Comparative account of diurnal and nocturnal behavioral alterations in CD and NASH

Introduction

Lifestyle plays an important role in maintaining the socio-behavioral and neural function of the individual. In particular, the diet, routine-exercise, feeding-fasting window and sleep-wake cycle contribute to the lifestyle of an individual (Boden-Albala & Sacco, 2000). Dietary intake, especially saturated fats and refined sugar (western diet or diet containing high levels of fat and sugar) are vital for induction of lifestyle disorders such as NAFLD/NASH along with cognitive decline and dementia (Stranahan et al., 2008). In clinical and animal model studies, an altered sleep-wake cycle alone; or in combination with a high calorie diet is associated with metabolic disorders, cardiovascular diseases and behavioral complications (Erion et al., 2014; Joshi et al., 2021; Khazen et al., 2019; Upadhyay et al., 2020; Younossi et al., 2019). Hippocampus is embedded deep inside the temporal lobe of the brain and is considered critical for learning and memory in mammals. Interestingly, rodents exposed to high calorie diet were reported to show reduced BDNF expression and an impaired hippocampal-dependent memory leading to decreased neuronal plasticity and deficits in learning and memory (Molteni et al., 2002; Stranahan et al., 2009). Depression and anxiety are also common in patients with NAFLD (Youssef et al., 2013). Patients with NASH had recorded significantly higher prevalence of major depressive disorder (MDD) and generalized anxiety disorders (GAD) (Elwing et al., 2006). Patient with NAFLD exhibit subclinical/clinical depression (53%/14%) and histological perturbations in liver (Youssef et al., 2013). Wistar rats (female) fed with cafeteria diet coupled with experimentally induced stress showed higher anxiety levels and depression-like behaviour in elevated plus maze and force swim tests (da Costa Estrela et al., 2015).

Desynchrony in the endogenous circadian clock affects the pathogenesis and development of various diseases (Joshi et al., 2021; Shirsath et al., 2021). Consequentially, depression, anxiety-like behaviour and mood disorders are the most prevalent symptoms. In C57BL/6 mice, aberrant light-dark (LD) conditions exacerbates corticosterones levels and induces physiological and psychological changes (Kawai et al., 2018). Sand rats subjected to a short photoperiod regime for 3 weeks displayed

significant depression-like behavior in force swim test (Einat et al., 2006). In adult male white-footed mice (*Peromyscus leucopus*), short photoperiods alter brain size, hippocampal dendritic morphology and cause impaired long-term spatial learning & memory (Pyter et al., 2005). Enhanced spatial learning & memory was displayed by long-day male rodents as compared to short-day sham rodents (Pyter et al., 2006). Also, long day-light (LD) conditions have been reported to induce an anxiety-like response in meadow voles (*Microtus pennsylvanicus*) (Ossenkopp et al., 2005). Diurnal fat sand rats subjected to short daylight or melatonin resulted in depressed/anxious-like behavioral phenotype including decreased activity in forced swim test, increased anxiety in elevated plus maze test, decreased aggression and reduced reward seeking behaviour suggesting that reduced light results in variety of behavioral changes and a putative regulatory role of melatonin (Ashkenazy et al., 2009).

Increasing evidence shows that altered photoperiod and high calorie diet lowers hippocampus volume, impairs learning & cognitive functioning, reduces recognition memory, and causes locomotor deficits, impaired learning & attention in humans (Einat et al., 2006; Gladding et al., 2018; Hu et al., 2014; Kesby et al., 2015; Krivisky et al., 2011; Moretto et al., 2017; Vagena et al., 2019; Workman et al., 2009). More importantly, an altered photoperiod and high calorie diet increases the vulnerability to depression and anxiety (Anderson et al., 2001; Jacka et al., 2015; Ossenkopp et al., 2005; Schachter et al., 2018). Effect of high calorie diet on mood, cognition and associated neuro-behavioral syndromes has been most extensively studied in various animal models (da Costa Estrela et al., 2015; Erion et al., 2014; Molteni et al., 2002). The aim of this work is to gain broad understanding of neurobehavioral alterations caused in combination with an altered photoperiodic regime and high calorie diet. The diurnal and nocturnal neuro-behavioral assessment was achieved via performing test for depression (force swim and tail suspension test), anxiety-related (marble burying, elevated plus maze, sucrose preference, hole board tests), spatial learning and memory (Morris water maze tests) and locomotion. Though, it remains unclear as to how CD/NASH impacts specific brain functioning, the findings indicate a clear shift in neurobehavioral traits. Further, moderate-to-significant corrections following exogenous melatonin administration to the experimental mice was also recorded in some groups. A comparison of diurnal and

nocturnal behavioural experiment also provides insights into the critical window for studying ethological shifts in the experimentally induced CD and NASH.

Materials and methods

Experimental model: C57BL/6J mice male mice aged 6-7 weeks each weighing 20-22 g. Particulars of animal maintenance and ethical statement are provided in Materials and methods section.

Experimental groups:

1. Control
2. Chronodisruption (CD)
3. High fat-high fructose diet (H)
4. High fat-high fructose diet + Chronodisruption (HCD)
5. Chronodisruption + Melatonin (CDM)
6. High fat-high fructose diet + Melatonin (HM)
7. High fat-high fructose diet + Chronodisruption + Melatonin (HCDM)

Behavioral test assessed:

1. Test for locomotor deficits (using infrared actimeter)
2. Tests for Anxiety (Hole-board, marble burying and elevated plus maze test)
3. Test for spatial learning and memory (Morris water maze test)
4. Tests for Depression (Force swim, tail suspension and sucrose preference test)

The experimental protocol for the present study is depicted in Fig. 4.1. Detailed methodology is described in materials and methods section.

