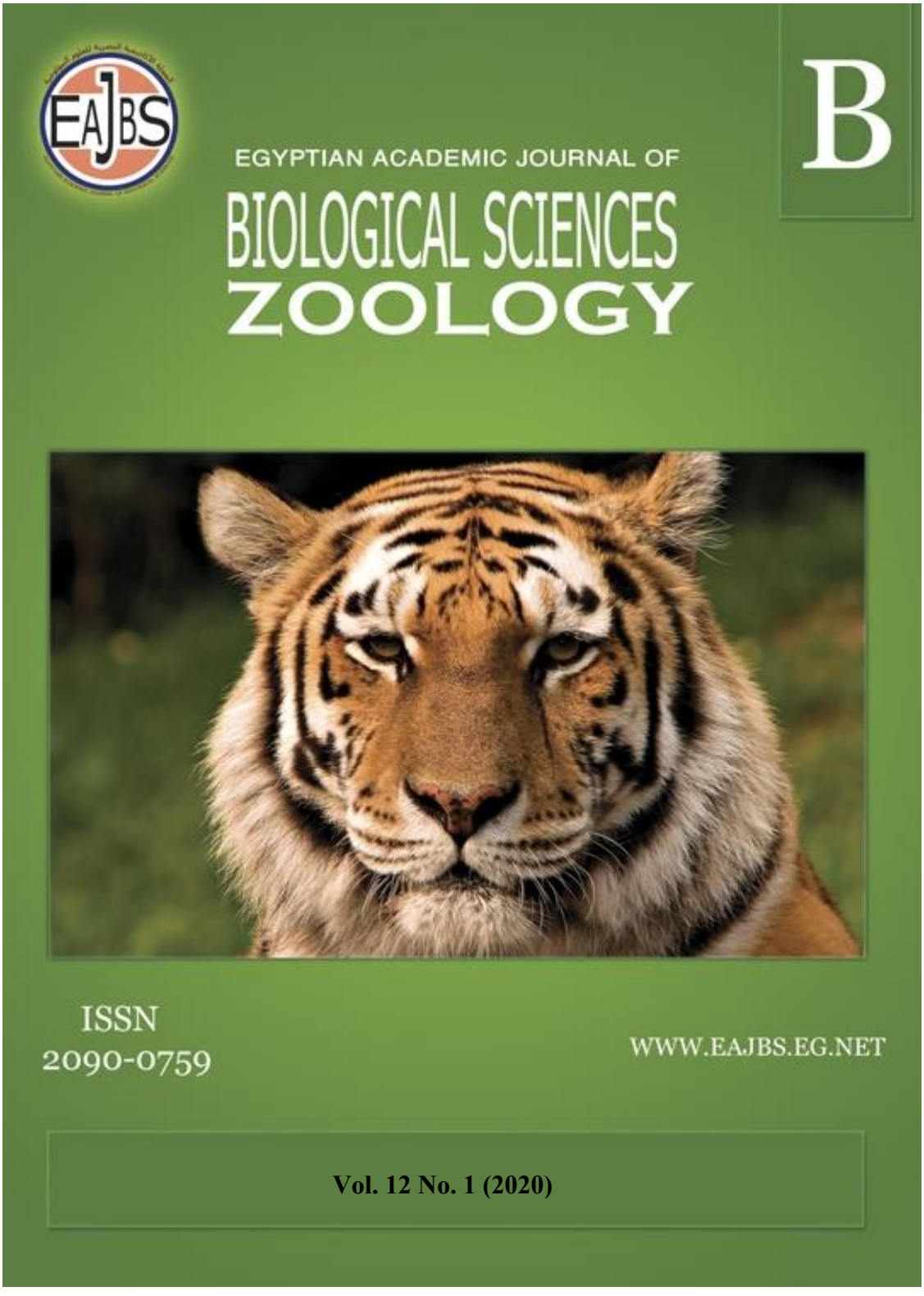

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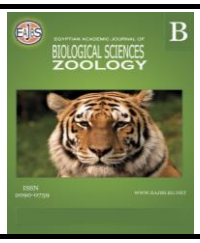

Timing of CCl_4-induced Liver Cirrhosis Modulates Locomotor And Melatonin Circadian Rhythms In Rats

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ABSTRACT

Patients with liver cirrhosis usually suffer from disturbances in sleep and melatonin rhythms. Since their homeostasis is tightly interconnected and regulated by the circadian clock, we aimed to use them as biomarkers to investigate if the time of exposure to a hepatotoxin would affect the level of haptic injury and possibilities of recovery. This is to simulate a situation where an organism is exposed to a hepatic toxin regularly at a specific time of day. Probably due to an environmental or lifestyle constraint. We monitored the circadian rhythms of locomotor activity and melatonin as biomarkers for liver health level in a chronic CCl_4-induced cirrhotic rat model. Cirrhotic rats expressed circadian locomotor activity that has shallower amplitudes compared to controls with higher activity during daytime and lower activity during the night compared to controls. Night CCl_4-treatment appeared correlated with more rhythm disturbance than daytime treatment. For melatonin, daytime CCl_4-treatment abolished circadian rhythmicity, but night treatment was correlated with 6-hour rhythm advance and reduction of the melatonin's night peak. Recovery was partial; however, it was better from daytime treatment than from night treatment. Histopathological evaluation of liver confirmed the above findings showing evidence of more severe liver lesions in the night than daytime treated rats. These results suggest that the CCl_4 hepatotoxin effects are clock modulated, which imposes careful consideration of the time factor in the design of research experiments and medical treatment programs for liver and sleep patients.

INTRODUCTION

Many patients with liver cirrhosis suffer from sleep disturbances, exhibiting delayed night sleep, insomnia, or excessive daytime sleepiness (Mabrouk *et al.*, 2012). Some also suffer from disturbances in cognitive and motor skills leading to a higher accident rate (Haeger *et al.*, 2019). These symptoms have been attributed to impaired hepatic melatonin metabolism (Montagnese *et al.*, 2014). Melatonin and sleep are tightly interconnected under the regulation of the circadian system. This system has evolved in most organisms in adaptation to the stress caused by the fluctuating environmental conditions due to the rotation of the earth around its own axis and the sun, represented primarily by the daily light-dark cycle. These endogenous clocks bestow their owner's fitness advantages by enabling them to

make the necessary physiological and behavioral adjustments in advance of the relatively predictable and stressful environmental changes (Pilorz *et al.*, 2018).

Melatonin is a highly versatile bioactive indoleamine, that in addition to its major role in sleep, plays important functions in homeostasis, free radical scavenging, thermoregulation, metabolism, immunity, sexual behavior, neuromodulation, and vasomotor responses (Manchester *et al.*, 2015; Sun *et al.*, 2015). In humans, and many vertebrates, it is synthesized mainly by the pineal gland and its levels fluctuate in synchronization with the daily light-dark cycle under the regulation of the hypothalamic suprachiasmatic nucleus (SCN); The master circadian oscillator in vertebrates (Anwar *et al.*, 2015; Asher and Schibler, 2011). Its biosynthesis peaks at night and is acutely suppressed by light in healthy individuals. Therefore, it is known as the endogenous signal of darkness and sleep and coincides with a decrease in core body temperature, alertness, and performance. It was even found that longer nights are associated with longer durations of high melatonin (Cardinali and Pévet, 1998). It encodes the time-of-day, length-of-day information, and is a good marker for the output of the circadian pacemaker.

