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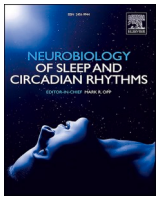

Investigating the resilience of kidneys in rats exposed to chronic partial sleep deprivation and circadian rhythm disruption as disruptive interventions

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ABSTRACT

Sleep is a vital biological function that significantly influences overall health. While sleep deprivation (SD) and circadian rhythm disruption are known to negatively impact various organs, their specific effects on kidney function remain understudied. This study aimed to investigate the impact of chronic partial sleep deprivation and circadian rhythm disruption on renal function in rats, providing insights into the relationship between sleep disturbances and kidney health. A total of 40 male Wistar rats were divided into five groups: a control group, a group with circadian rhythm disruption (CIR), a group with sleep deprivation during the light phase (SD-AM), a group with sleep deprivation during the dark phase (SD-PM), and a group with combined sleep deprivation and circadian rhythm disruption (SD-CIR). Sleep deprivation was induced using a specialized machine, depriving rats of sleep for 4 h daily, while circadian rhythm disruption was achieved through a 3.5-h light/dark cycle. After four weeks, kidney tissues and blood samples were collected for histological and biochemical analyses. The results showed that all experimental groups exhibited reduced water intake, with the CIR and SD-CIR groups also showing significantly lower food intake and reduced weight gain compared to controls. Oxidative stress markers revealed increased serum malondialdehyde (MDA) levels in the SD-PM and SD-CIR groups. Despite these metabolic and oxidative changes, histological examination of the kidneys revealed no significant alterations in renal structure or function across the groups. This study highlights the negative effects of chronic partial sleep deprivation and circadian rhythm disruption on feeding behavior, weight gain, and oxidative stress in rats. However, these interventions did not significantly alter renal structure or function. Further research is needed to explore the physiological mechanisms underlying these findings and the potential long-term effects of sleep disturbances on kidney health.

1. Introduction

Sleep is a fundamental physiological process characterized by a reversible state of reduced activity and decreased responsiveness to sensory stimuli. It is a vital aspect of human and animal life, serving essential functions in promoting physical and mental restoration, memory consolidation, and overall well-being. During sleep, the body undergoes various physiological changes that contribute to its

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restorative effects. Understanding the mechanisms and importance of sleep is crucial for comprehending the impact of sleep disturbances and developing strategies to promote healthy sleep patterns (Perrotta, 2019).

Sleep also plays a vital role in the survival, recovery, and rejuvenation of the body. It is essential for maintaining optimal health and well-being. In general, most adults require an average of 6–8 h of regular sleep each night to function at their best. This duration allows for the completion of essential sleep stages, including deep sleep and REM sleep, which are associated with physical and mental restoration, memory consolidation, and overall cognitive performance. Adequate and consistent sleep is crucial for supporting proper physiological functioning and promoting optimal daily functioning (J. H. Choi et al., 2016). Sleep and the circadian (24-h) clock system are closely interconnected, with each influencing the other. The circadian clocks regulate sleep to occur primarily during the dark phase, while sleep loss can have an impact on the function of tissue molecular clock rhythms (Archer and Oster, 2015).

Sleep deprivation (SD) is a condition characterized by insufficient quantity or poor quality of sleep. It occurs when an individual does not obtain an adequate amount of sleep needed for their optimal functioning and well-being. This can be due to various factors, such as shortened sleep duration, frequent awakenings during the night, or difficulty falling asleep. Sleep deprivation can have significant effects on physical, cognitive, and emotional functioning, and it is important to address and manage sleep deprivation to maintain overall health and well-being (Ashrafi et al., 2018; J. H. Choi et al., 2016). Both chronic sleep deprivation, which refers to a persistent reduction in either partial or total sleep time, and circadian rhythm misalignment, which involves inappropriate timing of sleep and wakefulness or a misalignment between central and peripheral rhythms, can have significant impacts on physical and mental health (Baron and Reid, 2014; Ramar et al., 2021; Seton and Fitzgerald, 2021).

Nowadays, technology and rotating work schedules, commonly referred to as shift work, have increasingly impacted both the quantity and quality of sleep (Kohn et al., 2020). Approximately 25% of wage and salary employees are engaged in shift work schedules, which can have a significant impact on their sleep patterns. Notably, up to 85% of shift workers experience sleep disorders, highlighting the need for strategies to mitigate the effects of shift work on sleep quality (Adimi Naghan et al., 2020). Shift work often results in a decrease in sleep duration of one to 4 h compared to non-shift workers. Consequently, individuals who work split shifts, early morning shifts, or rotating shifts frequently suffer from ongoing sleep deprivation, which can lead to the development of a condition known as shift work sleep disorder (SWSD).

SWSD is classified as a circadian rhythm disorder due to its manifestation of disrupted synchronization between internal sleep-wake rhythms and the natural light/dark cycle (Åkerstedt, 2003; Haile et al., 2019; Wickwire et al., 2017). Sleep disorders (SD) not only impact the brain but also have an influence on physiological functions in peripheral tissues. According to a recent study, there is evidence to suggest that sleep disorders are linked to a higher risk of developing cardiovascular diseases (Y. Choi and Choi, 2020). Patients with chronic kidney disease (CKD) commonly experience sleep disorders, such as insomnia and sleep apnea. These sleep disturbances are frequently observed in individuals with CKD (Nigam et al., 2018; Sen, 2018). Moreover, there is evidence indicating that sleep apnea, circadian rhythm disorders, hypersomnia, and insomnia may have an impact on the development of kidney diseases (Calero and Anderson, 2019; Fang et al., 2022). Clinical and experimental studies have provided evidence that sleep deprivation can trigger an inflammatory response, characterized by elevated levels of pro-inflammatory cytokines in the bloodstream(Rosa Neto et al., 2010; Shearer et al., 2001; Yehuda et al., 2009). Simultaneously, it is common for the majority of sleep disorders to be accompanied by sympathetic hyperactivity (López-Cano et al., 2019).