Experimental design

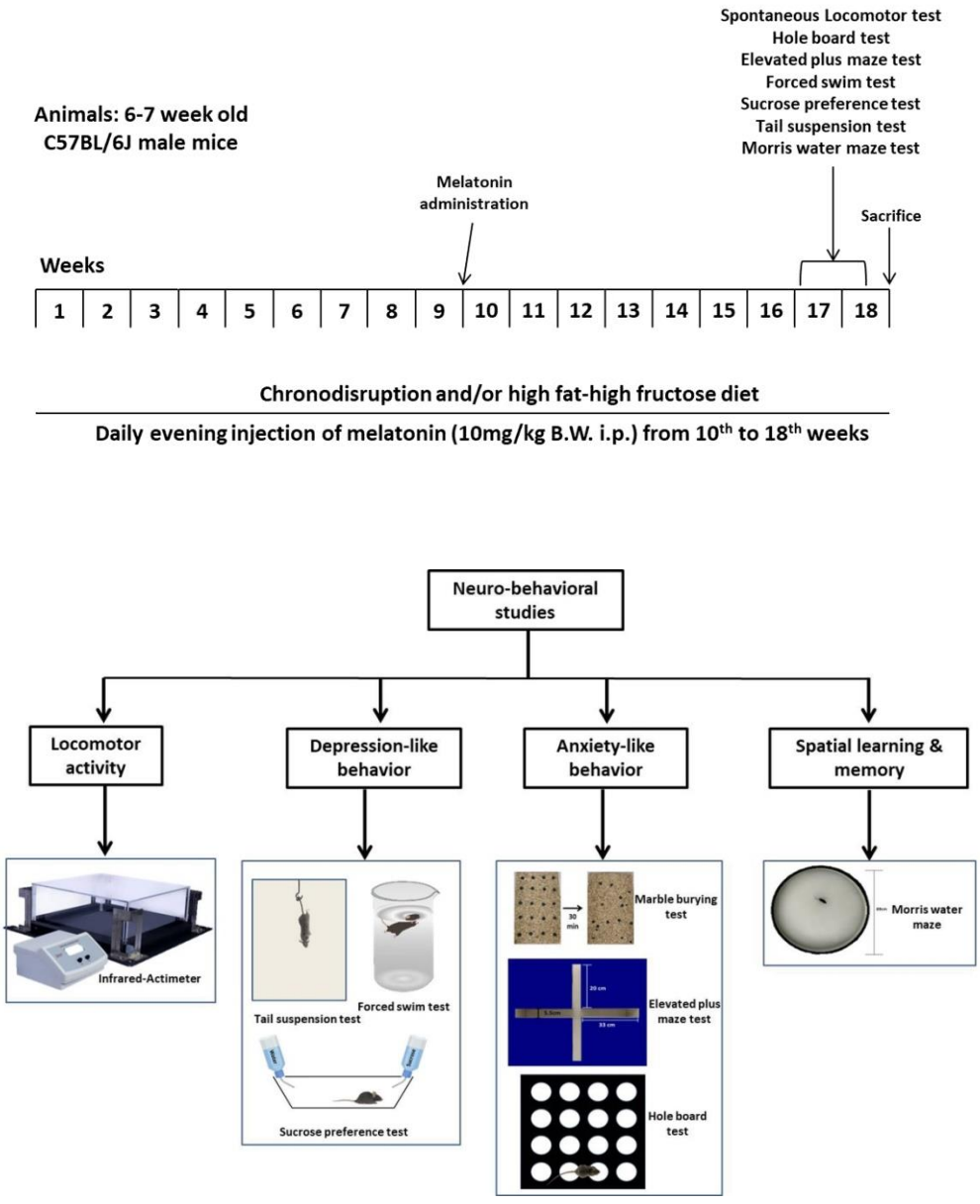

Figure 4.1: Experimental design for neuro-behavioral analysis in mice subjected to chronodisruption and high fat-high fructose diet fed C57BL/6J mice.

Results

Behavioral tests for locomotion (using infrared actimeter), anxiety-like (marble burying, elevated plus maze, hole board tests), depression (force swim and tail suspension test) and spatial learning and memory (Morris water maze tests) were performed at both diurnal and nocturnal timings. However, for depression-like behavior (sucrose preference test) was performed diurnally only. During the day-time, the experiments were performed from ZT3 to ZT9 (i.e. 10-00 a.m. to 4-00 p.m.) whereas; during night-hours from ZT15 to ZT21 (i.e. 10-00 p.m. to 4-00 a.m.). The behavioural changes were recorded (Logitech Webcam C270; 1280 x 720 pixels) and the data was analysed using ANYMaze software (Stoelting Co., Wood Dale, IL). In between the tests, the apparatus was cleaned with ethyl alcohol (40-50%), semi-dried with paper towel and then allowed to dry completely. Uniform conditions were maintained across the animal training and probe trials and care was taken to prevent olfactory/visual distractions during the experiments.

Effects of high fat-high fructose diet and/or chronodisruption on the diurnal and nocturnal locomotor activity in C57BL/6J mice

The effect of high fat-high fructose diet and/or chronodisruption in C57BL/6J mice on the spontaneous locomotor activity was assessed using infrared actimeter (Model: ACT-01, Orchid Scientific & Innovative India Pvt. Ltd., India). In diurnal experiment, a non-significant decrement in the locomotor counts was observed but in nocturnal experiment a significant decrease ($p<0.001$) was observed in CD, H and HCD groups as compared to the control group. But the same was reversed in melatonin treated groups in diurnal experiment. Further, in nocturnal experiment, in CDM group the locomotor counts were decreased as compared to CD, but the locomotor counts were significantly increased in HCDM ($p<0.001$) group and no change was observed in HM group (Fig. 4.2).

Effects of high fat-high fructose diet and/or chronodisruption on the diurnal and nocturnal anxiety-like behaviour in C57BL/6J mice

Diurnal and nocturnal anxiety levels in C57BL/6J mice in high fat-high fructose diet fed and/or chronodisruption subjected mice was analysed using hole board, elevated plus maze and marble-burying test. As an anxiety marker, the number of head dipping was decreased in CD and H groups as compared to control in both diurnal and nocturnal

period. However, no change was observed in the HCD group (Fig. 4.3). In melatonin treatment significantly higher head dipping score was observed in HM ($p<0.01$) group as compared to H, but non-significant increment was observed in CDM and HCDM groups as compared to their respective disease control groups in diurnal experiment. However, in nocturnal experiments the melatonin treated CDM group ($p<0.05$) shows significant increment in the head dipping number as compared to CD group and no change was observed in HM and HCDM groups.

In anxiety test (elevated plus maze) the number of entries and the time spent in the open arm zone was recorded (Fig. 4.7 & 4.8). Open arms duration was decreased in CD and HCD, however no change was observed in H group in diurnal experiments. But an increasing duration was observed in melatonin treated groups as compared to their disease control groups. In nocturnal experiments, a significant decrement ($p<0.001$) in the time spent in the open arms was observed in all three disease control groups i.e. CD, H and HCD groups. But in melatonin treated groups no major change was observed. In the same test, number of entries to the open arm zone was also recorded wherein; significantly less number ($p<0.01$) of entries were recorded in all the disease control groups in diurnal experiments, but the same was recovered in melatonin treated groups. A non-significant decrease in the number of entries to open arm zone in nocturnal experiments, but melatonin treatment recorded an improvement in the CDM ($p<0.05$) and HCDM ($p<0.05$) groups in diurnal experiments.

Since anxious mice bury more marbles, marble-burying behavior (Fig. 4.4) was performed to measure the anxiety levels in high fat-high fructose diet fed C57BL/6J mice (Handley, 1991; Snyder et al., 2011). In diurnal experiment, mice subjected to chronodisruption and high fat-high fructose diet buried non-significantly higher number of marbles as compared to control. Moreover, in HCD group there was significantly ($p<0.001$) higher number of buried marbles as compared to control. In nocturnal experiment, significantly higher numbers of marbles were buried in H ($p<0.05$) and HCD ($p<0.001$) group and non-significantly higher number was seen in CD group. In both diurnal and nocturnal experiments, melatonin treatment showed non-significant decrement in HM group only and no change was observed in CDM and HCDM groups.

Above observations in the hole board, elevated plus maze and marble burying tests suggest the development of the anxiety-like behavior in CD, H and HCD groups in both the diurnal and nocturnal experiments in C57BL/6J mice subjected to high fat-high fructose diet and/or chronodisruption subjected animals. However, timed administration of melatonin shows moderate to significant improvement in anxiety levels of C57BL/6J mice.

Effects of high fat-high fructose diet and/or chronodisruption on the diurnal and nocturnal depression-like behaviour in C57BL/6J mice

To determine whether the consumption of high fat-high fructose diet and altered photoperiodic regime plays a causative role in the development of depression, we evaluated depression like behavior by force swim, tail suspension and sucrose preference test. The depression-like behavior in C57BL/6J mice was assessed by recording the immobility time in Force swim test (FST) (Fig. 4.10) and Tail suspension test (TST) (Fig. 4.11), during diurnal and nocturnal periods. In both diurnal and nocturnal test, non-significant increment in the immobility time was observed in all the three diseases control groups i.e. CD, H and HCD groups. Intraperitoneal administration of melatonin was able to decrease the immobility time in CDM and HM groups in diurnal experiments and an increment in the immobility time was observed in HCDM group. However, in nocturnal experiment, a significant decrement was observed only in the CDM ($p<0.05$) group and no change was observed in HM and HCDM groups.

Tail suspension test was performed for assessing depression-like behavior in high fat-high fructose diet and/or chronodisruption subjected C57BL/6J mice for 18 weeks. During diurnal test, a significant increment in immobility time was observed in all the three disease control groups i.e. CD ($p<0.01$), H ($p<0.001$) and HCD ($p<0.001$); however melatonin supplementation accounted for a non-significant decrement. In nocturnal experiments no significant changes were observed in all the experimental groups (Fig. 4.11).

The sucrose preference test (SPT) is a reward-based test, which is used as an indicator of decreased ability to experience pleasure or anhedonia (Fig. 4.12). The condition of anhedonia is a characteristic feeling of depressed patients that indicates their inability to

experience enjoyable activities. In this study, the results of sucrose preference test were noted at the end of 24 h. CD mice had recorded a significantly ($p<0.001$) lower sucrose preference as compared to other experimental groups whereas, other experimental groups (barring CDM and HM) had recorded a non-significant increment.