Because the circadian clock is regulating the interconnected melatonin and sleep rhythms, and melatonin is protecting the liver from peroxidation (Mukherji *et al.*, 2019); the main cause of fibrosis from hepatotoxins. This study aimed to evaluate the effect of time-of-exposure to a hepatic toxin on the subsequent disturbance of circadian plasma melatonin and behavioral activity, as well as recovery possibilities. This is to simulate a situation where an organism is exposed to a hepatic toxin on a regular basis at a specific time of day. Probably due to an environmental or lifestyle constraint. A variation in the liver intoxication outcome at different time points would denote the implication of the circadian clock in modulating those harmful effects.

MATERIALS AND METHODS

Experimental Animals:

Healthy male Sprague-Dawley rats (120-180 g, 6-8 weeks old) obtained from the Research Institute of Theodor Bilharz in Egypt were used as experimental animals. At the end of experiments, the remaining rats were killed by an overdose of isoflurane (Seymour and Nagamine, 2016) followed by autopsy to collect livers for histological examination. Animal care and experimental procedures followed the ethics guidelines of the animal care committee of the Faculty of Science, Suez Canal University, Ismailia, Egypt.

Animal Housing and Care:

Animals were kept under natural light-dark cycles of 14-hour light and 10-hour dark (LD 14:10) (Lights-on = 5:00 am (ZT0) and lights-off = 7:00 pm (ZT14), respectively; Egyptian local time). The housing room temperature was kept at 25±2 °C and relative humidity 55±15%. Rats were housed in wire-mesh cages (34 × 28 × 40 cm) with waste drawers, lined with sand and sawdust to absorb and reduce odor. Regulated drinking water-system and external food bowls were used to avoid direct handling of animals and minimizing stress and disturbance. Food and water were given ad libitum, and cleaning cages occurred at random times to prevent animals from entrainment by these potential *Zeitgeber* events. Light intensities, measured by a digital illumination meter (INS DX-200), were at a maximum illuminance of (100 lux) during the light phase and a minimum illuminance of (0.01 lux) during the dark phase.

Chemicals:

Carbon Tetrachloride (CCl_4) (Sigma-Aldrich Chemicals Co.) was obtained from local commercial suppliers. It was used to induce liver cirrhosis in the olive oil solvent. Chronic

CCl_4 administration causes an increase in collagen synthesis, followed by the development of liver fibrosis and eventually cirrhosis (Jiménez *et al.*, 1992).

Experimental Design:

Experiments were conducted under natural light-dark cycles of LD 14:10. Rats were injected with 40% CCl_4/olive oil (1 ml/kg body weight per day, twice per week) for 6 weeks to induce chronic chemical liver cirrhosis. Followed by a 4 weeks recovery period in which CCl_4-treatment was ceased. Night treatment with CCl_4 (Night-CCl_4) was administered at ZT15-17 for groups 2 and 5, while daytime treatment (Day-CCl_4) at ZT3-5 for groups 3 and 6.Sixty-three rats were divided into six groups. Groups (1-3, n=5 in each) were for analyzing locomotor activity using running wheels. Groups (4-6, n=16 in each) were for monitoring serum melatonin levels four times a day (every 6 hours at ZT5, 11, 17, 23, n=4 each). Details of the groups are summarized in (Fig. 1). Before the experiments, rats were allowed two habituation weeks to settle in the animal house conditions.

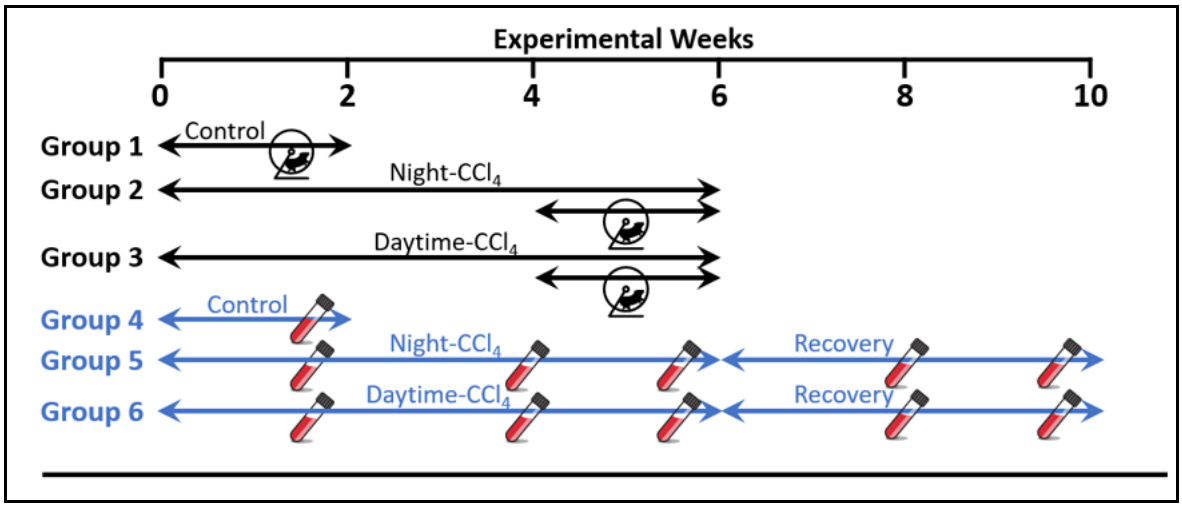

Fig. 1: Schematic representation of the experimental design.

= Locomotor activity recording. = Serum melatonin sampling (End of every 2 weeks)

Locomotor Activity Assays:

Rats were kept separately in a tower (height = 183 cm) of five mesh-wire cages (50 × 35 × 37 cm). Each cage is provided with a running wheel (23.5 cm in diameter and 13 cm wide) equipped with electronic switch sensors for counting wheel revolutions. Details of the setup are described in (Hassan and Zaki, 2015). Locomotor activity expressed in the number of wheel revolutions every 3-minutes was continuously recorded and automatically saved to a computer. Data were then analyzed using Microsoft Excel 2016.

Melatonin Level Determination:

Enzyme-Linked Immunosorbent Assay (ELISA) of serum melatonin was used according to the manufacturer's instructions (Rat Melatonin ELISA Kit, MyBioSource, USA). This kit can detect melatonin concentrations as low as 2 pg/ml with high sensitivity, specificity, and no significant cross-reactivityy or interference between melatonin and analogues.

Liver Histological Examination:

After behavioral recordings and blood sampling, rats were sacrificed, and their livers were immediately fixed in 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed, dehydrated, and embedded in Paraffin, then stained with haematoxylin-eosin (HE) stain and Masson's trichrome (Amin and Mahmoud-Ghoneim, 2011).

