immersed in 10% neutral buffered formaldehyde for a duration of 48 hours. The specimens were further dehydrated by exposure to increasing concentrations of ethanol (50%, 60%, 70%, 90%, and 100%), followed by a minimum of three stages of xylene clearance. The tissue samples were infiltrated and embedded in molten paraffin blocks at 60-70°C. The tissue blocks were sliced into 5 µm sections utilizing a rotary microtome. The pieces were affixed to glass slides, dried with a tissue dryer, and subsequently deparaffinized with xylene for 30 minutes. Following drying in an oven at 50°C, the slides were stained with Harris's hematoxylin and eosin solution. Final Preparation: The stained sections were covered with a slide and analyzed using a bright-field light microscope.

Brain lesion scoring

In general, lesion grading and scoring for the rats were assessed semi-quantitatively using image analysis software (Am Scope, 3.7) in conjunction with a microscope eyepiece camera (MD500, 2019), while tissue samples were examined under a light microscope (NOVEL XSZ-N107T, China). In summary, pycnotic neuroglial cells in the cephalic tissues were enumerated in ten randomly selected microscopic fields at high-power magnification (100X), and the mean percentage of the counted cell counts was statistically calculated. The areas of vascular congestion and perivascular edema were measured in micrometers, after which the mean average was determined as a percentage and subjected to statistical comparison. The average percentage of all computed data was represented using the following lesion scoring system: a score of 0-10% indicates no lesions; a score of 10-25% denotes mild lesions; a score of 25-50% signifies moderate lesions; a score of 50-75% reflects severe lesions; and a score of 75-100% represents critical lesions.

Statistical analysis

The statistical analysis was performed using GraphPad Prism 10. Measured parameters were provided as mean±SEM. For group comparisons, a one-way ANOVA was used, followed by Tukey's multiple comparison test. The results were statistically significant when the p-value was less than 0.05. The Shapiro-Wilk test was used to determine normality.

RESULTS

Sleep deprivation had varying effects on the three neurotransmitters examined. Serotonin levels were significantly affected after 18 hours of sleep deprivation but returned to normal with longer deprivation durations of 24 and 72 hours. GABA levels remained stable regardless of sleep deprivation duration (Figure 2A and B).

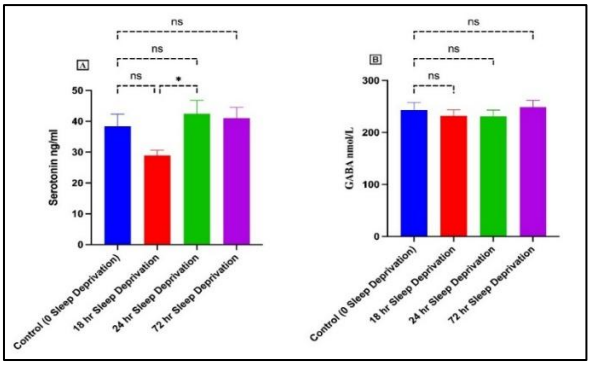

Figure 2: Effect of sleep deprivation on **A**) serotonin level and **B**) GABA level (n=8).

In contrast, glutamate levels increased progressively with longer sleep deprivation periods. Total Antioxidant Capacity remained stable across all sleep deprivation durations (Figure 3A and B).

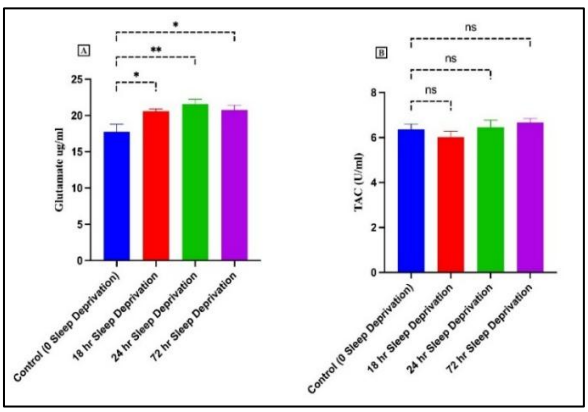

Figure 3: Effect of sleep deprivation on **A**) Glutamate level and **B**) TAC level (n=8).