Effects of high fat-high fructose diet and/or chronodisruption on the diurnal and nocturnal spatial learning and memory in Morris water maze test of C57BL/6J mice

The result of morris water maze showed a decrement in time spent and distance travelled in the zone of the platform by CD, H and HCD groups as compared to control group. In both diurnal and nocturnal experiments, there was a non-significant decrease in time spent in the platform zone by all the three disease control groups i.e. CD, H and HCD (Fig. 4.13). However, timed administration of melatonin could reverse the time spent in the platform zone in CDM, HM and HCDM groups in diurnal experiment, but no change was observed in CDM and HM in nocturnal experiment. Similarly melatonin treatment could reverse the distance travelled in the platform zone in HM and HCDM group in diurnal and nocturnal groups (Fig. 4.13). The number of entries to platform zone was non-significantly improved in HM and HCDM groups in both diurnal and nocturnal studies (Fig. 4.14).

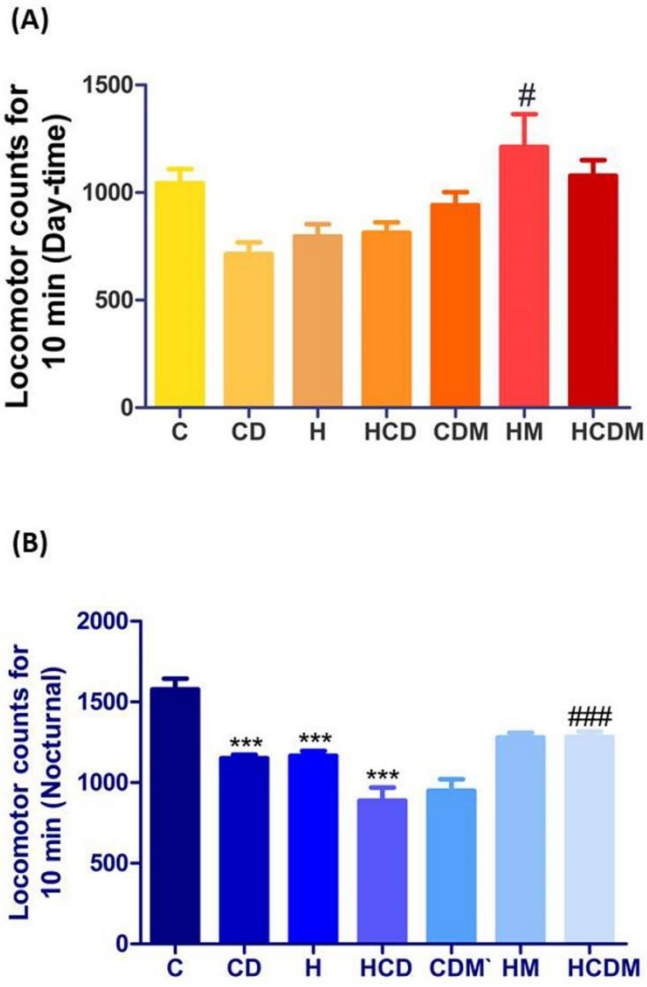

Figure 4.2: Locomotor counts (10 min) using infrared actimeter in mice subjected to high fat high fructose diet and/or chronodisruption in both (A) diurnal and (B) nocturnal experiments. Corrective role of melatonin was observed in (CDM, HM and HCDM) groups. Results are expressed as mean ± S.E.M. ***$p < 0.001$ is when CD, H and HCD compared to Control (C). #$p < 0.05$, and ###$p < 0.001$ is when HM compared with H and HCDM with HCD group respectively.

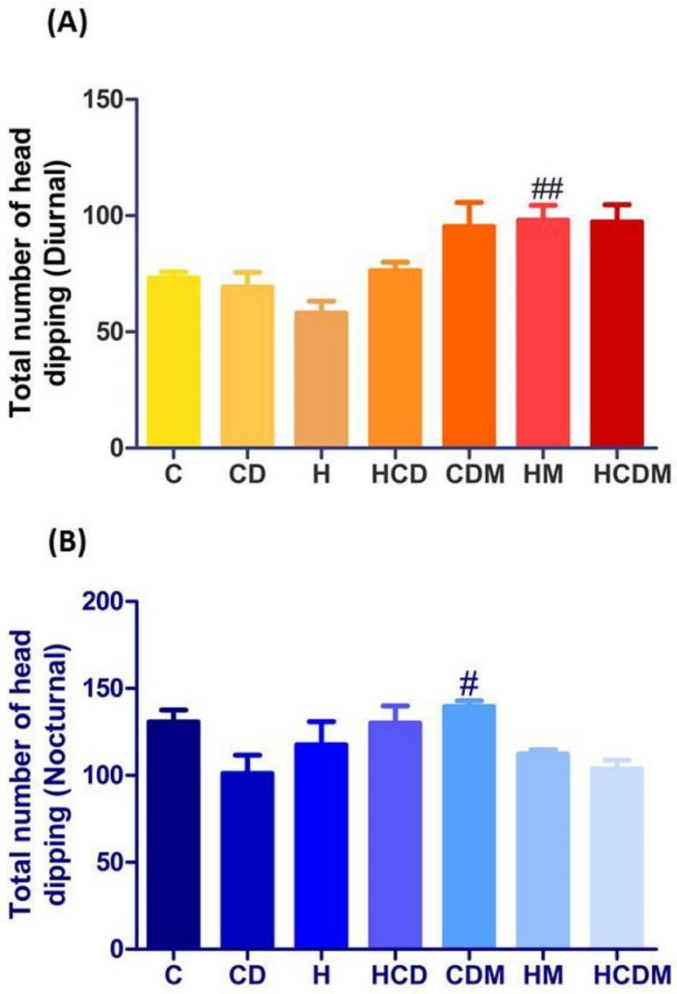

Figure 4.3: C57BL/6J mice performance in the hole-board test for the evaluation of anxiety-like behavior. Non-significant decrement was observed in CD and H group as compared to control group. Timed administration of exogenous melatonin improved the anxiolytic behavior in CDM, HM and HCDM groups in diurnal experiment and significant improvement in CDM group was observed in nocturnal experiment. Results are expressed as mean ± S.E.M. #$p < 0.05$ and ##$p < 0.01$, is when CDM compared with CD and HM with H group respectively.

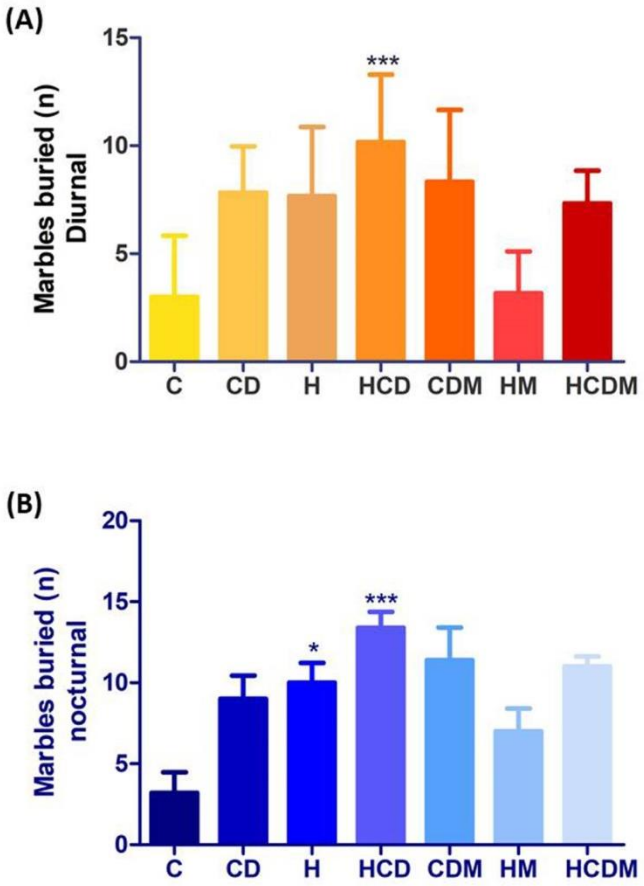

Figure 4.4: Anxiety-like behavior tested in marble burying in high fat-high fructose diet and/or chronodisruption subjected C57BL/6J mice. In disease control (CD, H and HCD) group's mice buried more marbles than control animals in both diurnal and nocturnal experiments. Results are expressed as mean ± S.E.M. *p < 0.05 and ***p < 0.001 is when H and HCD compared to Control (C).