Statistical Data Analysis:

Data were expressed as mean ± SD (Standard deviation) and analyzed by SPSS 21.0 software using a one-way analysis of variance (ANOVA) followed by post hoc Tukey test. The statistical significance level was considered at $p<0.05$. Actograms were plotted using ActogramJ (http://actogramj.neurofly.de/) (Schmid *et al.*, 2011); an open-source plug-in for ImageJ Software (http://rsb.info.nih.gov/ij), both are free.

RESULTS

Effect of CCl_4-induced liver cirrhosis on circadian locomotor activity:

Both control (Group 1) and cirrhotic (Groups 2 and 3) rats displayed nocturnal locomotor activity with clear circadian rhythm under (LD 14:10) cycles (ANOVA, $p<0.05$). However, activity levels of cirrhotic rats were higher during daytime and lower during the night compared to controls (ANOVA, $p<0.05$) (Figs. 2 & 3). Activity's acrophase was in the first half of the night around ZT17, while its trough was in the first half of the day around ZT5 in all groups. There was no significant difference in the total locomotor activity between the three groups.

Comparing the daytime and night treatments revealed that the locomotor activity of daytime treated rats was significantly lower at the end of daytime (ZT11), but significantly higher at the end of the night (ZT23) (Fig. 2).

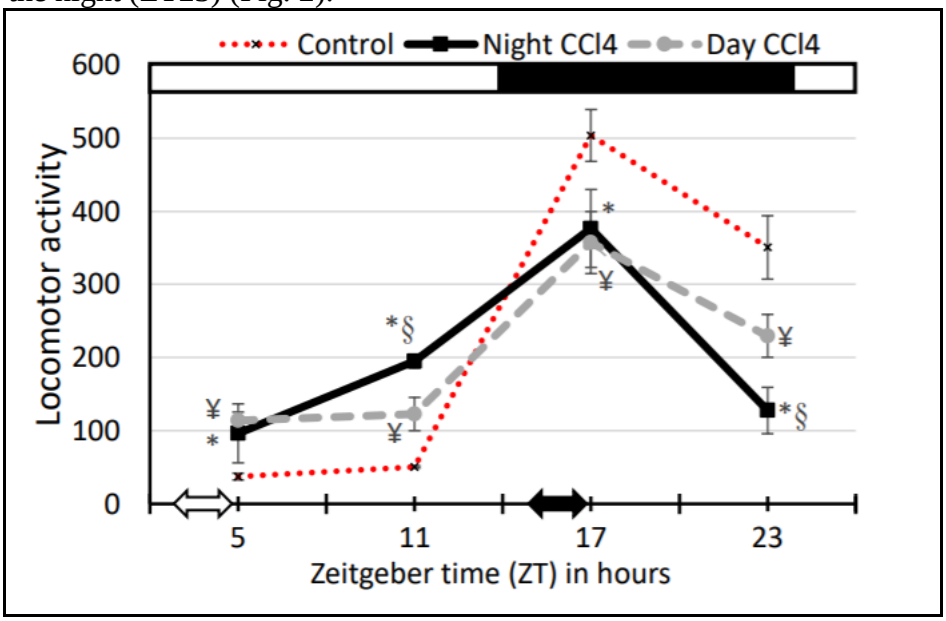

Fig. 2: Circadian locomotor activity rhythms of control and CCl_4-induced cirrhotic rats. Locomotor activity is represented as means±SD of running wheel revolutions at 6-hour intervals. White and black bars above the figure indicate daytime and nighttime, respectively, under a natural cycle of LD 14:10 h. Double-head arrows on the x-axis indicate the timing of CCl_4-treatment at daytime ZT3-ZT5 (white arrow), and at nighttime ZT15-ZT17 (black arrow). Significant difference from control is indicated by ¥ for daytime and * for nighttime treatments, while § indicates a significant difference between the two treatment timings using one-way ANOVA followed by post-hoc Tukey test at $p<0.05$.

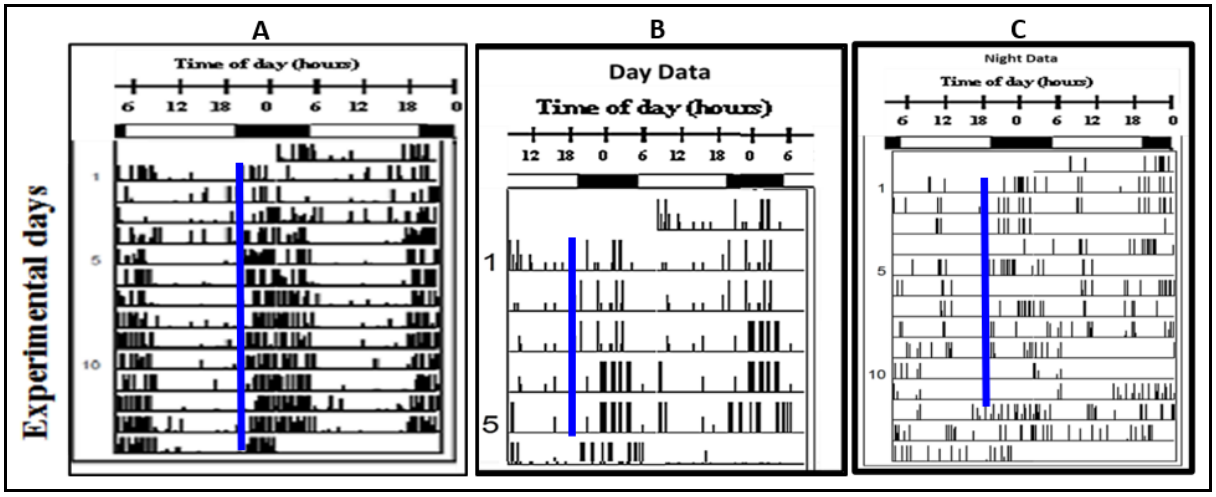

Fig. 3: Double-plotted actograms of circadian locomotor activity of representative control (A), daytime CCl_4-treated (B), and night CCl_4-treated (C) rats. Activity counts were recorded in 3-minute intervals. White and black bars above the figures indicate daytime and nighttime, respectively, under natural cycles of LD 14:10 h. Vertical guidelines inside the figures indicate the entrainment phase.

Effect of CCl_4-induced liver cirrhosis on melatonin levels:

Control rats exhibited normal nocturnal circadian melatonin rhythm that peaks at late night around ZT23 (46.5 ± 1.5 pg/ml) while staying at a baseline level during daytime around (13.5 ± 0.6 pg/ml) (Fig. 4). Rats treated with CCl_4 at daytime exhibited reduced serum melatonin levels at subjective night after two weeks and continued to six weeks at a level equal to normal daytime baseline level, practically abolishing its circadian rhythmicity (Fig. 4A). When these rats were allowed to recover by ceasing CCl_4-treatment, subjective night levels of melatonin started to recover and significantly increased. However, although two weeks were enough for the initial night rise in melatonin at ZT17 to recover to its normal level, even four weeks were not enough for a full recovery of the night peak at ZT23. At this point It is only partially recovered by about 50% then 75% after two and four weeks, respectively (Fig. 4B).