IL-6 levels were not significantly affected by sleep deprivation at any duration, and TNF-α levels remained unchanged after 18 and 24 hours but significantly increased after 72 hours of deprivation (Figure 4 A and B). Histopathological analysis of brain tissue revealed structural changes and varying lesion severity across all groups.

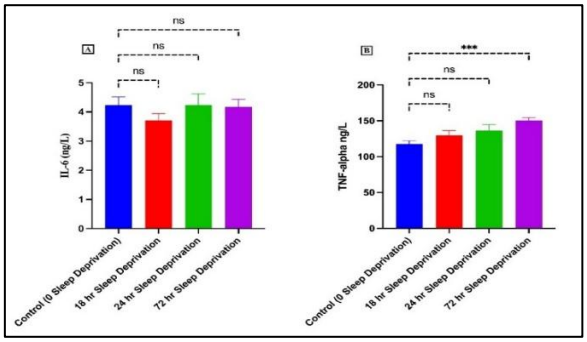

Figure 4: Effect of sleep deprivation on **A**) IL-6 level and **B**) TNF-α level (n=8).

Lesion severity increased progressively with longer durations of sleep deprivation but remained below critical levels. Animals subjected to 24 and 72 hours of sleep deprivation exhibited moderate lesions (Table 1) (Figures 5-8). Significant alterations are observed in markers like systemic inflammatory index (SII) (B), neutrophil-to-lymphocyte ratio (NLR) (C), platelet-to-lymphocyte ratio (PLR) (D), platelet-to-neutrophil ratio (PNR) (E), lymphocyte-to-monocyte ratio (LMR) (H), and monocyte-to-lymphocyte ratio (MLR) (I), particularly in the 24h and 72h sleep-deprived groups, suggesting a time-dependent inflammatory response.

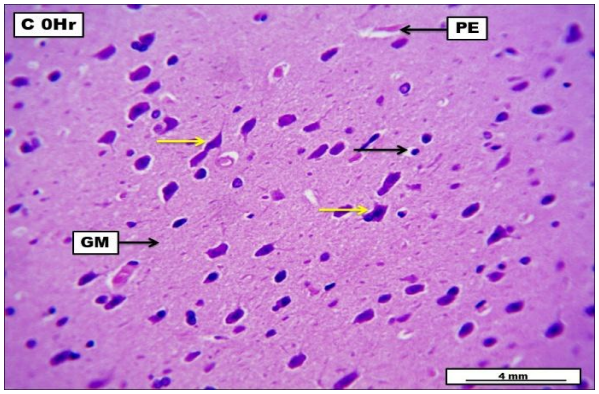

Figure 5: Photomicrograph of brain from a rat in the control group (C 0Hr) with zero hour of sleep deprivation, present virtually typical morphological structures, represented by intact neural cells (yellow arrows) with deep purplish nuclei distributed normally within the light pink, gray matter (GM). Besides, the section shows some non-significant pyknotic glial cells (black arrow), together with the presence of slight perineural edema (PE). H&E. Scale bar: 4 mm.

The 24h group often shows the lowest inflammatory indices, while 72h deprivation elevates them, notably in SII, NLR, and MLR, indicating a rebound or compensatory pro-inflammatory shift. Markers such as red cell distribution width (RDW) (A), red cell-to-platelet ratio (RPR) (F), and neutrophil-to-eosinophil ratio (NER) (G) show no significant differences (ns) cross groups, implying they are less sensitive to sleep deprivation effects (Figure 9. A-I).

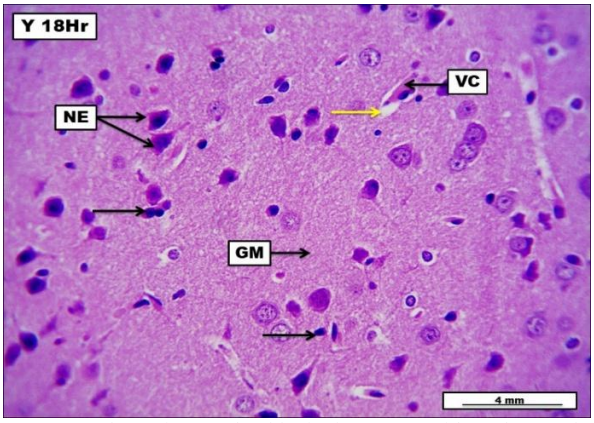

Figure 6: Photomicrograph of brain from a rat with 18 hours of sleep deprivation (18Hr), demonstrate low grade of vascular congestion (VC) mutual with trivial perivascular edema (yellow arrows). Occurrence of typical pyramidal cell neurons (NE) disseminated classically within a pinkish gray matter (GM). The section also reveals the presence of numerous pycnotic glial cells (black arrows). H&E. Scale bar: 4 mm.