There is a hypothesis suggesting that the presence of an inflammatory milieu and increased sympathetic activity may contribute to changes in the kidney tubular apparatus and the glomerular basement membrane (Calero and Anderson, 2019; Poonit et al., 2018; Zhang et al., 1997). Conversely, research conducted on mice has shown that inducing chronic kidney disease can impair clock function, leading to disrupted behavioral circadian rhythms characterized by instability (Myung et al., 2019). Nevertheless, the impact of sleep deprivation on kidney function has received comparatively less attention in scientific research (Maung et al., 2016).

To the best of our knowledge, there has been no study conducted thus far that specifically examines the effects of chronic sleep deprivation, in conjunction with circadian rhythm disruption, on renal structure and function in rats. In this particular study, an animal model was created to simulate partial chronic sleep deprivation while also introducing alterations to the light/dark cycle. This model aimed to mimic conditions similar to chronic insomnia and shiftwork experienced by humans.

2. Methods & Materials

2.1. Animals

All the study protocols, including the animal experiments, were approved by the Birjand University of Medical Sciences ethics committee (ethics code: IR.BUMS.REC.1400.222). All protocols for the animal experiments adhered to the guidelines and regulations set forth by the Laboratory Animal Care Committee of the Iranian Ministry of Health and Medical Education. These guidelines aim to minimize the number of animals used and reduce any potential suffering they may experience during the study. The study was conducted following the ARRIVE (Animal Research: Reporting of *in vivo* Experiments) guidelines, which provide a set of recommendations to enhance the reporting and transparency of animal research.

Forty male adult Wistar rats, weighing between 200 and 250 g and at 8 weeks of age, were obtained from the Research Centre of Experimental Medicine at Birjand University of Medical Sciences. The rats were housed in two distinct temperature-controlled rooms, maintained at a temperature of 20 ± 2 °C, with a relative humidity of 35–45%. Additionally, the two rooms were subjected to different light/dark (LD) cycles. In one of the rooms, the LD cycle was set to 12 h of light and 12 h of darkness. Specifically, the lights were turned on from 6:00 a.m. to 6:00 p.m., while darkness prevailed from 6:00 p.m. to 6:00 a.m. In the second room, the LD cycle was designed to follow a pattern of 3.5 h of light followed by 3.5 h of darkness. This intentional disruption of the natural circadian cycle was introduced. It is crucial to note that this particular schedule, with alternating 3.5-h periods of light and darkness, did not repeat at the same time within each subsequent 24-h period. Throughout the study period, all rats were provided with ad-libitum access to tap water and standard laboratory animal chow from Pars Dam Co., Iran.

2.2. induction of sleep deprivation and study design

The rats were randomly divided into five groups, with each group consisting of eight rats (n = 8/group). The groupings were as follows.

1) **Control (C) group**: Rats kept in a 12-h LD cycle with unrestricted sleep.
2) **Circadian disruption (CIR) group**: Rats exposed to a 3.5-h LD cycle with unrestricted sleep. This specific LD cycle duration was chosen to prevent frequency de-multiplication of LD cycles (Stephenson et al., 2012).
3) **Sleep deprivation during the light phase (SD-AM) group**: Rats were subjected to a 12-h LD cycle with sleep deprivation from 8 to 12 a.m., corresponding to the first 6 h of the light phase.

4) **Sleep deprivation during the dark phase (SD-PM) group**: Rats were subjected to a 12-h LD cycle with sleep deprivation from 8 to 12 p.m., corresponding to the first 6 h of the dark phase.
5) **SD plus CIR (SD-CIR) group**: Rats exposed to a 3.5-h LD cycle with an additional 4 h of sleep deprivation per day. The timing of sleep deprivation in this group varied based on the rotation of the LD cycle, resulting in sleep deprivation occurring either during the dark or light phase. Fig. 1 illustrates the study design.

SD was induced using a sleep deprivation machine, as described in a previous study (Rezaei et al., 2020) (Fig. 2). The machine operated with a cycle of 3 min of immobility followed by a 5-s transition time repeatedly. Also, the partial and chronic sleep deprivation protocols utilized in this study were selected based on previous studies conducted in the field (Stephenson et al., 2012). To induce partial and chronic sleep deprivation, a duration of 4 h of daily sleep deprivation was chosen for 28 consecutive days. This protocol was implemented consistently throughout the study to ensure a sustained and prolonged sleep restriction condition (Opperhuizen et al., 2015; Stephenson et al., 2012).

2.3. blood and tissue sampling

At the end of the 28-day period, all animals were weighed and subjected to a fasting period of 12 h. Following the fasting period, the animals were sacrificed under anesthesia using a combination of ketamine and xylazine administered intraperitoneally at a dosage of 80 mg/kg and 10 mg/kg, respectively. This anesthesia protocol ensured a humane and painless procedure for sacrificing the animals (Veilleux-Lemieux et al., 2013).

Blood samples were obtained through cardiac puncture, and the collected samples were then subjected to centrifugation at 3000×*g* for 10 min. The resulting serum was carefully separated and stored at a temperature of −80 °C for subsequent analysis. Following blood collection, the kidneys were isolated and weighed. Subsequently, the right kidney was fixed in a 4% paraformaldehyde solution, which served as a fixative for histological procedures. Blood and tissue samples were collected from all the study groups within a specific time window between 8:00 a.m. and 12:00 p.m. This approach was implemented to ensure uniformity in the sampling time across all the groups under investigation. By adhering to a standardized sampling strategy, potential variations in biological rhythms and diurnal fluctuations were minimized, thus enhancing the reliability and comparability of the collected samples. To facilitate simultaneous sampling from all groups, a specific color label was assigned to each animal within each group. This labeling system enabled efficient and coordinated sampling across the studied groups. For example, all rats designated as number one (identified by a right-hand stain) across the groups were sampled initially, followed by rats numbered two and three, and so on. This sequential approach ensured systematic and organized sampling, allowing for streamlined data collection and analysis.