On the other hand, rats treated with CCl_4 at night expressed a significant increase in melatonin level at late daytime (ZT11), starting after four weeks of treatment and a significant decrease of about 50% at late night (ZT23), starting from two weeks of treatment compared to controls (Fig. 4C). The melatonin rhythm in these rats appeared 6-hours advanced with a significant rise at ZT11 and significant amplitude reduction during the night phase. When these rats were allowed to recover, a normal low level at ZT11 was fully restored after two weeks. However, their early night (ZT17) melatonin level increased significantly compared to control, only after four weeks (Fig. 4D). However, even four weeks of recovery was not enough for the subjective late-night peak melatonin at (ZT23) to restore its normal level and stayed significantly lower (66%) compared to controls (Fig. 4D).

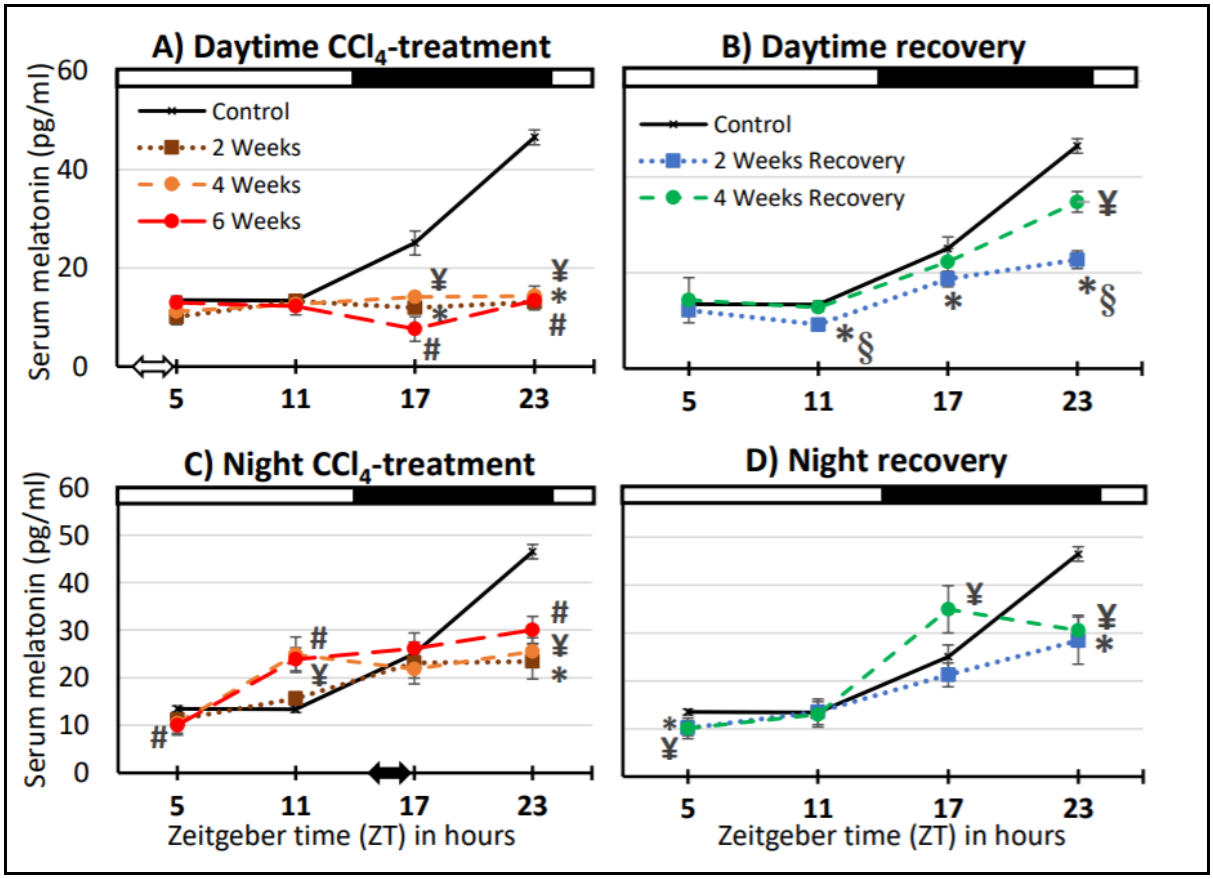

Fig. 4: Circadian rhythm of serum melatonin levels of (A) control and daytime CCl_4-treated rats and (B) their recovery versus (C) night CCl_4-treated rats and (D) their recovery. Data is showing the effect of chronic cirrhosis induction after 2, 4, and 6 weeks of CCl_4-treatment and the recovery after 2 and 4 weeks. White and black bars above the figures indicate daytime and nighttime phases, respectively, under a natural LD 14:10. Double-headed arrows on the x-axis mark the time of CCl_4-treatment in (A) daytime (ZT3-5) and in (C) night (ZT15-17). Blood was sampled after the end of every 2-week period. Serum melatonin level is presented as mean±SD at 6 h intervals (n=4 rats/time point). Significant difference from control is marked with * for 2 weeks, ¥ for 4 weeks, # for 6 weeks, whereas, § marks a significant difference between the daytime and night treatments.

Histopathological evaluation of CCl_4-induced liver cirrhosis:

Histopathological evaluation of liver samples of control animals revealed normal histo-architecture and normal appearance of the central vein with well-formed cord arrangement of hepatocytes with well-preserved cytoplasm, prominent nucleus and nucleolus, and normal portal area (Fig. 5). On the other hand, livers of rats treated with CCl_4 at daytime (Fig. 6) and night (Fig. 7) exhibited a strong yellow color, apparently manifesting severe abnormality characterized by the portal and intra-lobular inflammatory infiltrates, Kupffer cell hyperplasia, vesicular steatosis, portal area congestion, ballooning degeneration with swollen liver cells, and clearly visible focal necrosis. These livers had fibrous septa with a heterogenous population of non-parenchymal cells. However, the severity of histopathological hepatic lesions was markedly lower in rats treated with CCl_4 at night compared to rats treated in the daytime.

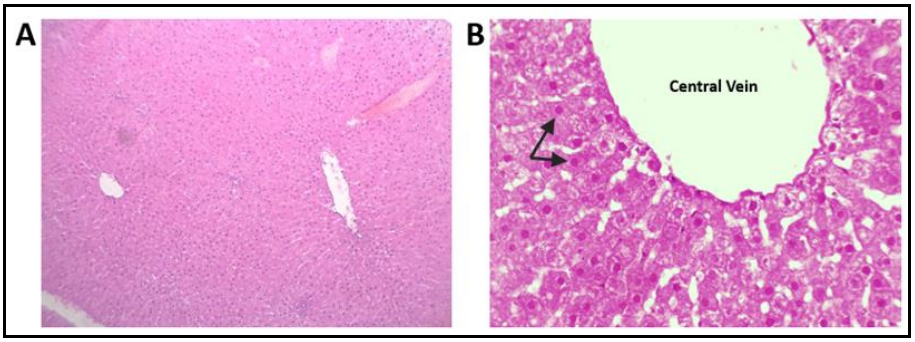

Fig. 5: Histopathological evaluation of the liver tissue of control rats. (A) Normal liver architecture. (B) Normal appearance of central vein with well-formed cord arrangement of hepatocytes having conspicuous nucleus as marked by arrows (HE, 10x and 40x).