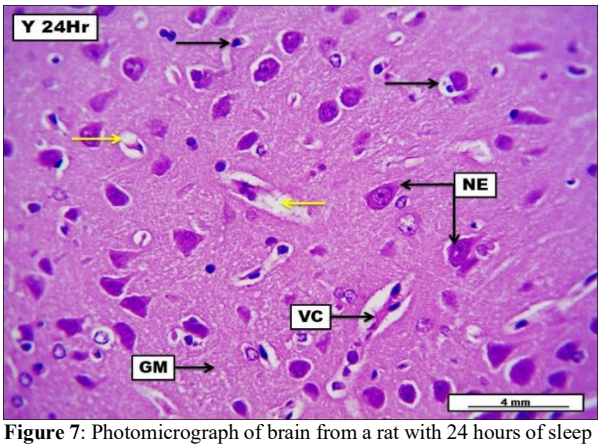

Figure 7: Photomicrograph of brain from a rat with 24 hours of sleep deprivation (24Hr), display slightly significant vascular dilation and congestion (VC) with evident perivascular edema (yellow arrows), in addition to the presence of low grade of pycnotic glial cells with slight pericellular edematous transudation (black arrows). Furthermore, the section expresses typically structured neural cell bodies (NE). H&E. Scale bar: 4 mm.

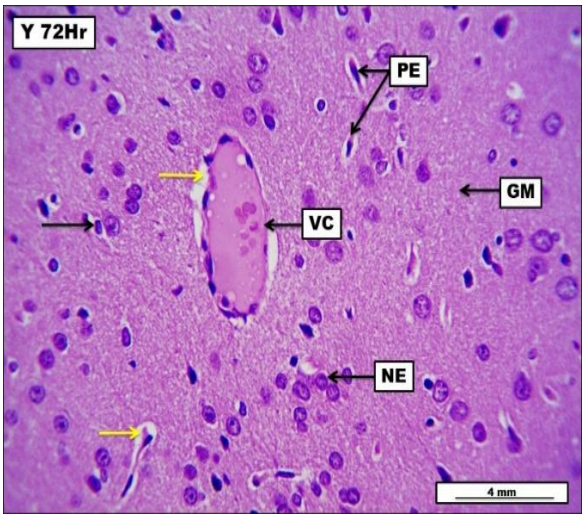

Figure 8: Photomicrograph of brain from a rat with 72 hours of sleep deprivation (72Hr), reveal the presence of moderately significant vascular engorgement and congestion (VC), together with perivascular edema (yellow arrows). In addition to the presence of perineuronal edema (PE), on the other hand, some neurons (NE) bare normal morphological structures distributed with gray matter (GM). H&E. Scale bar: 4 mm.

Table 1: Semi quantitative assay of brain sections of the rats (n=8)

	Perivascular Edema * (Mean%)**	Vascular Congestion (Mean %)**	Glial Pycnotic Cells (Mean %)**	Lesion Scoring (0 -100%)	Lesion Grading
(0 hr) C†	3.72 % A#	3.84 % A	6.37 % A	0-10 %	No lesion
(18 hrs)	14.63 % B	13.42 % B	19.52 % B	10-25 %	Mild
(24 hrs)	28.82 % C	32.29 % C	36.79 % C	25-50 %	Moderate
(72 hrs)	32.45 % C	42.67 % C	41.61 % C	25-50 %	Moderate

*Area of perivascular and vascular congestion edema were quantified in micrometers (µm). Pyknotic cells were estimated in (%) of calculated cell number. All morphometric parameters for lesion scoring were calculated as mean averages. ** Each value indicates the average proportion of eight animals (n=8). # Statistical comparison across groups: Mean values with different capital letters show significant differences ($p< 0.05$). †(0 hr.) C: Control group with zero time of sleep deprivation; (18 hrs.): Animal group with 18 hours of sleep deprivation; (24 hrs.): Animal group with 24 hours of sleep deprivation; (72 hrs.): Animal group with 72 hours sleep deprivation.