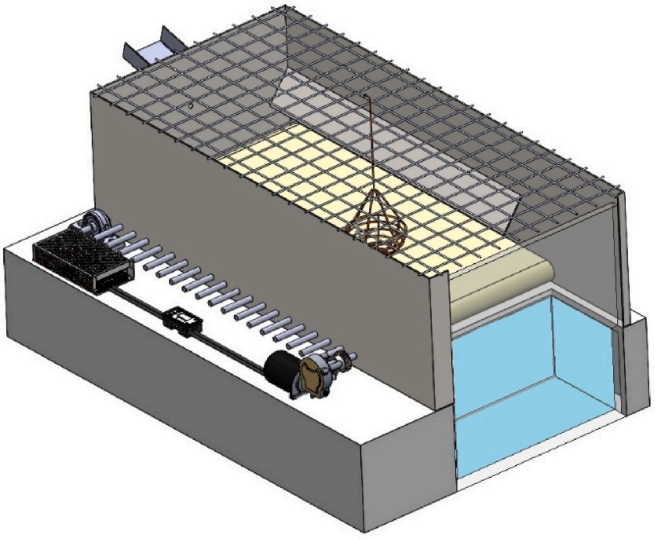

Fig. 2. The sleep deprivation machine used in the study consisted of a water chamber and an automatic conveyor belt. The machine's operation involved moving the animals, which resulted in them being compelled to either walk or fall into the water. This mechanism was employed to induce sleep deprivation by interrupting the animals' sleep and forcing them to remain awake.

To assess kidney function, serum urea and creatinine (Cr) levels were determined using standard diagnostic kits obtained from Bionick, Iran. These kits are commonly used in clinical and research settings to measure and quantify urea and creatinine levels in biological samples. The kits provide reliable and standardized methods for evaluating kidney function by analyzing the concentration of these biomarkers in the serum samples.

To assess lipid peroxidation, levels of malondialdehyde (MDA) in serum samples were measured using a commercially available kit from Nasdox, Navand Salamat, Iran. This kit is specifically designed to quantify MDA, which is a widely accepted biomarker for evaluating

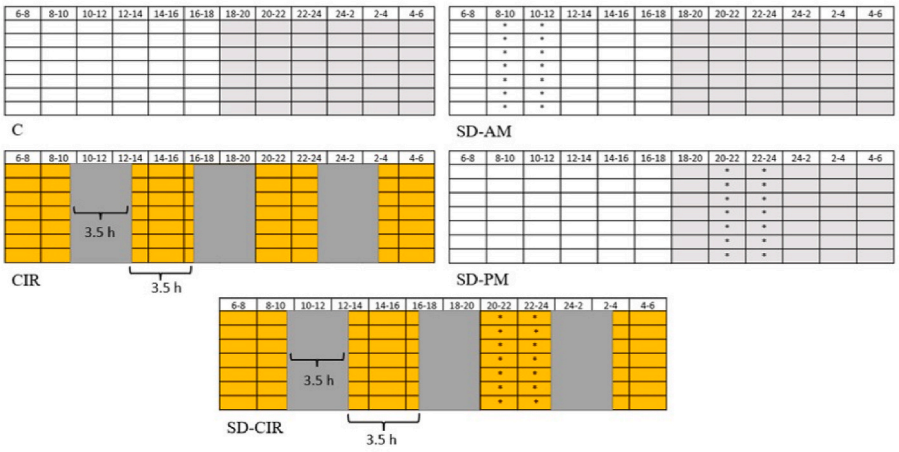

Fig. 1. The experimental conditions are presented in a chart, illustrating the light/dark (LD) cycles and sleep deprivation (SD) intervals for the different study groups. The chart shows white/gray tables representing normal LD cycles of 12 h of light followed by 12 h of darkness. In contrast, yellow/gray tables represent LD cycles that disrupt the circadian rhythm, with 3.5 h of light followed by 3.5 h of darkness. The asterisk (*) indicates the specific times of sleep deprivation in each of the studied groups.

oxidative stress and lipid peroxidation. By utilizing the kit, the MDA levels in the serum samples were determined, providing valuable insights into the extent of oxidative damage in the lipid components of the cells. In this method, the plasma lipids present in the serum samples undergo a reaction with thiobarbituric acid at elevated temperatures. This reaction results in the formation of a pink-colored adduct known as the MDA-thiobarbituric acid adduct. The intensity of the pink color is directly proportional to the concentration of MDA in the sample. The MDA-thiobarbituric acid adduct is then quantified colorimetrically at a wavelength of 530 nm using a spectrophotometer. By comparing the absorbance of the sample against a standard curve, the MDA levels in the serum samples can be determined accurately (Mohammadifard et al., 2021).

Superoxide Dismutase (SOD) activity was assessed using a WST-1 based SOD inhibition assay kit (Navand Salamat, Iran). In brief, 50 μL of each serum sample was added (in duplicate) to individual wells of a 96-well plate. Subsequently, 250 μL of the kit's reaction mixture was added to each well, and the plate was incubated at room temperature for 5 min. After incubation, the plate was read at 405 nm using a spectrophotometer. SOD activity was expressed in units per milliliter (U/mL), reflecting the level of SOD enzyme activity in the serum sample (Ahmadi-Zohan et al., 2021).