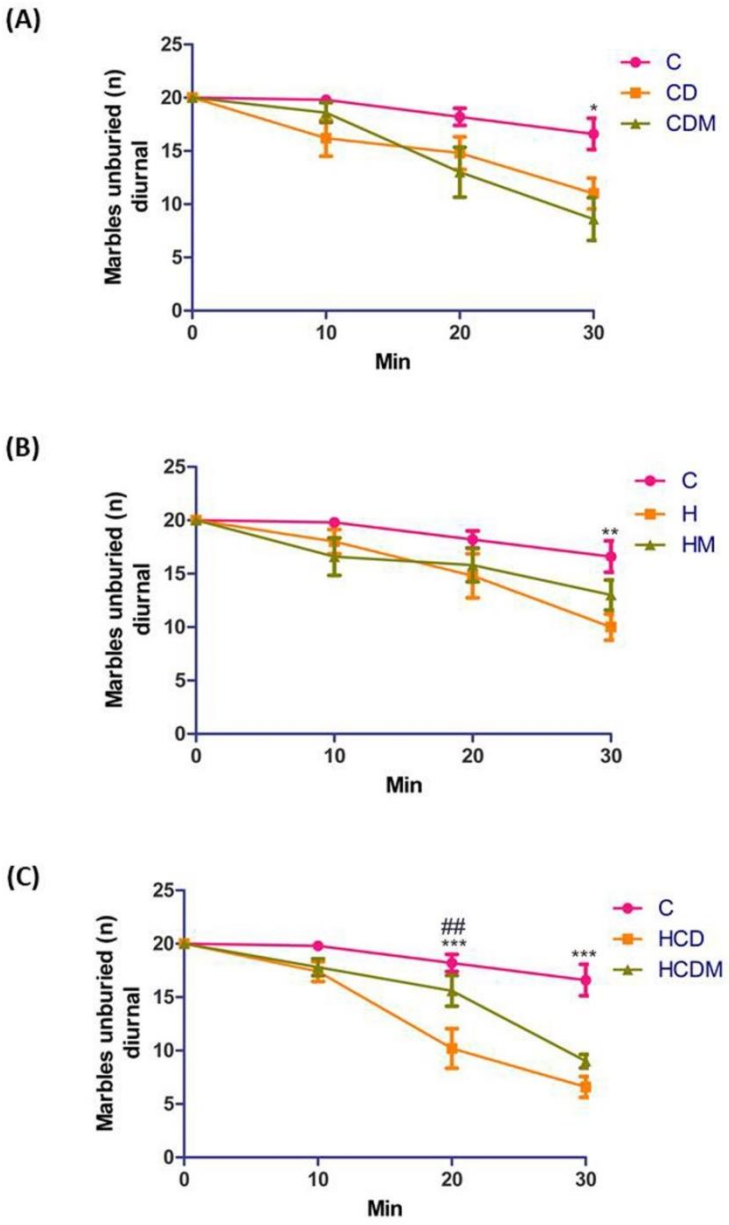

Figure 4.5: Anxiety-like behavior tested in marble burying in high fat-high fructose diet and/or chronodisruption subjected C57BL/6J mice in diurnal experiment. Total number of marbles buried (n) at different intervals of 10, 20 and 30 minutes respectively. Results are expressed as mean ± S.E.M. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ is when CD, H and HCD compared to Control (C). ##$p < 0.01$ is when HCDM compared with HCD.

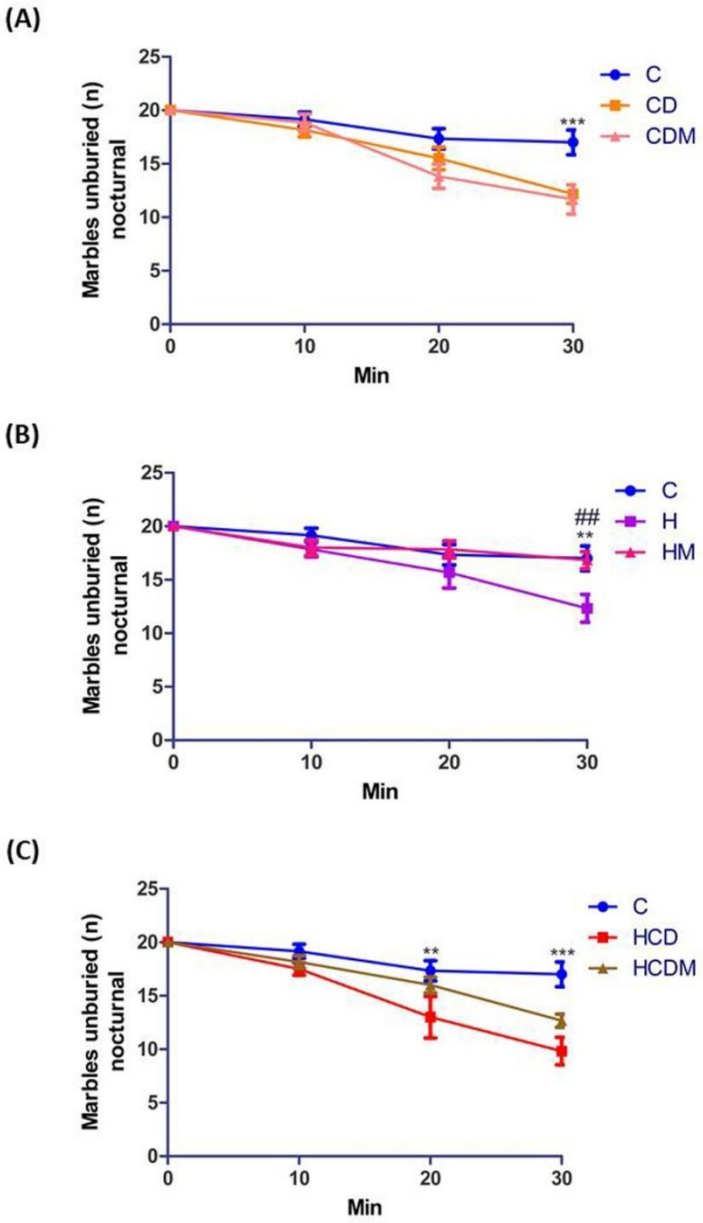

Figure 4.6: Anxiety-like behavior tested in marble burying in high fat-high fructose diet and/or chronodisruption subjected C57BL/6J mice in nocturnal experiment. Total number of marbles buried (n) at different intervals of 10, 20 and 30 minutes respectively. Results are expressed as mean ± S.E.M. **p < 0.01, and ***p < 0.001 is when CD, H and HCD compared to Control (C). ##p < 0.01is when HM compared with H group respectively.