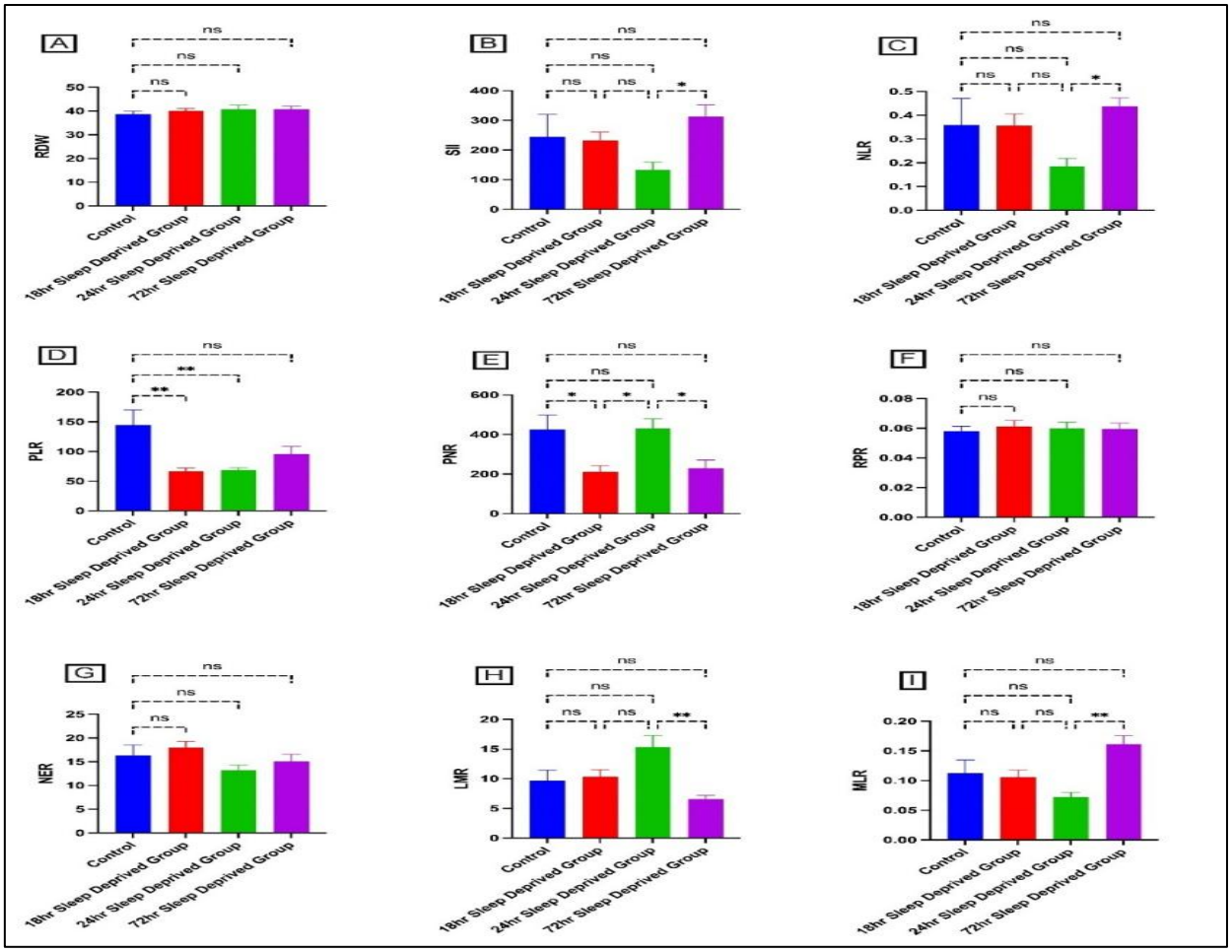

Figure 9 (A-I): Changes in inflammatory cell ratios following different durations of sleep deprivation. Parameters include Red Cell Distribution Width (RDW), Systemic Inflammatory Index (SII), Platelet-to-Lymphocyte Ratio (PLR), Platelet-to-Neutrophil Ratio (PNR), Red Blood Cell-to-Platelet Ratio (RPR), Neutrophil-to-Eosinophil Ratio (NER), Lymphocyte-to-Monocyte Ratio (LMR), and Monocyte-to-Lymphocyte Ratio (MLR). One-way ANOVA was used to analyze statistical differences followed by Tukey's post hoc test. **significant differences ($p< 0.05$); ns: non-significant differences.