The fixed kidney samples were processed by embedding them in paraffin wax. Subsequently, the samples were sectioned at a thickness of 5 μm and stained using the hematoxylin/eosin (H&E) staining technique. For evaluation, three slides were randomly selected from each rat. The slides were examined under a light microscope, specifically the Euromex-CMEX-10 model, to visualize and analyze the kidney tissue. The quantitative parameters, namely glomerular area, glomerular tuft area, urinary space area (in μm^2), and cortex thickness (in μm), were measured using Image J Software (version 1.44p) developed by the National Institute of Health (NIH) in the United States. The software was employed as described in previous studies to analyze the kidney tissue sections (Hassanzadeh-Taheri et al., 2016, 2019; Zarezadeh et al., 2017).

The pathological lesions observed in the kidney tissue sections were evaluated and scored using a predefined scoring checklist. The checklist included various pathological features such as degeneration, congestion, infiltration, and hemorrhage. Indeed, a total of 10 microscopic fields were examined from each slide. The aforementioned pathological lesions were individually scored based on their extent in each microscopic field. The scoring system used was as follows: 1 for normal, 2 for slight injury involving up to 25% of the microscopic field, 3 for moderate injury involving 25–50% of the microscopic field, 4 for severe damage involving 50–75% of the microscopic field, and 5 for very severe damage involving more than 75% of the microscopic field. To calculate the final score for each group, the scores of all samples within the group were summed together (Abedini et al., 2021; Hassanzadeh-Taheri et al., 2018, 2021).

2.4. statistical Analyses

The data are presented as means ± standard deviations. Statistical analyses were conducted using SPSS software, version 22. The homogeneity of the data was assessed using the Shapiro-Wilk test. Differences between groups were determined using ANOVA (analysis of variance) followed by post-hoc tests, such as Tukey and Dunnett's T3 tests. A significance level of $p < 0.05$ was used to determine statistical significance, indicating that p-values less than 0.05 were considered significant.

3. Results

3.1. Effects on feeding and weight change

During the final week of the experiment, we assessed and compared the water intake and food consumption of the rats (Fig. 3).

Notably, disrupting the circadian cycle, either independently or in combination with sleep deprivation, led to a significant reduction in both food and water intake among the animals. Interestingly, when examining the impact of sleep deprivation during both daytime and nighttime, we observed that it did not significantly affect the animals' food consumption. However, there was a notable decrease in water consumption among the sleep-deprived animals.

The findings presented in this study suggest that mechanisms associated with the circadian rhythm play a significant role in regulating the intake of water and food, with a particular emphasis on food consumption. These results highlight the influence of the internal biological clock on the feeding behavior of the animals, emphasizing the importance of circadian rhythms in modulating physiological processes related to nutrient intake.

The results indicate that disrupting the circadian cycle has a more pronounced effect on the pattern of water and food consumption in animals compared to the impact of sleep deprivation alone. This suggests that the timing and synchronization of the internal biological clock have a stronger influence on feeding behavior than sleep deprivation alone. These findings highlight the significance of circadian rhythms in regulating nutrient intake and emphasize the complex interplay between sleep, circadian rhythms, and feeding behaviors.

Despite the absence of a significant difference in starting body weights among the groups studied ($p = 0.90$), there was a significant disparity in final body weights ($p = 0.01$). The mean body weight changes throughout the experimental period are depicted in Fig. 3C. Rats in the SD-CIR group exhibited a significantly lower weight gain compared to the control group ($p = 0.01$). These findings suggest that sleep deprivation, particularly in conjunction with circadian rhythm disruption, may lead to increased energy expenditure and subsequent weight loss. This raises the possibility that these factors play a role in promoting weight reduction.

3.2. Effects on plasma MDA and SOD

Serum MDA levels, serving as an oxidative stress marker, were measured in this study. Additionally, SOD activity, an indicator of the antioxidant defense system, was evaluated. Comparing the results to the control group, it was found that the plasma MDA levels in both the SD-PM ($p = 0.01$) and SD-CIR ($p = 0.003$) groups of rats were significantly increased (Fig. 4A). These findings suggest that sleep deprivation during dark conditions (SD-PM) and sleep deprivation combined with circadian rhythm disruption (SD-CIR) may contribute to higher oxidative stress levels, as indicated by elevated MDA levels in the plasma. No significant difference was observed between the SD-PM and SD-CIR groups in terms of serum MDA levels. Furthermore, when comparing SOD levels among the studied groups, no statistically significant differences were found (Fig. 4B).

3.3. Effects on plasma urea and Cr

Renal function was evaluated by assessing biochemical parameters, specifically the plasma concentrations of urea and creatinine (Cr). The test results revealed no significant differences in plasma urea and Cr levels among all the study groups (Fig. 5). This suggests that the experimental interventions, including sleep deprivation and circadian rhythm disruption, did not have a significant impact on these renal function markers in the tested animals.

3.4. Effects on renal morphology

The results of the morphological examination of kidneys, including the kidney index (kidney weight/body weight), cortex area, glomerular area, glomerular tuft area, and urinary space area, are summarized in Table 1. The assessment of kidney weight index in the different study