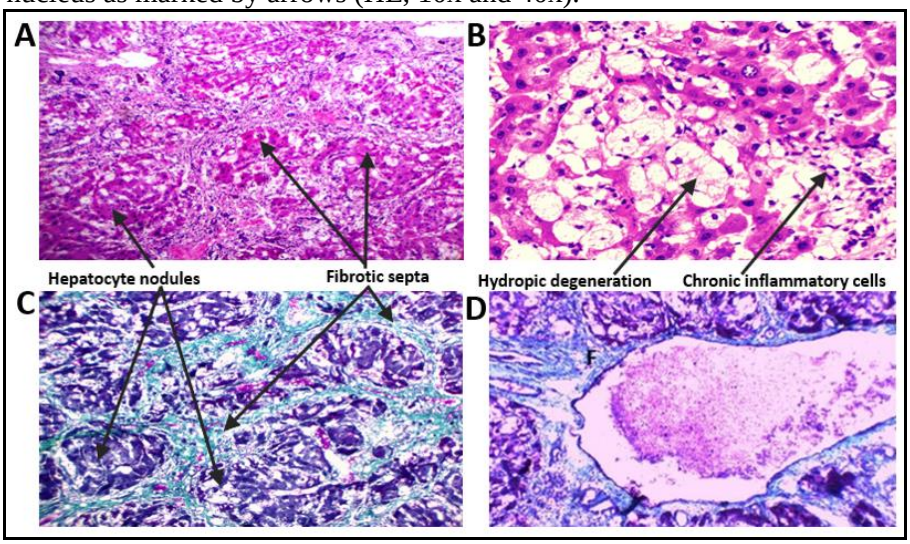

Fig. 6: Histopathological evaluation of the liver tissue of CCl_4-treated rats at daytime (ZT3-5) showing (A) and (B) Loss of cell architecture by extending fibrous septa with chronic inflammatory cells, and forming cirrhotic nodules (HE, 10x and 40x). (C) and (D) Show cirrhotic nodules (Masson Trichrome, 10x and 40x).

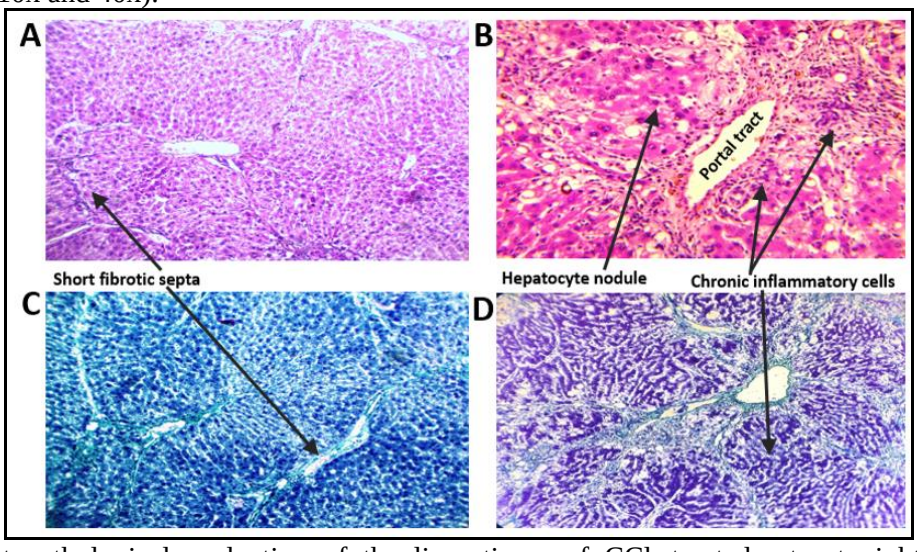

Fig. 7: Histopathological evaluation of the liver tissue of CCl_4-treated rats at night (ZT15-17) showing (A) and (B) expansion of the portal tracts with chronic inflammatory cells and extension of short fibrous septa (HE, 10x and 40x). (C) and (D) Extension of short fibrous septa from portal tracts (Masson Trichrome, 10x and 40x). However, the severity of lesions here is lower than in livers of rats treated with CCl_4 at daytime.

DISCUSSION

This study aimed to investigate if the time-of-exposure to a cirrhosis-inducing agent would affect the level of liver damage and the possibilities of recovery after ceasing exposure. This is to simulate a situation where an organism is exposed to a hepatic toxin regularly at a specific time of day. Probably due to an environmental or lifestyle constraint such as exposure to toxicants during night shift work. Several lines of evidence suggest that drug-induced hepatotoxicity and injury, including fatty degeneration and hepatocellular death, are clock-modulated (Bruckner *et al.*, 2002; Chen *et al.*, 2009; Mukherji *et al.*, 2019; Yoshioka *et al.*, 2017). For example, absence of the Period 2 (*Per2*) core clock gene in *Per2*-Null mice that modulates the uncoupling protein-2 (*Ucp2*) gene expression in the liver leads to a decrease in intracellular levels of ATP and consequently increased production of toxic CCl_4 derivatives through a clock-controlled PPAR-α signal transduction pathway (Chen *et al.*, 2009). Overall results of this study illustrated that exposure at night was correlated with higher disturbances in locomotion and melatonin rhythms that were harder to recover compared to daytime exposure.

Experimental Design:

In this study, we have chosen to induce liver cirrhosis by CCl_4 because it is a widely used classic model in biomedical research as its underlying biochemical mechanisms and histopathological changes are comparable to those observed in humans (Domitrović *et al.*, 2009; Nhung *et al.*, 2014). Metabolism of CCl_4 produces trichloromethyl (CCl_3*)-free radical by cytochrome P450 isozymes. The free radical then reacts with various vital cellular biomolecules leading to peroxidation, membrane damage, and disturbed calcium homeostasis (Chen *et al.*, 2009; Weber *et al.*, 2003). Chronic exposure to the potent CCl_4 hepatotoxin induces inflammation, oxidative stress, and cell death. This translates into centrilobular necrosis that induces fibrosis as part of the wound healing process within 5-7 weeks (Oleshchuk *et al.*, 2019). It might progress to cirrhosis in case of more prolonged exposure. However, that is dose-, species- and strain-dependent (Starkel and Leclercq, 2011). Here, CCl_4 was administered twice per week for six weeks (Fortea *et al.*, 2018) via intraperitoneal injections to reduce cirrhosis-induction time (Jiménez *et al.*, 1992).