DISCUSSION

In this study we evaluated the impacts of sleep deprivation on neurotransmitters (serotonin, GABA, and glutamate) in the brain tissue and inflammatory markers (TNF-alpha and IL-6) in the blood after different short-term sleep-deprived periods (18, 24, and 72 hours). This study demonstrated that sleep deprivation (SD) induces significant neurochemical alterations, particularly in serotonin (5-HT), glutamate, and GABA levels in the brain. These findings highlight the dynamic interplay between excitatory and inhibitory neurotransmission during different durations of SD, as well as the brain's compensatory mechanisms to maintain homeostasis. The analysis of brain serotonin levels revealed a transient decrease at 18 hours of SD, with normalization observed at 24 and 72 hours. This initial decline may be attributed to reduced availability of tryptophan, the precursor for serotonin synthesis; increased monoamine oxidase (MAO) activity leading to accelerated breakdown of serotonin into 5-HIAA [25]; disruption of the circadian regulation of serotonergic neurons; and elevated cortisol levels that suppress tryptophan hydroxylase. Supporting this, previous studies have observed significantly elevated 5-HIAA levels during and after SD, indicating enhanced serotonin turnover [26]. Interestingly,

serotonin levels returned to baseline after 24 hours of SD, suggesting the activation of compensatory mechanisms. These may include upregulation of tryptophan hydroxylase and serotonin transporter (SERT) recycling pathways [27], the modulatory role of orexinergic neurons on serotonergic tone in the dorsal raphe nucleus [28], and HPA axis adaptation, leading to reduced cortisol and restored serotonin synthesis. In addition, increased dopaminergic and noradrenergic activity during prolonged wakefulness may further support serotonergic function [29]. By 72 hours of SD, serotonin levels stabilized, indicating long-term neuroadaptive responses. These may involve enhanced brain-derived neurotrophic factor (BDNF) expression, which promotes synaptic plasticity and serotonergic neuron resilience [30]; desensitization of 5-HT1A autoreceptors, thereby reducing inhibitory feedback on serotonin release [31]; and microglial adaptation that shifts from a pro-inflammatory to a regulatory phenotype supporting neuronal function [32]. In parallel, our findings showed a consistent increase in glutamate levels in all SD groups (18, 24, and 72 hours), with no significant changes in GABA levels compared to the control. These results are in agreement with prior studies showing elevated glutamate and glutamine content in the cerebral cortex, hippocampus, and thalamus following sleep loss. The elevated glutamate levels likely reflect increased neuronal activity and excitability [33]. Despite this excitatory shift, GABA levels remained stable, suggesting the presence of compensatory mechanisms aimed at preserving excitatory-inhibitory balance. These may include increased activity of GABA-synthesizing enzymes (e.g., glutamic acid decarboxylase) and reduced GABA degradation [34], as well as sustained GABAergic tone despite rising excitatory drive [35]. Additionally, SD may impair astrocytic function and disrupt the glutamate-glutamine cycle, leading to glutamate accumulation in the synaptic cleft without altering GABA concentrations [36]. The elevated glutamate levels may also be influenced by increased neuronal firing during SD and by stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis, which preferentially enhances glutamate release [37]. These findings support the concept that the brain employs adaptive plasticity, maintaining GABA homeostasis to prevent over-inhibition while allowing for elevated glutamatergic activity during prolonged wakefulness [35]. Together, these results suggest that neurotransmitter systems respond to sleep deprivation in a temporally dynamic and compensatory manner, highlighting their critical roles in regulating brain function under physiological stress. Serum TAC levels remained stable despite varying durations of sleep deprivation, likely due to compensatory mechanisms that preserve redox balance. One such mechanism is the upregulation of enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which work to neutralize excess reactive oxygen species (ROS) generated during wakefulness [38]. Furthermore, glutathione (GSH) metabolism may adapt to oxidative stress through enhanced synthesis, maintaining antioxidant capacity despite cellular stress [39]. Notably, oxidative stress from sleep