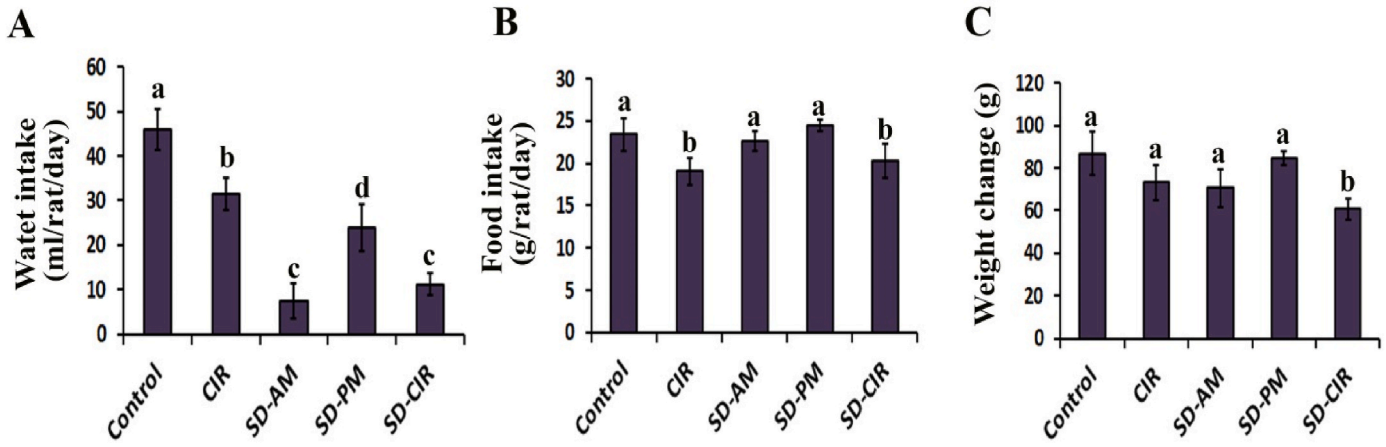

Fig. 3. This figure illustrates the effects of the 28-day study on water intake (A), food consumption (B), and weight change (C). The experimental groups include CIR (circadian rhythm disrupted), SD-AM (sleep deprived during light conditions, 8–12 a.m.), SD-PM (sleep deprived during dark conditions, 8–12 p.m.), and SD-CIR (sleep deprived plus circadian rhythm disruption). Each value presented in the figure represents the mean ± standard deviation (S.D.). Significant differences ($p < 0.05$) between groups are indicated by different letters (a–d), while the same letters denote non-significant differences between groups.

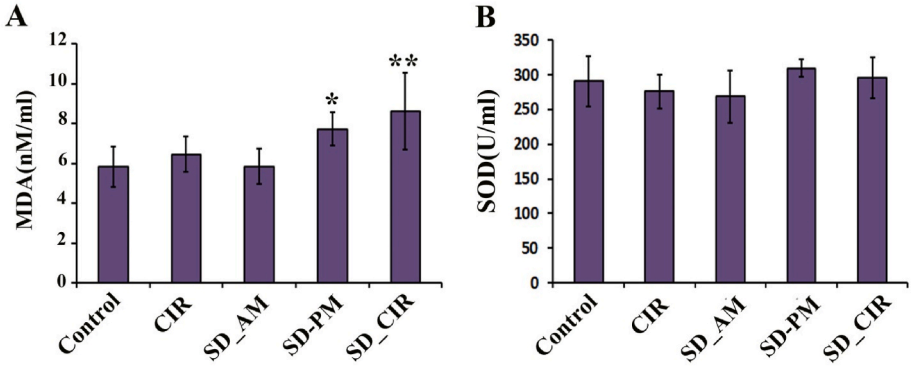

Fig. 4. This figure displays the effects of the 28-day study on the plasma levels of malondialdehyde (MDA) (A) and superoxide dismutase (SOD) (B). The experimental groups include CIR (circadian rhythm disrupted), SD-AM (sleep deprived during light conditions, 8–12 a.m.), SD-PM (sleep deprived during dark conditions, 8–12 p.m.), and SD-CIR (sleep deprived plus circadian rhythm disruption). Each value presented in the figure represents the mean ± standard deviation. Statistical differences are indicated by asterisks, with * representing $p < 0.05$ and ** representing $p < 0.01$ compared to the control group.

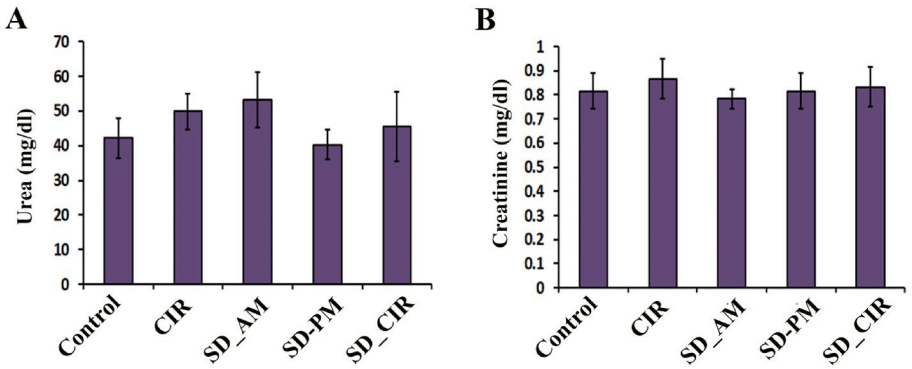

Fig. 5. Presents the effects of the current study on plasma levels of urea (A) and creatinine (B). The experimental groups include CIR (circadian rhythm disrupted), SD-AM (sleep deprived during light conditions, 8–12 a.m.), SD-PM (sleep deprived during dark conditions, 8–12 p.m.), and SD-CIR (sleep deprived plus circadian rhythm disruption). Each value displayed in the figure represents the mean ± standard deviation.

groups did not reveal any statistically significant differences among the groups. Similarly, no statistically significant differences were observed in the cortex area ($p = 0.94$), glomerular area ($p = 0.089$), tuft area ($p = 0.052$), and urinary space area ($p = 0.30$) among the study groups. These findings indicate that the experimental interventions did not have a significant impact on the measured morphological parameters of the kidneys.

Group 1 represents the control group, Group 2 represents the sleep-deprived during light conditions (8–12 a.m.) group, Group 3 represents the sleep-deprived during dark conditions (8–12 p.m.) group, and Group 4 represents the sleep-deprived plus circadian rhythm disruption group. The values presented in the table are the measured parameters for each

Table 1
Effects of chronic partial sleep deprivation and circadian cycle disruption on renal morphology of rats.