Effect of CCl_4-induced Cirrhosis On Locomotor Activity:

CCl_4-treated rats in this study exhibited higher than normal locomotor activity during the light phase (rest phase for the nocturnal rats), regardless of the time of treatment. This is comparable to human patients with cirrhosis complaining from fragmented night sleep due to frequent waking and difficulty of falling asleep at night (Ghabril *et al.*, 2017) or under the effect of some stressors (Sallam *et al.*, 2016). However, night treatment resulted in more severe effects expressed as higher activity at late daytime (ZT11) (Fig. 2). On the other hand, CCl_4-treatment decreased activity during the night phase (activity phase for the nocturnal rats) regardless of the time of treatment, with night treatment again causing more severe effects but here expressed as lower than normal activity level at late night (ZT23). This is also comparable to cirrhotic patients complaining of sleepiness during the daytime (Jiménez-Anguiano *et al.*, 2009).

These results are in accordance with previous studies that demonstrated that different models of chronic liver disease are associated with locomotor dysfunction, especially suppressed locomotor activity (Cauli *et al.*, 2007). Likewise, Montagnese *et al.* (2013) explained that patients with cirrhosis exhibited alterations in sleep quality, and sleep timing worsened in parallel with the degree of hepatic dysfunction and reduced ability to produce restorative sleep (Formentin *et al.*, 2018). Suppression of melatonin at night in the CCl_4-treated rats might be the reason behind reduced night activity in these nocturnal animals. As melatonin administration was found to shift circadian rhythms in both humans (Arendt and

Skene, 2005; Cardinali, *et al.*, 2006) and rodents (Pévet *et al.*, 2006). On the other hand, the increased activity during daytime in treated rats might have triggered the "homeostatic sleep" regulation mechanism that is responsible for the increase in sleep propensity at next night when sleep is curtailed or absent. It has been shown that sleep deprivation produces an increase in slow-wave activity in the recovery night. In contrast, a daytime nap attenuates slow-wave activity in the subsequent sleep episode (Montagnese and Bajaj, 2019).

Ceasing CCl_4-treatment seemed to help rats to gradually recover close to their normal rhythmicity. However, recovery from daytime treatment was better than night treatment, started after two weeks, and increased after 4 weeks. Meaning that night exposure to the CCl_4 hepatotoxin is more deleterious and is more likely to produce a seemingly decompensated cirrhosis compared to daytime treatment (Bruyneel and Sersté, 2018).

Effect of CCl_4-induced Cirrhosis On Melatonin:

Melatonin is metabolized in the liver to be quickly excreted as 6-sulfatoxymelatonin in the urine. Its levels were found disturbed in liver cirrhosis patients depending on the grade of liver failure (Chojnacki *et al.*, 2012). It was proposed that melatonin disturbances might be due to intoxication of the pineal gland by excess ammonia that results from hepatic damage, or a dysfunction of the suprachiasmatic nucleus; the central circadian pacemaker (Bruyneel and Sersté, 2018). These disturbances might also be caused by the altered rhythmicity of hepatic clock genes caused by CCl_4 intoxication, since master clock dysfunction and liver disorders were found to interchangeably affect each other (Tahara and Shibata, 2016). For example, impaired rhythmicity of *Cry2* in fibrotic livers or the loss of rhythmicity of the two clock-related genes; peroxisome proliferator-activated receptor alpha and cytochrome P450 oxidoreductase (Chen *et al.*, 2010), that might affect rhythmicity of melatonin metabolism in the liver.

In this study, rats that received night CCl_4 expressed morning elevations of melatonin at late daytime (ZT11) (Fig. 4C). Similar elevations have long been known in patients with hepatic failure (Montagnese *et al.*, 2014; Steindl *et al.*, 1997). Here they were also concurrent with the elevation of locomotor activity (Fig. 2). On the other hand, daytime CCl_4-treatment had a lesser effect on the locomotor activity but had a major impact on melatonin since its level was completely suppressed during the night, practically abolishing its rhythmicity (Fig. 4A). This is reminiscent of some cirrhosis patients reported by Montagnese *et al.* 2010, who have lost melatonin rhythmicity.

Pathohistological Evaluation of CCl_4-induced Cirrhotic Livers:

Liver cirrhosis in CCl_4-treated rats was confirmed using a pathohistological study of liver tissue to eschew any possibility of unpredictable acute damage caused by CCl_4 (particularly in initial doses) and the highly variable yield of cirrhosis (Fortea *et al.*, 2018). Liver tissue of CCl_4-treated rats exhibited necrosis, fibrosis, mononuclear cell infiltration, hemorrhage, fatty degeneration, and formation of regenerative nodules. Additionally, apoptotic figures, microvesicular steatosis, and hydropic degeneration in hepatocytes were also noticed (Figs. 6 & 7). We graded them as (2/5) degrees of cirrhosis that didn't reach decompensated cirrhosis according to Doi *et al*. (1991). The partial recovery of locomotor and melatonin rhythms after four weeks in this experiment attests that the protocol used here produced a level of reversible liver damage.

Conclusion:

The findings of this study suggest the involvement of the circadian clock in modulating the effect of the CCl_4 hepatotoxin. Because different exposure times were found correlated with different effect levels. This would have significant implications in the health and research fields. In research, they add a behavioral dimension to the already established physiological and histological utilities of this rat model of induced cirrhosis. Nevertheless, they add more demands to the temporal design of research experiments involving chemical

induction of diseases in experimental models. Those experimental designs must carefully consider the timing of experimental treatments to avoid any possible confounding effects due to the involvement of the circadian clock in modulating treatment results. Experiments acknowledging those factors are expected to produce more accurate, reliable, and consistent results. On the health side, there is now a well-recognized scientific branch known as "Chronopharmacology" that is concerned with determining the best timing of drug administration as timing greatly affects absorption, distribution, metabolism and excretion of the administered xenobiotics (Elsaey *et al.*, 2016; Ferrell and Chiang, 2015). Awareness of rhythmic modulation of drug metabolism and susceptibility to injurious agents enables achieving higher desirable effects and lower adverse effects. It is also expected to help identifying specific high-risk times of the day.

The results also highlighted the involvement of the circadian clock and melatonin in the degradation of health-related quality of life in liver patients (Montagnese and Bajaj, 2019; Mukherji *et al.*, 2019). Poor sleep patterns, chronic fatigue, and reduced ability to work led to the usage of exogenous melatonin for treatment (Cruz *et al.*, 2005). This could alleviate liver fibrosis induced by many drugs via preventing peroxidation and necrosis-associated inflammatory signaling, and can even reverse cirrhosis in its early stage (Hu *et al.*, 2019; Zhang *et al.*, 2017). However, we agree with many authors (Córdoba *et al.*, 2010; Montagnese *et al.*, 2010) that circadian abnormalities of melatonin in mammals are difficult to explain due to their complex networking, and further research on a finer time scale is warranted to elucidate their intricacies.

REFERENCES

Amin, A. and Mahmoud-Ghoneim, D. (2011): Texture analysis of liver fibrosis microscopic images: a study on the effect of biomarkers. Acta biochimica et biophysica Sinica, 43: 193-203.

Anwar, M. J.; Muhammad, B. Y.; Bader, A. A.; Abdulghani, M.; Mahmood, D. and Haider, M. (2015): An insight into the scientific background and future perspectives for the potential uses of melatonin. Egyptian Journal of Basic and Applied Sciences, 2: 139-152.