deprivation often affects specific brain regions such as the hippocampus and cortex, while systemic antioxidant levels remain unchanged [40]. Finally, evidence suggests a redistribution of antioxidant resources, with increased activity in high-demand brain areas potentially supported by peripheral antioxidant mobilization [40]. This study demonstrated that prolonged sleep deprivation, particularly for 72 hours, significantly increases serum TNFα levels. The elevation of this pro-inflammatory cytokine correlates with cognitive deficits and anxiety-like behaviors observed in sleep-deprived animals, suggesting that TNFα is crucial in the modulation of sleep, neuroinflammation, and cognitive function [41]. The increase in TNFα is associated with several mechanisms. First, sleep deprivation triggers a strong inflammatory response, characterized by TNFα elevation in the hippocampus and activation of pro-inflammatory pathways. Second, microglial activation in response to sleep loss contributes to TNFα production, potentially disrupting the balance between pro- and anti-inflammatory signals and leading to emotional and cognitive disturbances. Third, TNFα is known to impair the integrity of the blood-brain barrier (BBB), increasing its permeability and facilitating neuroinflammation [41]. Unlike TNFα, serum IL-6 levels did not show significant changes across different durations of sleep deprivation. This finding indicates a complex regulation of cytokine responses to prolonged stress. One plausible explanation is the compensatory activity of anti-inflammatory cytokines, particularly IL-10, which suppresses IL-6 production by inhibiting immune cell activation [42]. Additionally, stimulation of the hypothalamus-pituitary-adrenal (HPA) axis during stress leads to corticosterone release, which modulates cytokine production and may blunt IL-6 responses [43]. The differential sensitivity of cytokines also plays a role; TNFα is an earlier and more robust responder to stress compared to IL-6, which may require more severe or chronic conditions to increase significantly. Finally, IL-6 exhibits circadian fluctuations, and the timing of blood sampling may have influenced detection, potentially masking changes due to sleep deprivation [44]. Sleep deprivation leads to significant immune alterations, with total white blood cell (WBC) counts increasing across all deprivation durations (18, 24, and 72 hrs), indicating general immune activation. Lymphocyte counts were elevated at 18 hr and 24 hr but returned to baseline at 72 hr, suggesting an initial immune mobilization followed by possible suppression during prolonged stress. Monocyte, eosinophil, and basophil counts increased notably in the 72 hr group, supporting a shift toward a pro-inflammatory state, in line with elevated TNF-α levels [45]. Inflammatory ratios revealed dynamic immune changes. While the systemic inflammation index (SII) and neutrophil-to-lymphocyte ratio (NLR) remained stable, the platelet-to-lymphocyte ratio (PLR) decreased at 18h and 24h due to lymphocyte elevation. Conversely, at 72 hr, the lymphocyte-to-monocyte

ratio (LMR) declined, and the monocyte-to-lymphocyte ratio (MLR) increased, further indicating systemic inflammation. These findings are consistent with prior reports on immune dysregulation from prolonged sleep loss [46]. Histopathological analysis revealed progressive brain damage with longer sleep deprivation. After 72 hr, marked vascular dilation, perivascular edema, and glial cell pyknosis were noted, though neurons remained structurally intact. In contrast, 18 hr of deprivation showed only mild alterations. These structural changes parallel the biochemical markers of inflammation and reinforce the time-dependent vulnerability of the brain to sleep deprivation [47].

Conclusion

This study shows that total sleep deprivation selectively alters neurotransmitter balance, immune activity, and oxidative stress in a duration-dependent manner. Serotonin showed transient changes, while glutamate and TNF-α increased with prolonged deprivation. Inflammatory cell ratios and monocytes rose after 72 hours, reflecting systemic immune activation. Future studies should assess additional biomarkers (e.g., dopamine, IL-1β, ROS) and the recovery phase to better understand the full scope and reversibility of sleep deprivation-induced physiological changes.

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Conflict of interests

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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