Groups	Kidney Index (mg/g)	Cortex Area (μm)	Glomerular Area (μm ^{2})	Tuft Area (μm ^{2})	Urinary Space (μm ^{2})
Control	4.09 ± 0.16	3598 ± 355	9289 ± 2615	6917 ± 1622	2896 ± 792
CIR	4.16 ± 0.25	3546 ± 370	8835 ± 2400	5192 ± 1823	2883 ± 602
SD-AM	4.16 ± 0.32	3578 ± 373	10228 ± 2839	6380 ± 1970	2988 ± 732
SD-PM	3.93 ± 0.24	3657 ± 280	8528 ± 2924	6169 ± 2516	2885 ± 574
SD-CIR	3.83 ± 0.35	3504 ± 230	8557 ± 2453	6283 ± 2113	2492 ± 651
p-value	0.19	0.94	0.089	0.052	0.30

group.

3.5. Effects on renal pathology

The kidney sections from the different study groups were evaluated for histopathological lesions using a semi-quantitative scoring checklist. The presence of main pathological lesions, including degenerative changes, hemorrhage, infiltration, and congestion, was assessed (Fig. 6). The histopathological grading scores of the kidney sections did not show any significant differences between the groups. This indicates that the experimental interventions, including chronic partial sleep deprivation and circadian cycle disruption, did not lead to noticeable differences in the observed histopathological lesions in the kidneys.

4. Discussion

The objective of the present study was to examine the effects of sleep deprivation and circadian rhythm disruption on renal function and structure in rats. The results indicated that when sleep deprivation and circadian rhythm disruption were combined, there were notable changes in the animals' food consumption, water intake, and weight gain. Additionally, the combination of sleep deprivation and circadian rhythm disruption led to increased oxidative stress. However, despite these observed effects, the study did not find substantial alterations in renal function or structure as a result of partial sleep deprivation, circadian rhythm disruption, or a combination of both.

The interpretation of the impact of the T7 cycle, a 3.5-h light/dark cycle used in this study is a subject of debate. Some studies suggest that exposure to the T7 cycle induces circadian misalignment without causing complete arrhythmicity (Duy and Hattar, 2017; Fernandez et al., 2018). Conversely, other research has shown that 12 days of exposure to the T7 cycle can result in a complete inversion of the circadian phase (Fuchs et al., 2023). While the T7 cycle may not eliminate circadian function, it does extend the circadian period to approximately 25 h, leading to a daily phase delay in rhythms governing activity and sleep (LeGates et al., 2012; Moriya et al., 2015). Fuchs et al. observed that the T7 cycle produces a 7-h rhythm of activity, characterized by heightened activity during dark phases and increased sleep during light phases. This alternation of light and dark influences both activity and sleep patterns, entraining the circadian system to a 25-h cycle. Notably, if light exposure were the sole determinant, stronger correlations between activity and sleep patterns would occur, leading to desynchronization. However, the milder differences in activity levels under the T7 cycle suggest preserved circadian variation, with greater activity during subjective nighttime (Fuchs et al., 2023). Abnormal circadian rhythms, even without inducing arrhythmicity, can lead to misalignment, which may disrupt normal kidney function and contribute to chronic kidney disease, particularly in older populations (Firsov and Bonny, 2018; Mohandas et al., 2022). However, this study cannot conclusively determine whether the T7 model effectively disrupts circadian rhythms. Future research should prioritize investigating