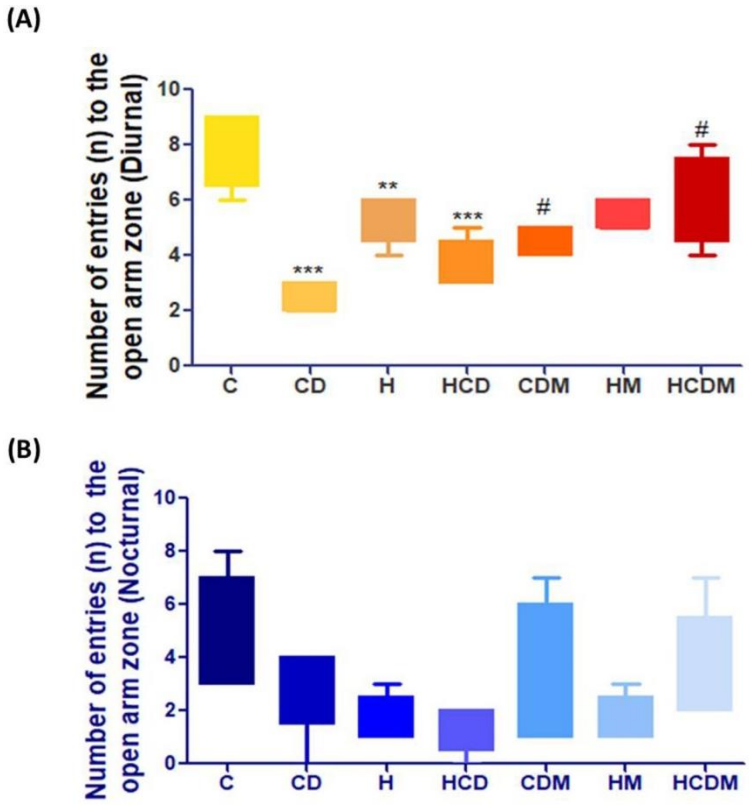

Figure 4.7: Decreased number of entries in elevated plus maze test of mice subjected to high fat high fructose diet and/or chronodisruption. Number of entries in the open arm zone (n) in both the (A) diurnal and (B) nocturnal experimental regime. Anxiolytic effect of timed administration of melatonin on number of entries in elevated plus maze test in both diurnal and nocturnal experiments. Results are expressed as mean ± S.E.M. *$p < 0.05$, and ***$p < 0.001$ is when CD, H and HCD compared to Control (C) and #$p < 0.05$, is when CDM and HCDM is compared with CD and HCD groups respectively.

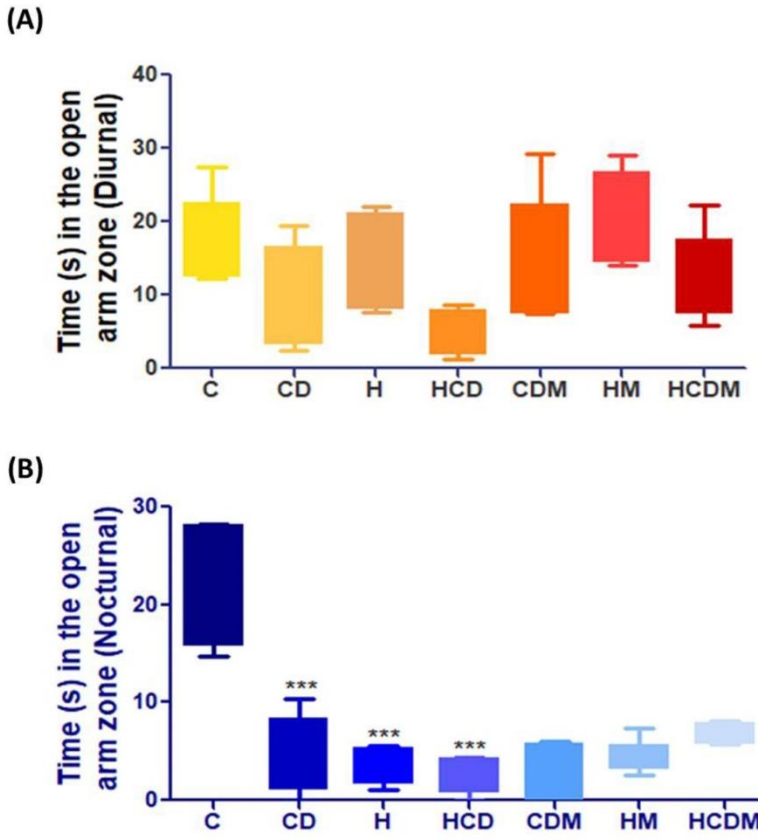

Figure 4.8: Time spent (s) in elevated plus maze test in mice subjected to 18 weeks of High fat high fructose diet and/or chronodisruption induced anxiety-like behavior in both the diurnal and nocturnal experimental regime. Anxiolytic effect of timed administration of melatonin was observed in diurnal experiment and no change was observed in nocturnal experimental regime. Results are expressed as mean ± S.E.M. ***$p < 0.001$ is when CD, H and HCD compared to Control (C) group.

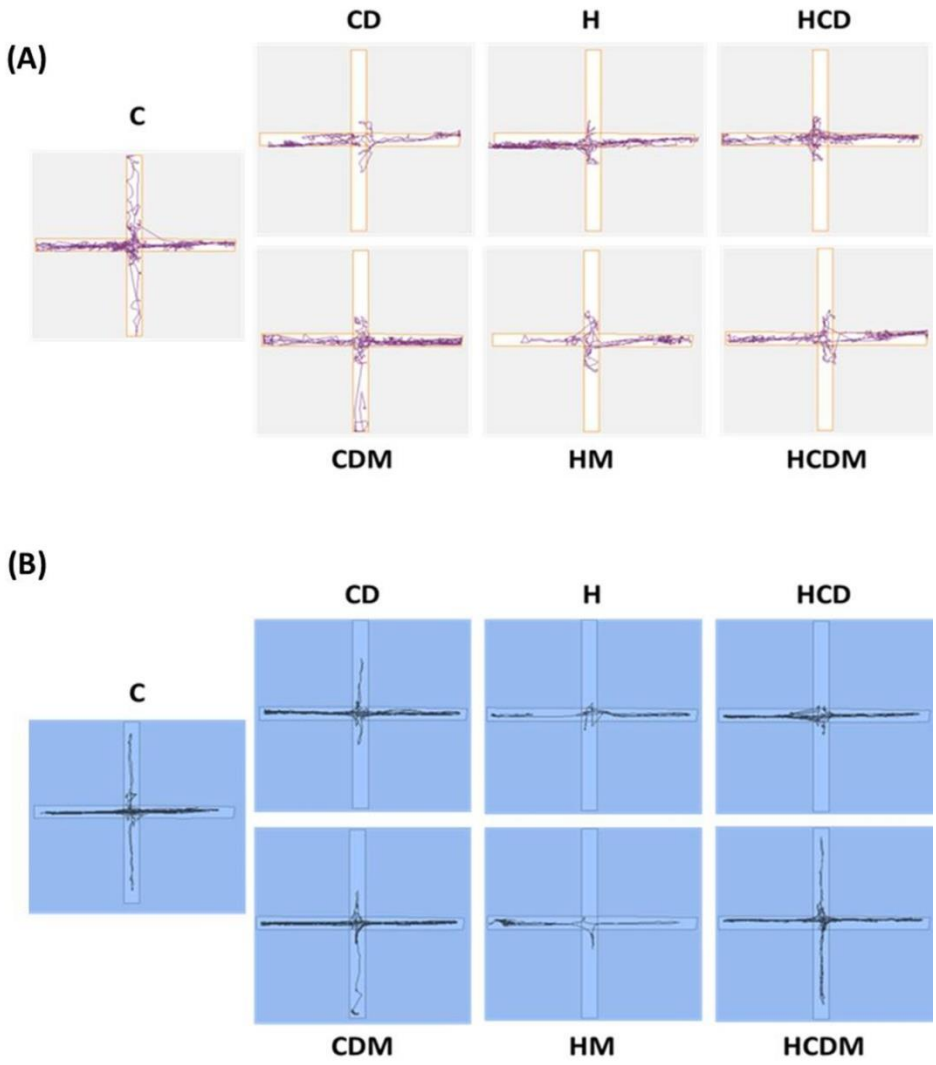

Figure 4.9: Representative tracking images of mice performance in elevated plus maze test in diurnal (white colour) and nocturnal (blue colour) experimental regime. Mice were tracked during the experiment using ANYMaze software.