Arendt, J. and Skene, D. J. (2005): Melatonin as a chronobiotic. Sleep Medicine Reviews, 9: 25-39.

Asher, G. and Schibler, U. (2011): Crosstalk between components of circadian and metabolic cycles in mammals. Cell Metabolism, 13: 125-137.

Bruckner, J. V.; Ramanathan, R.; Lee, K. M. and Muralidhara, S. (2002): Mechanisms of circadian rhythmicity of carbon tetrachloride hepatotoxicity. Journal of Pharmacology and Experimental Therapeutics, 300: 273-281.

Bruyneel, M. and Sersté, T. (2018): Sleep disturbances in patients with liver cirrhosis: prevalence, impact, and management challenges. Nature and Science of Sleep, 10: 369-375.

Cardinali, D. P.; Furio, A. M.; Reyes, M. P. and Brusco, L. I. (2006): The use of chronobiotics in the resynchronization of the sleep-wake cycle. Cancer Causes & Control, 17: 601-609.

Cardinali, D. P. and Pévet, P. (1998): Basic aspects of melatonin action. Sleep Medicine Reviews, 2: 175-190.

Cauli, O.; Mlili, N.; Llansola, M. and Felipo, V. (2007): Motor activity is modulated via different neuronal circuits in rats with chronic liver failure than in normal rats. The European Journal of Neuroscience, 25: 2112-2122.

Chen, P.; Kakan, X. and Zhang, J. F. (2010): Altered circadian rhythm of the clock genes in fibrotic livers induced by carbon tetrachloride. FEBS Letters, 584: 1597-1601.

Chen, P.; Li, C. Y.; Pang, W. Q.; Zhao, Y.; Dong, W.; Wang, S. M. and Zhang, J. F. (2009): The protective role of *Per2* against carbon tetrachloride-induced hepatotoxicity. American Journal of Pathology, 174: 63-70.

Chojnacki, C.; Walecka-Kapica, E.; Klupinska, G.; Wachowska-Kelly, P.; Zylinska, K.; Winczyk, K. and Chojnacki, J. (2012): Serotonin and melatonin secretion and metabolism in patients with liver cirrhosis. Polskie Archiwum Medycyny Wewnetrznej-Polish Archives of Internal Medicine, 122: 392-397.

Córdoba, J.; Steindl, P. and Blei, A. T. (2010): Melatonin rhythms in patients with cirrhosis response. American Journal of Gastroenterology, 105: 222-222.

Cruz, A.; Padillo, F. J.; Torres, E.; Navarrete, C. M.; Muñoz-Castañeda, J. R.; Caballero, F. J.; Briceño, J.; Marchal, T.; Túnez, I.; Montilla, P.; Pera, C. and Muntané, J. (2005): Melatonin prevents experimental liver cirrhosis induced by thioacetamide in rats. Journal of Pineal Research, 39: 143-150.

Doi, K.; Kurabe, S.; Shimazu, N. and Inagaki, M. (1991): Systemic histopathology of rats with CCl_4-induced hepatic cirrhosis. Laboratory Animals, 25: 21-25.

Domitrović, R.; Jakovac, H.; Tomac, J. and Šain, I. (2009): Liver fibrosis in mice induced by carbon tetrachloride and its reversion by luteolin. Toxicology and Applied Pharmacology, 241: 311-321.

Elsaey, M. A.; Sallam, A. E.-D.; Hassaneen, E. and Zaghloul, M. S. (2016): Circadian phase modulates the enhancing effect of the Egyptian *Moringa peregrina* extract on learning and memory in mice. Biological Rhythm Research, 47: 703-715.

Ferrell, J. M. and Chiang, J. Y. L. (2015): Circadian rhythms in liver metabolism and disease. Acta Pharmaceutica Sinica B, 5: 113-122.

Formentin, C.; Garrido, M. and Montagnese, S. (2018): Assessment and management of sleep disturbance in cirrhosis. Current Hepatology Reports, 17: 52-69.

Fortea, J. I.; Fernández-Mena, C.; Puerto, M.; Ripoll, C.; Almagro, J.; Bañares, J.; Bellón, J. M.; Bañares, R. and Vaquero, J. (2018): Comparison of two protocols of carbon tetrachloride-induced cirrhosis in rats - improving yield and reproducibility. Scientific Reports, 8: 9163-9173.

Ghabril, M.; Jackson, M.; Gotur, R.; Weber, R.; Orman, E.; Vuppalanchi, R. and Chalasani, N. (2017): Most individuals with advanced cirrhosis have sleep disturbances, which are associated with poor quality of life. Clinical Gastroenterology and Hepatology, 15: 1271-1278.

Haeger, P.; Bouchet, A.; Ossandon, C. and Bresky, G. (2019): Treatment with melatonin improves cognitive behavior and motor skills in a rat model of liver fibrosis. Annals of Hepatology, 18: 101-108.

Hassan, S. A. and Zaki, A. N. (2015): Monitoring and evaluation of circadian locomotor activity rhythms in rodents. The Egyptian Journal of Experimental Biology (Zoology), 11: 219-224.

Hu, C.; Zhao, L.; Tao, J. and Li, L. (2019): Protective role of melatonin in early-stage and end-stage liver cirrhosis. Journal of Cellular and Molecular Medicine, 23: 7151-7162.

Jiménez-Anguiano, A.; Díaz-Medina, V.; Farfán-Labonne, B. E.; Giono-Chiang, G.; Kersenobich, D.; García-Lorenzana, M.; Gutiérrez-Ruiz, M. C. and Velázquez-Moctezuma, J. (2009): Modification of sleep architecture in an animal model of experimental cirrhosis. World Journal of Gastroenterology, 15: 5176-5180.

Jiménez, W.; Clária, J.; Arroyo, V. and Rodés, J. (1992): Carbon tetrachloride induced cirrhosis in rats: a useful tool for investigating the pathogenesis of ascites in chronic liver disease. Journal of Gastroenterology and Hepatology, 7: 90-97.

Mabrouk, A. A.; Nooh, M. A.; Azab, N. Y.; Elmahallawy, I. I. and Elshenawy, R. H. M. (2012): Sleep pattern changes in patients with liver cirrhosis. Egyptian Journal of Chest Diseases and Tuberculosis, 61: 447-451.

Manchester, L. C.; Coto-Montes, A.; Boga, J. A.; Andersen, L. P. H.; Zhou, Z.; Galano, A.; Vriend, J.; Tan, D.-X. and Reiter, R. J. (2015): Melatonin: an ancient molecule that makes oxygen metabolically tolerable. Journal of Pineal Research, 59: 403-419.

Montagnese, S. and Bajaj, J. S. (2019): Impact of hepatic encephalopathy in cirrhosis on quality-of-life issues. Drugs, 79: 11-16.

Montagnese, S.; De Pittà, C.; De Rui, M.; Corrias, M.; Turco, M.; Merkel, C.; Amodio, P.; Costa, R.; Skene, D. J. and Gatta, A. (2014): Sleep-wake abnormalities in patients with cirrhosis. Hepatology: 705-712.