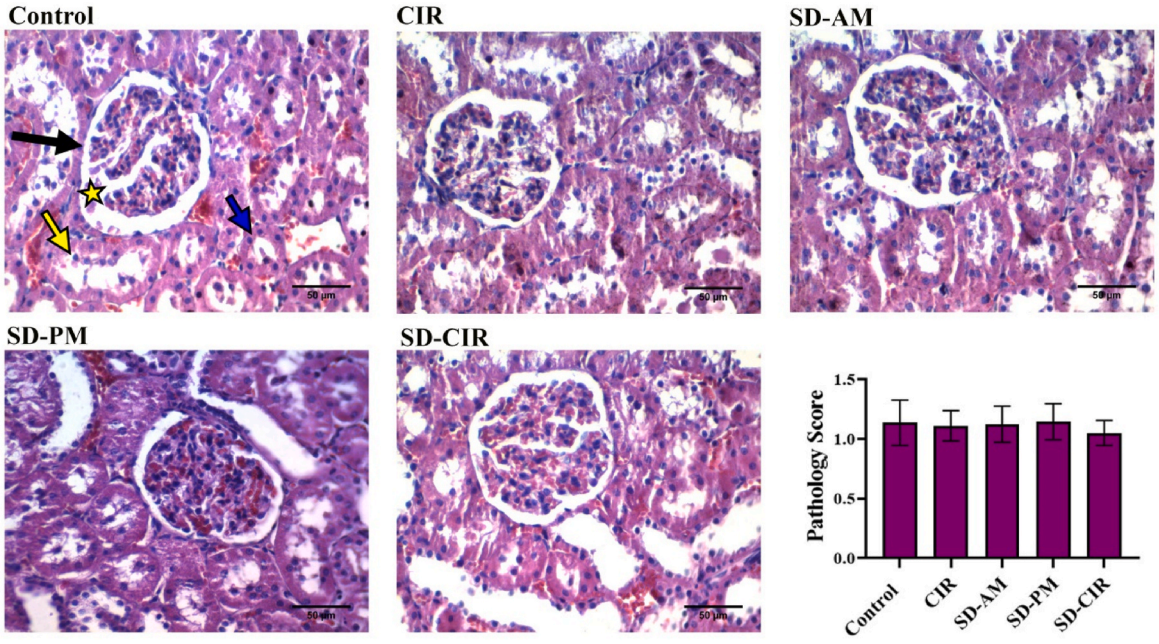

Fig. 6. Kidney micrographs and histopathological results of chronic partial sleep deprivation with/without circadian rhythm disruption. The kidney sections were stained with hematoxylin and eosin (H&E) and observed under a microscope at a magnification of 400×. The scale bars in the micrographs represent 50 μm. In the control micrographs, specific structures such as the proximal convoluted tubule (marked with a yellow arrow), distal convoluted tubule (marked with a blue arrow), glomerulus (marked with a black arrow), and the urinary space (marked with a yellow star) are indicated. The histopathological assessment was performed using a scoring system, where scores were assigned as follows: 1 (normal), 2 (slight damage), 3 (moderate injury), 4 (severe damage), and 5 (very severe damage). The sum of the scores is presented as the mean ± standard deviation (S.D.). The evaluated groups include CIR (circadian rhythm disrupted), SD-AM (sleep deprived during light conditions, 8–12 a.m.), SD-PM (sleep deprived during dark conditions, 8–12 p.m.), and SD-CIR (sleep deprived plus circadian rhythm disruption).

the physiological and behavioral impacts of the T7 cycle to validate its potential role in circadian disruption.

In this study, researchers found that disrupting the circadian rhythm in animals, either independently or in conjunction with sleep deprivation, significantly reduced their food and water intake. Surprisingly, while sleep deprivation did not notably affect food consumption during the day or night, it did decrease water intake. Physiologically, both food intake and energy metabolism follow daily patterns regulated by the circadian timing system, similar to sleep cycles. For instance, when rats undergo a 24-h fast at various times, they do not proportionally increase their food consumption during refeeding. Instead, their feeding behavior is rhythmic, peaking at dawn, highlighting the circadian influence on feeding (Rivera-Estrada et al., 2018). Humans also experience daily variations in hunger and satiety, with increased hunger and reduced satiety in the evening compared to the morning, highlighting the influence of the circadian rhythm on the regulation of appetite and feeding behaviors in humans (Sargent et al., 2016; Scheer et al., 2013). This diurnal fluctuation is partly regulated by hormones like ghrelin and gastrointestinal peptide YY (Rynders et al., 2020).

In our study, also, water consumption in rats is influenced by food availability and time of day, peaking during dark hours when food is present after 24 h of water deprivation. It has been reported that the lowest water consumption in rats occurs when food is unavailable, particularly during light hours (Ang et al., 2001). The study revealed that sleep-deprived rats exhibited significantly decreased water intake, indicating that sleep disruption also affects hydration. However, one study showed that in both US and Chinese adults, shorter sleep duration was linked to a greater likelihood of inadequate hydration compared to those who slept for 8 h (Rosinger et al., 2019). Furthermore, rats subjected to sleep deprivation with disrupted circadian cycles showed a weight decrease compared to controls, although this difference was not statistically significant. This suggests that circadian disruption and altered sleep/wake patterns may influence food consumption and metabolism, potentially leading to weight loss. Overall, these findings suggest that circadian rhythm disruption appears to have a more significant influence on regulating feeding behavior, leading to systematic changes in food intake due to metabolic synchronization, whereas sleep deprivation primarily impacts water intake and affects feeding behavior through hormonal changes and recovery eating patterns (Arble et al., 2010; Marcheva et al., 2013; Pickel and Sung, 2020; Serin and Acar Tek, 2019).

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize or repair their harmful effects. In this context, changes in the activity levels of MDA and SOD can indicate oxidative damage to tissues, potentially leading to cell injury and death (Bajpai et al., 2017; Cetinkaya et al., 2005). In the current experiment using our animal model, the levels of SOD were not affected. However, a significant increase in the levels of MDA was observed in both the SD-PM and SD-CIR groups after 28 days of sleep restriction. This indicates that sleep restriction led to increased oxidative stress as reflected by elevated MDA levels. Likewise, in clinical practice, a study involving 24-h acute sleep deprivation in young adults demonstrated significantly elevated levels of plasma MDA concentration. In contrast, decreased levels of plasma SOD were observed (Marzany et al., 2018; Wei et al., 2017). Recent studies have indeed confirmed that the formation of serum MDA levels is associated with the circadian rhythm and exhibits a 24-h periodicity in both humans and animals (Morera et al., 2007; Sani et al., 2007, 2015). According to the research conducted by Sani et al., it was found that the levels of MDA in both serum and kidney are higher during the dark phase compared to the light phase (Sani et al., 2007). These findings align well with our own research observations.

The increase in stress levels observed in the rats, despite sleep deprivation occurring during their peak activity hours, may be attributed to the chronic nature of the intervention, even if it is relatively mild. Furthermore, in other studies where sleep deprivation was implemented for a shorter duration of 4 h, certain alterations have been reported (Jha et al., 2016). Even if it is timely and mild, forced locomotion itself can act as a stressor for rats (Nollet et al., 2020).

The evaluation of renal function, as assessed by plasma concentrations of biochemical factors such as urea and Cr, did not reveal any significant differences among the groups studied. To the best of our knowledge, only a limited number of studies have investigated the effects of chronic sleep deprivation and circadian rhythm disruption on the parameters mentioned. Previous reports have indicated that acute sleep deprivation for 24, 48, and 72 h in mice with normal circadian rhythm can lead to increased plasma levels of urea, with the highest elevation observed in the 72-h sleep-deprived group (Periasamy et al., 2015). Additionally, in clinical practice, sleep deprivation has been found to increase plasma levels of uric acid. However, it appears to have minimal effect on plasma levels of Cr (Chou et al., 2020). Normal patterns of circadian rhythms have a significant impact on organ function and play a crucial role in preventing various health problems. When the molecular pathways involved in circadian rhythms are disrupted in hamsters, it can lead to an increase in serum levels of Cr, which is an indicator of renal damage (Egstrand et al., 2020; Martino et al., 2008). These findings present a discrepancy compared to our research. It is worth noting that various factors, such as age, diet, and previous medical history, can indeed influence biochemical parameters, and their potential impact may not have been accounted for in the current study. Hence, the disparity in findings could potentially be attributed to differences in the design of our study or the relatively short duration of the intervention.