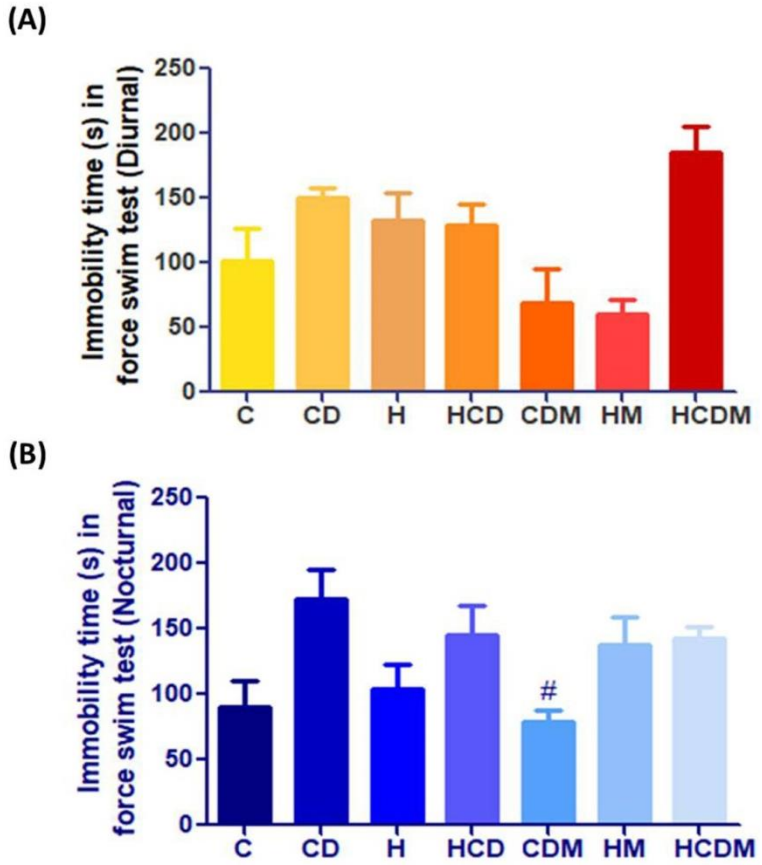

Figure 4.10: Effect of chronodisruption and/or high fat high fructose diet on immobility time in forced swim test during (A) diurnal and (B) nocturnal regime. Results are expressed as mean ± S.E.M. #$p < 0.05$ is when CDM compared with CD.

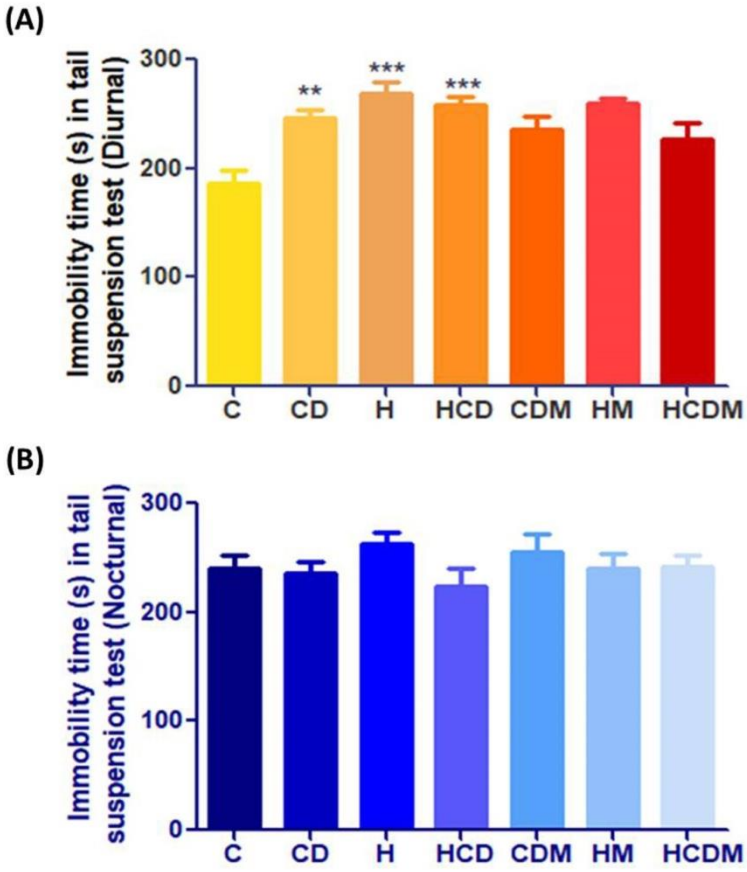

Figure 4.11: Effect of chronodisruption and/or high fat high fructose diet on immobility time in tail suspension test during (A) diurnal and (B) nocturnal regimes. Overall decrease in immobility time was observed in melatonin treated (CDM, HM and HCDM) groups. Results are expressed as mean ± S.E.M. **p < 0.01 and ***p < 0.001 is when CD, H and HCD compared to Control (C).

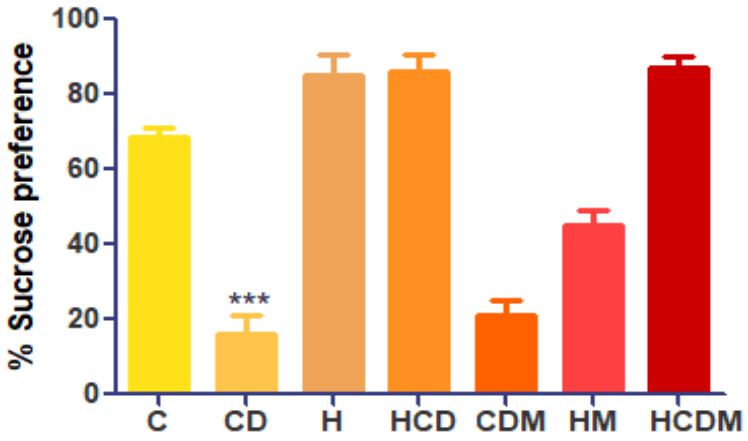

Figure 4.12: Effect of high fat-high fructose diet and/or chronodisruption in mice on sucrose preference test. Sucrose preference was measured by liquid consumption in two-bottle preference: one containing water and other containing 2% sucrose water. Sucrose preference was calculated as a percentage: volume of sucrose consumed/total volume of fluid consumed X 100. Mice in CD group showed significant reduction in the sucrose preference over water compared with control and were considered to be depressed. Exogenous melatonin administration was not showing any improvement in any of the treatment groups. Results are expressed as mean ± S.E.M. ***$p < 0.001$ is when CD compared to Control (C).

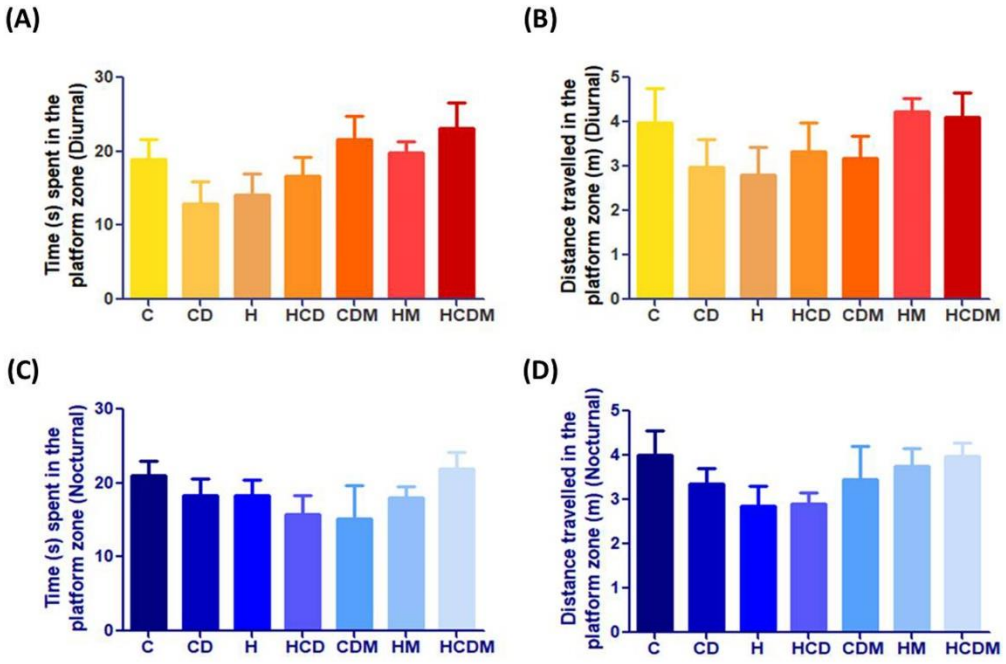

Figure 4.13: The learning and memory abilities in different experimental groups were assessed by morris water maze test. High fat-high fructose diet and/or chronodisruption impair spatial learning & memory in morris water maze test. Time spent (s) and distance travelled (m) in platform zone in diurnal and nocturnal regime. Corrective changes of timed administration of melatonin on spatial learning and memory performance in morris water maze test in both diurnal and nocturnal experiments.