Montagnese, S.; Middleton, B.; Mani, A. R.; Skene, D. J. and Morgan, M. Y. (2010): Melatonin rhythms in patients with cirrhosis. The American Journal of Gastroenterology, 105: 220-222.

Montagnese, S.; Nsemi, L. M.; Cazzagon, N.; Facchini, S.; Costa, L.; Bergasa, N. V.; Amodio, P. and Floreani, A. (2013): Sleep-wake profiles in patients with primary biliary cirrhosis. Liver International, 33: 203-209.

Mukherji, A.; Bailey, S. M.; Staels, B. and Baumert, T. F. (2019): The circadian clock and liver function in health and disease. Journal of Hepatology, 71: 200-211.

Nhung, T. H.; Nam, N. H.; Nguyen, N. T. K.; Huy, L. M.; Giang, T. H.; Nghia, H. and Thanh, N. V. (2014): Establishment of a standardized mouse model of hepatic fibrosis for biomedical research. Biomedical Research and Therapy, 1: 43-49.

Oleshchuk, O.; Ivankiv, Y.; Falfushynska, H.; Mudra, A. and Lisnychuk, N. (2019): Hepatoprotective effect of melatonin in toxic liver injury in rats. Medicina, 55: 304.

Pévet, P.; Agez, L.; Bothorel, B.; Saboureau, M.; Gauer, F.; Laurent, V. and Masson-Pévet, M. (2006): Melatonin in the multi-oscillatory mammalian circadian world. Chronobiology International, 23: 39-51.

Pilorz, V.; Helfrich-Förster, C. and Oster, H. (2018): The role of the circadian clock system in physiology. Pflüegers Archiv - European Journal of Physiology, 470: 227-239.

Sallam, A. E.-D.; Hassan, S. A.; Hassaneen, E. and Ali, E. M. (2016): Environmental stress of mobile phone EM radiation on locomotor activity and melatonin circadian rhythms of rats. Biological Rhythm Research, 47: 597-607.

Schmid, B.; Helfrich-Förster, C. and Yoshii, T. (2011): A new ImageJ plug-in "ActogramJ" for chronobiological analyses. Journal of Biological Rhythms, 26: 464-467.

Seymour, T. L. and Nagamine, C. M. (2016): Evaluation of isoflurane overdose for euthanasia of neonatal mice. Journal of the American Association for Laboratory Animal Science, 55: 321-323.

Starkel, P. and Leclercq, I. A. (2011): Animal models for the study of hepatic fibrosis. Best Practice & Research Clinical Gastroenterology, 25: 319-333.

Steindl, P. E.; Ferenci, P. and Marktl, W. (1997): Impaired hepatic catabolism of melatonin in cirrhosis. Annals of Internal Medicine, 127: 494.

Sun, H.; Huang, F.-f. and Qu, S. (2015): Melatonin: a potential intervention for hepatic steatosis. Lipids in Health and Disease, 14: 75-75.

Tahara, Y. and Shibata, S. (2016): Circadian rhythms of liver physiology and disease: experimental and clinical evidence. Nature Reviews. Gastroenterology & Hepatology, 13: 217-226.

Weber, L. W. D.; Boll, M. and Stampfl, A. (2003): Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Critical Reviews in Toxicology, 33: 105-136.

Yoshioka, H.; Nonogaki, T.; Fukuishi, N.; Shinohara, Y.; Hwang, G.-W.; Ohtani, K. and Miura, N. (2017): Chronotoxicity of bromobenzene-induced hepatic injury in mice. The Journal of Toxicological Sciences, 42: 251-258.

Zhang, J.-J.; Meng, X.; Li, Y.; Zhou, Y.; Xu, D. P.; Li, S. and Li, H. B. (2017): Effects of melatonin on liver injuries and diseases. International Journal of Molecular Sciences, 18: 27.

ARABIC SUMMARY

إختلاف توقيت تليف الكبد المستحث برابع كلوريد الكربون مرتبط بتغييرات متباينة في الروتين اليومي للنشاط الحركي وللميلاتونين في الفئران

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يعاني مرضى التليف الكبدي عادة من اضطرابات في الروتين اليومي للنوم والميلاتونين. وحيث أن الاستقرار الحيوي لكليهما يعتمد على الآخر، بالإضافة إلى أن كليهما يخضع لتنظيم الساعة البيولوجية، فقد هدفنا في هذه الدراسة إلى استغلالهما كدلائل حيوية لاستكشاف ما إذا كان وقت تعرض الكبد لمادة سامة يؤثر على مدى التلف الكبدي الناتج وعلى احتمالات التعافي منه. وتحاول الدراسة هنا أن تحاكي المواقف التي من الممكن أن يتعرض الكائن فيها لمادة سامة للكبد بشكل متكرر في نفس الوقت من اليوم، الأمر الذي قد يحدث في أنماط روتين حياتية أو بيئية معينة. قمنا في هذه الدراسة برصد الإيقاع اليومي للنشاط الحركي ومستويات هرمون الميلاتونين كدلائل حيوية على مدى صحة الكبد في نموذج تجريبي لفئران التليف الكبدي المزمن المستحث بواسطة رابع كلوريد الكربون. وتمت فيها مقارنة تأثير المعالجة الليلية بالنهارية لرابع كلوريد الكربون. وقد أظهرت النتائج أن إيقاع نشاط الحركة اليومي لفئران التليف الكبدي تميز بموجات ضحلة السعة مقارنة بفئران المجموعة الضابطة، الأمر الذي نتج من زيادة النشاط الحركي نهاراً وانخفاضه ليلاً، الأمر الذي قلص الفارق الكبير المعتاد بين ذروة النشاط ليلا وفترة الراحة نهاراً في هذه الحيوانات الليلية. وقد نتج عن المعالجة برابع كلوريد الكربون ليلاً اضطرابات أكبر في إيقاع النشاط الحركي من المعالجة النهارية. أما بالنسبة للميلاتونين، فقد أدت المعالجة النهارية إلى إبطال الإيقاع، أما المعالجة الليلية فقد أدت إلى تبكير الإيقاع بمعدل ست ساعات تقريبا بالإضافة إلى خفض مستوى الميلاتونين ليلاً مقارنة بفئران المجموعة الضابطة. أما التعافي، بعد توقف المعالجة برابع كلوريد الكربون، فقد كان جزئياً، وإن كان أفضل في الفئران المعالجة نهاراً عن ليلاً. وقد أكد الفحص النسيجي للكبد النتائج مظهراً مستويات أعلى من التلف النسيجي في أكباد الفئران المعالجة ليلاً عن نهاراً. هذه النتائج تدعم فرضية أن مستوى سمية رابع كلوريد الكربون للكبد يخضع لتعديل الساعة البيولوجية اليومية. الأمر الذي يفرض المراعاة الدقيقة لعامل الوقت في تصميم التجارب المعملية وبرامج العلاج الدوائي لمرضى اضطرابات الكبد والنوم.