The renal weight-to-body weight ratio, often referred to as the kidney index, is a significant marker used to assess the normal structural characteristics of the kidneys (Nirogi et al., 2014). In the current study, no significant differences were found in the kidney index among the groups under investigation. This finding contradicts previous studies that have reported an increase in kidney weight in sleep-deprived rats without accompanying pathological changes (Everson et al., 1989; Kushida et al., 1989). It is important to acknowledge that no single report can fully investigate the relationship between chronic sleep deprivation or circadian rhythm disruption and the specific parameter of interest, such as the kidney index.

In this study, renal structural changes were assessed, specifically focusing on parameters such as glomerular area, glomerular tuft area, urinary space area, and cortex thickness. These measurements were utilized to evaluate and analyze the structural characteristics of the kidneys. During the 4 weeks of SD and/or circadian rhythm disruption, the experimental groups did not exhibit significant differences in the assessed parameters, including glomerular area, glomerular tuft area, urinary space area, and cortex thickness. It is important to note that there is currently a lack of sufficient studies in the literature that specifically evaluate morphological parameters in kidney samples. According to a study conducted by Thomal et al., acute sleep deprivation during pregnancy was found to have negative effects on rat offspring. The study revealed that newborn rats born to sleep-deprived mothers exhibited a decrease in the number of glomeruli, which are the functional units responsible for filtration in the kidneys. Additionally, the study showed an increase in glomerular area in these offspring (Thomal et al., 2010). Indeed, comparing findings across studies exploring the effects of sleep deprivation on renal parameters can be challenging due to variations in sleep deprivation models and the use of different animal species. However, it is important to note that alterations in renal cortex thickness have been associated with kidney diseases characterized by reduced renal function (Hoi et al., 2018; O'Neill, 2000). As previously mentioned, changes in renal cortex thickness are typically regarded as late indicators of progressive kidney damage. These changes often manifest in advanced stages of renal disease, rather than early or subtle alterations.

Histopathological examinations of the kidneys did not reveal any pathological lesions, indicating that the kidneys appeared normal.

However, in contrast to these findings, acute sleep deprivation for consecutive days (1, 3, and 5 days, referred to as SD1, SD3, and SD5 respectively) in rats resulted in negative impacts on renal pathology. Accordingly, in the group subjected to one day of sleep deprivation (SD1), histopathological assessments revealed glomerular congestion and swelling of the tubular epithelium. In the group subjected to three days of sleep deprivation (SD3), findings included glomerular tuft congestion and apoptosis of tubular epithelium. Additionally, in the group subjected to five days of sleep deprivation (SD5), glomerular tuft congestion and the formation of casts were reported (Taha et al., 2018). Additionally, Martino et al. conducted a study reporting that alterations in circadian rhythms in hamsters resulted in increased renal pathology. The observed renal pathology included dilation of proximal tubules, along with degenerative and regenerative changes in the kidney. Furthermore, the study found collagen deposition in the renal cortex and ischemic changes in the glomeruli (Martino et al., 2008). Indeed, the use of different sleep deprivation methods, including variations in duration and timing, as well as the degree of circadian rhythm disruption, can be potential explanations for the divergent outcomes observed in our study.

To our knowledge, this is the first study to investigate the combined effects of chronic sleep deprivation and circadian rhythm disruption on both renal structure and function in rats. Thus, it is recommended to conduct further studies with varying intensities to explore this topic more comprehensively. In summary, our findings indicate that kidney tissue rhythms are relatively less impacted by circadian rhythm disruption compared to other organs, such as the reproductive system. This observation suggests that the treatment of circadian disorders becomes more complex when targeting the kidneys. On one hand, there is evidence suggesting that the kidney, much like the choroid plexus, plays a significant role in the master circadian timekeeping of the body as a whole (Firsov and Bonny, 2018). This implies that the kidney is involved in regulating the body's internal circadian rhythms and maintaining overall circadian synchronization (Stow and Gumz, 2011).

This study has some limitations that should be considered. First, the four-week duration may not fully capture the long-term effects of sleep deprivation and circadian disruption on renal health. Second, the lack of locomotor activity measurements limits insights into behavioral changes associated with the interventions. Future research should include longer study durations and behavioral assessments to provide a more comprehensive understanding of the interactions between sleep, circadian rhythms, and renal health.

5. Conclusion

The findings of the study suggest that chronic partial sleep deprivation, in combination with circadian rhythm disruption, has negative effects on feeding behavior, and weight gain, and results in elevated levels of a marker of oxidative stress, MDA. However, it is noteworthy that this condition does not appear to have a significant impact on renal structure and function. Ultimately, the authors emphasize that future research should focus on the physiological and behavioral aspects to validate any potential circadian disruption, thereby confirming the T7 model in animals.

CRediT authorship contribution statement

Shirin Rezazadeh: Writing – review & editing, Writing – original draft, Formal analysis. **Saeed Rastgoo Salami:** Writing – review & editing, Writing – original draft, Formal analysis. **Mehran Hosseini:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Henrik Oster:** Methodology, Conceptualization. **Mohammad Reza Saebipour:** Validation, Supervision, Methodology, Conceptualization. **Mohammad Mehdi Hassanzadeh-Taheri:** Supervision, Project administration, Investigation, Conceptualization. **Hamed Shoorei:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization.

Informed consent statement

Not applicable.

Availability of data and Materials

The dataset generated and/or analyzed during the current study is not publicly available due to restrictions or privacy concerns. However, it can be obtained from the corresponding author upon a reasonable request.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Birjand University of Medical Sciences. The ethical code is IR.BUMS.REC.1400.222.

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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