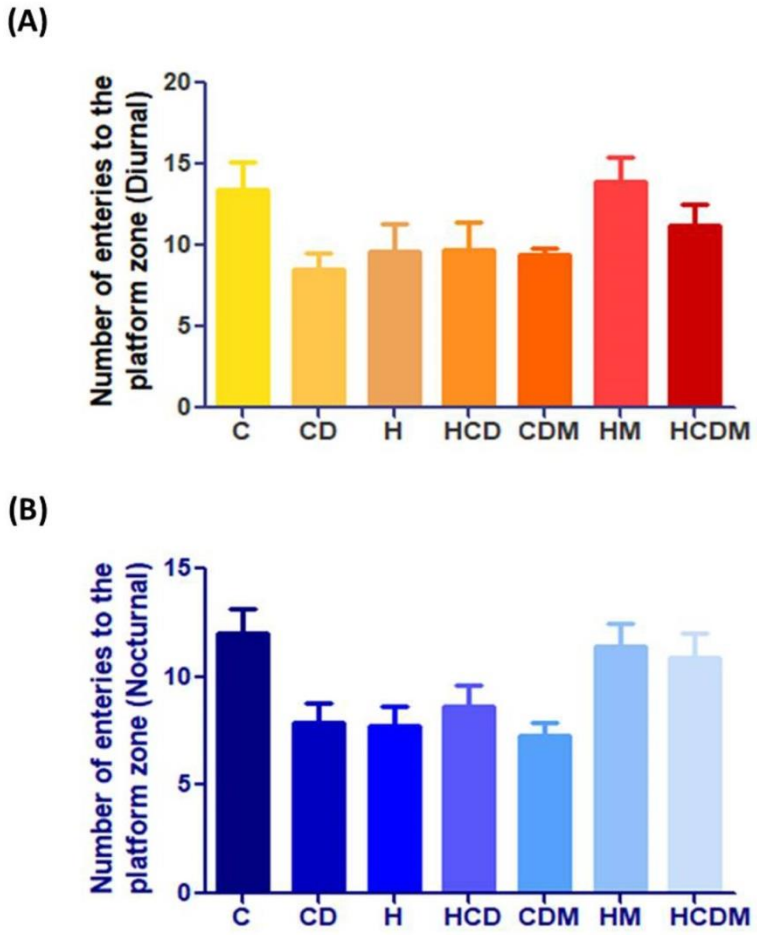

Figure 4.14: Number of entries in the platform zone in morris water maze test during the (A) diurnal and (B) nocturnal experimental regime. In CD, H and HCD groups impaired spatial learning & memory was recorded in morris water maze test. Administration of exogenous melatonin improves performance in morris water maze test in (CDM, HM and HCDM) groups in both diurnal and nocturnal experiments.

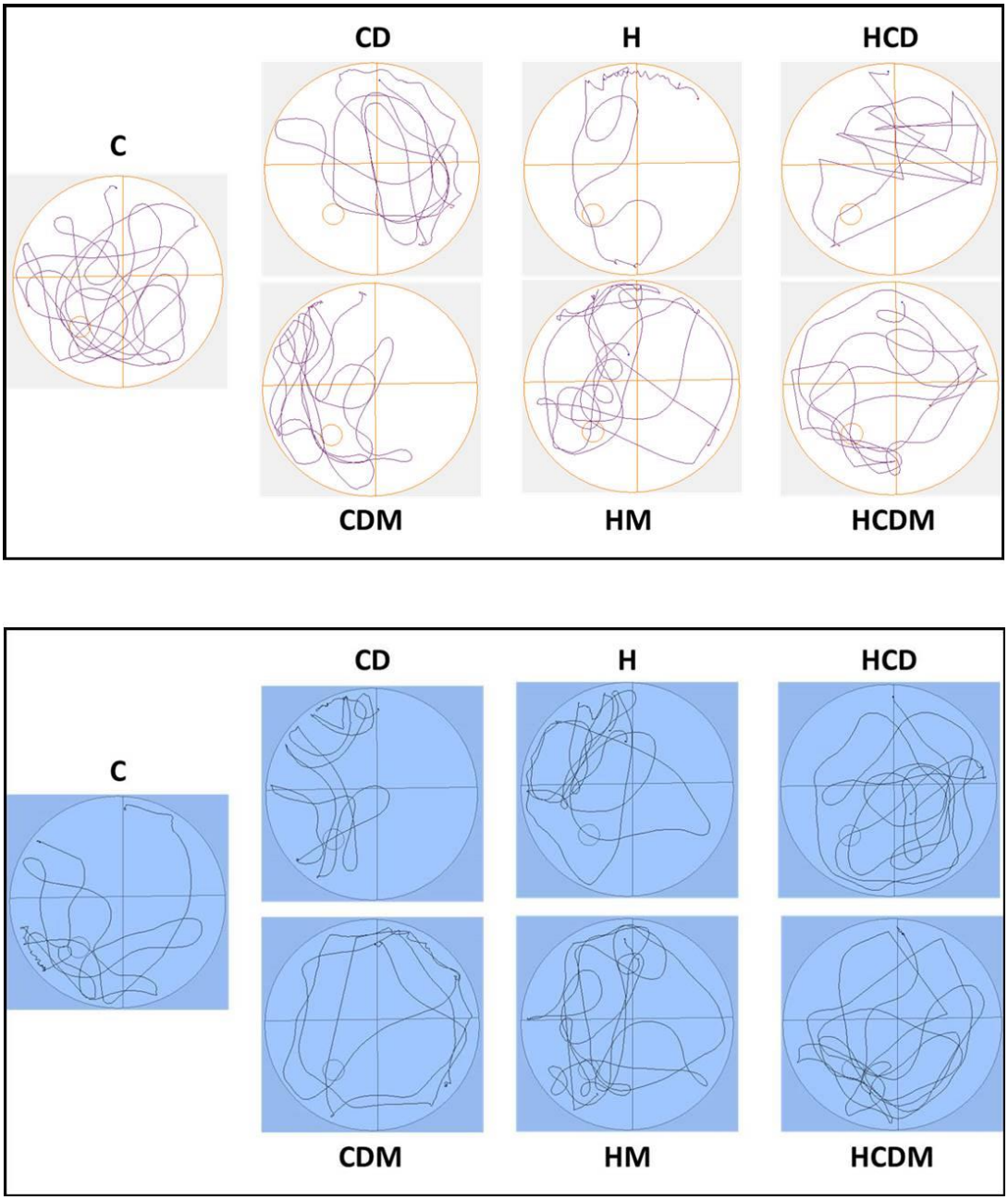

Figure 4.15: Representative tracking images of mice movement in the morris water maze test during the probe trial in diurnal (white colour) and nocturnal (blue colour) experiments. Mice were tracked during the experiment using ANYMaze software.

Discussion

The surge in prevalence of NASH primarily due to high calorie diet, altered feeding-fasting and delayed sleep-wake cycles has also been reported to cause behavioural changes viz. depression, anxiety and mood disorders (Elwing et al., 2006; Youssef et al., 2013). The said psychological changes can be resultant of circadian misalignment due to alterations in photoperiod alone, or a synergistic effect with consumption of a high calorie diet (High fat-high fructose or western diet) (Anderson et al., 2001; Baron & Reid, 2014; Courtet & Olié, 2012; da Costa Estrela et al., 2015; Nguyen et al., 2019; Schachter et al., 2018; Youssef et al., 2013). Many clinical studies have shown that obesity, type 2 diabetes and NASH are associated with neurobehavioral disorders such as mood swings, depression, anxiety and an increased risk for cognitive decline (Elwing et al., 2006; Holt et al., 2014; Jantaratnotai et al., 2017; Roberts et al., 2003; Smith et al., 2013; Surdea-Blaga & Dumitraşcu, 2011; Youssef et al., 2013). Therefore, we examined the behavioral alterations in C57BL/6J mice subjected to high fat-high fructose diet (H) and/or subjected to photoperiodic manipulation induced chronodisruption (CD). Overall, the results of this chapter demonstrate that mice subjected to H or CD have progressively higher anxiety-like behaviour, deficits in locomotor activity and impaired learning in both the diurnal and nocturnal phases of experiment. Also, these experimental groups showed varying degrees of depression and deficit in spatial learning and memory. Overall, we had shown for the first time, a combined effect of photoperiodic manipulation induced chronodisruption and high fat-high fructose diet induced neurobehavioral perturbations in C57BL/6J mice and also the role of exogenous melatonin as an important hormone in management of neurobehavioral alterations in lifestyle disorders.

In animals, locomotor behavior is a basic behavior and an important indicator of circadian rhythms, which are coordinated by the nervous and endocrine systems (Pierce & Kalivas, 2007). In CD, H and HCD groups, a non-significant decrement in diurnal experiment; whereas in nocturnal experiment, significant decrement in the locomotors counts was observed in the disease control groups (CD, H & HCD), suggesting that the overall motor activity was altered. The data of decreased locomotor counts revealed deficits in spontaneous motor behaviour in our study that is comparable to the loss of

alertness or sedative-like effects in mice (Trivedi et al., 2022). These results are also in accordance with other studies on high fat diet (Bravo et al., 2014; Moretto et al., 2017) and circadian misalignment (Sauvet et al., 2019). Melatonin, the pineal gland hormone regulates the sleep-wake pattern of an individual and its rhythmicity is involved in the light-dark cycle controlled synchronization of locomotor activity in animals (Underwood & Goldman, 1987). It has been reported in house sparrow and Japanese quail, that melatonin release from the pineal gland entrains the circadian rhythm of locomotor activity (Murakami et al., 2001). In our study, melatonin treated groups has significantly higher locomotor counts in HM (diurnal experiment) and HCDM (nocturnal) experiments; however no major change was found in CDM group in both the experiments.

Researchers had employed a variety of stressors such as anxiogenic drugs (Basso et al., 1999; Rogerio & Takahashi, 1992), high fat diet (Yoshizaki et al., 2020), high fat-high cholesterol diet (Hu et al., 2014; Mukherjee & Banerjee, 2018) and photoperiodic manipulation induced circadian desynchrony to induce anxiety like behaviour (Cleary-Gaffney & Coogan, 2018; Fonken et al., 2009; Hogan et al., 2015; Nagy et al., 2015). In our study, CD, H and HCD treated groups accounted for a progressive increment in anxiety-like behavior in C57BL/6J mice (hole-board, elevated plus maze and marble burying tests). Further, melatonin treatment had accounted for corrective changes in the indices and the same was in accordance with the melatonin mediated improvement in anxiolytic properties in experimental models (Corrales et al., 2013; Pierrefiche et al., 1993) and in patients groups (Hansen et al., 2015). However, no major change in marble burying test was recorded in CDM group in our diurnal and nocturnal studies.

Depression is a well-known neuro-behavioral problem in patients with metabolic syndrome and especially in those with a lifestyle disorder (Anderson et al., 2001; Elwing et al., 2006; Holt et al., 2014; Nyer et al., 2013; Roberts et al., 2003; Youssef et al., 2013) and remains undertreated and underdiagnosed in many patients with NAFLD or NASH (Surdea-Blaga & Dumitraşcu, 2011). Depressive symptoms have adverse effects on feeding-fasting, sleep-wake cycle and also on the quality of life and it may impact the quality of treatment in the condition of NAFLD or NASH (Fang et al., 2019; Nyer et al., 2013; Xiao et al., 2021). Force swim test (FST), Tail suspension test (TST) and Sucrose

preference test (SPT) are effective in predicting for depression-like behavior despair (Liu et al., 2018; Porsolt et al., 1977; Serchov et al., 2016; Steru et al., 1985; Yin et al., 2021). In our study, animals subjected to CD, H and HCD had recorded moderate to significant increase in immobility time in FST and TST. These results are in agreement with other studies with photoperiodic manipulation (Bedrosian et al., 2013; Fonken et al., 2009; Fonken & Nelson, 2013) and high calorie diet in C57BL/6J mice (Almeida-Suhett et al., 2017; Krishna et al., 2015). Further, daily administration of melatonin was able to improve the depression-like behavior in (CDM and HM) groups (diurnal) and in CDM group (nocturnal) as evidenced by FST. A counter-argument to this fact is that, melatonin increases struggling behavior of rodents in FST (Nagy et al., 2015; Raghavendra et al., 2000; G. Ramírez-Rodríguez et al., 2009; G. B. Ramírez-Rodríguez et al., 2020). The results of our disease control groups treated with melatonin (CDM & HM) had also recorded a similar increment in struggling behaviour (low immobility index) following melatonin treatment. It is interesting to note that, the observed gross changes did not follow the same pattern in the nocturnal study. Further, HCDM group showed an increment in immobility time that corroborates the reports of other research groups wherein; melatonin has been implicated for sedative-like effect resulting in higher immobility indices (Bourin et al., 2004; Brotto et al., 2000; Dubocovich et al., 1990). Chronic administration of melatonin in Long-Evans rats for 14 days (in drinking water) also had accounted higher immobility indices that supports our observations (Brotto et al., 2000). Anhedonia is referred as reduced ability to experience pleasures and it has been studied in different neuropsychiatric disorders and is a major symptom of depression (Gorwood, 2008). In rodents, the consumption of sweet solution gives pleasure with normal working hedonic system whereas; depression-like condition results in less craving for sugar (Kang et al., 2016; Liu et al., 2018). According to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria the anhedonic behavior is considered as a core feature of major depression (Bell, 1994). Numerous studies have reported reduced sucrose uptake following various stress exposure in rodent models (Fonken et al., 2009; Haridas et al., 2013; Liu et al., 2018; Serchov et al., 2016). In our study, CD mice showed significantly less preference to sucrose as compared to control group and melatonin treatment (CDM) was not able to improve the condition. This experiment does not have any similar replicate in literature and hence, reason for the

same could not be established. Other experimental groups H, HCD, HM and HCDM recorded higher indices of sucrose preference possibly due to the supplementation of 20% fructose in their high fat-high fructose diet.

Assessment of spatial learning and memory of experimental animals using distal cues to navigate around the perimeter of swimming arena and to locate a submerged escaped platform defines the morris water maze test (Morris, 1981). Multiple trials and reference memory is tested by preference for the platform area when the platform is absent on the probe day (Morris, 1981; Vorhees & Williams, 2006). In the present study, CD, H and HCD groups showed lower number of entries, distance travelled and time spent in the platform zone as compared to control. These results are in accordance with reports on high calorie diet and alterations in photoperiodic regime in mice that accounts an impaired spatial learning and memory (Cassell, 2020; Klein et al., 2016; Pathan et al., 2008; Workman et al., 2009). However melatonin treatment was able to make corrective changes in the HM and HCDM groups, but no change was observed in CDM group. Melatonin is known to reversed HFD-induced cognitive decline and have a positive effect on spatial learning in probe trial performance (Lin et al., 2020; Xu et al., 2019) and our observations are in agreement with the same.

Taken together, data from diurnal and nocturnal neurobehavioral studies suggest that timed administration of exogenous melatonin showed moderate to significant improvement in majority of the parameters that investigate locomotor activity, anxiety, learning and memory. Findings of this study emphasize the role of melatonin as an important hormone in management of neurobehavioral perturbations in NAFLD/NASH.