Introduction

Lifestyle disorders are defined as diseases caused by the way people eat, live and work. The occurrence of lifestyle diseases like hypertension, diabetes mellitus, dyslipidemia, and overweight/obesity associated with gastrointestinal and cardiovascular diseases is high on the rise. Lifestyle disease and behavioral anomalies are common conditions with major public health implications that tend to co-occur within individuals and the presence of any one increase the risk for developing depression or any behavioral anomalies. Lifestyle diseases are associated with an increased risk of behavioral disorders such as depression and anxiety, but the association between lifestyle and mental disorders lacks clarity. Obesity contributes to significant health impairments and increases the risk of the prevalence of neurological problems like mild cognitive impairment, dementia, and Alzheimer's diseases are similarly increased with obesity (Elias *et al*., 2005; Whitmer *et al*., 2005). Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease afflicting about one third of the world's population and has been recognized as a major health burden. NAFLD can progress to non-alcoholic steatohepatitis (NASH), characterized by inflammation, apoptosis, and ballooning degeneration (Thounaojam *et al*., 2012). The development of NAFLD or NASH is not only related to excess energy intake but more importantly with unhealthy dietary pattern, sedentary lifestyle, altered sleep wake cycle and is estimated to be around 20-30% in Western countries and 9-32% in the Indian population and about 80-90% among obese adults (Bellentani *et al*., 2010).

Lifestyle disorder is a growing problem worldwide and is associated with a range of comorbidities, including cognitive dysfunction. Consumption of a high fat diet induces obesity and chronic neuro-inflammation that may affect brain physiology and alter mood and behavior (Schachter *et al*., 2018). Studies on animal models of obesity, factors like peripheral low-grade inflammation, dysbiosis and other inflammatory processes affect the development of emotional and cognitive alterations (Castanon *et al*., 2015). Increased levels of oxidative stress, dysregulated production and release of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β results in insulin resistance, learning and memory deficits (Gomaa *et al*., 2019). In mouse model of Alzheimer diseases (AD), diet induced NAFLD contributes to the progression of brain abnormalities through an increased expression of *Tnfα, Cox2, p21,* and *Nox2,* unbalanced brain MUFAs and PUFAs metabolism in brain contributing the pathogenesis of AD (Pinçon *et al*., 2019). Studies have reported hippocampal dependent

deficits in learning and memory, cognitive functions in the rats fed with diets high in saturated fats and sugar (Davidson *et al*., 2012). Study by Mondal (2020) using murine model of NASH, increased pro-inflammatory cytokines IL-1β, IL-6 and decreased expression of tight junction protein Claudin 5 caused neuro-inflammation and induction of blood-brain barrier dysfunction (Mondal *et al*., 2020). Numerous studies have shown that brain function is sensitive to various inflammatory pathways and mediators. Inflammatory cytokines such as IL-1β and IL-6 are at peak in obesity and various liver diseases including NASH (Felipo *et al*., 2012). In genetic models of obesity such as *db/db* mice, decreased BDNF levels, impaired hippocampus dependent spatial memory performances, altered hippocampal neurogenesis and synaptic plasticity was found (Erion *et al*., 2014; Stranahan *et al*., 2009). In *db/db* mice, blockade of IL-1β expression normalizes the hippocampal dendritic spine density and improves synaptic function and cognitive impairment (Erion *et al*., 2014). In overweight and obese elderly patients higher body mass index (BMI) was associated with lower brain volumes causing deficits in cognition like behavior (Raji *et al*., 2010). Various regression studies associated with increased BMI, decreased brain volume and alterations of brain morphology in obese young adult's shows that clinical obesity is associated with enlarged orbito-frontal white matter and reductions in focal gray matter volume (Pannacciulli *et al*., 2006). Clinical studies had shown that circulatory inflammatory lipopolysaccharides-binding protein (LBP) concentration associates with brain white matter integrity and working memory in both obese and non-obese subjects (Moreno-Navarrete *et al*., 2017). In obesity mediated behavioral distortions hippocampus is a key brain area for mediating performances of spatial learning, memory and cognitive impairments.

The endogenous circadian clock influences key physiological functions, such as body temperature, cognitive performance, heart rate, appetite, gastrointestinal motility, hormone levels and sleep-wake cycle. Apart from their major role in controlling circadian rhythms, circadian clock genes have a widespread influence on various emotions such as anxiety, cognition, depression, mood and reward-related behaviors (Wulff *et al*., 2010). Disruption of the sleep wake cycle destabilizes various physiological functions and develops a range of metabolic disorders and cognitive defects (Wulff *et al*., 2010). Neurobehavioral or cognitive functioning are closely associated with endogenous circadian clock and sleep/wake cycle (Cajochen *et al*., 2004). Disruption in the circadian rhythms impairs memory in rodents and humans.

Brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin receptor kinase-B (TrkB) signalling pathway regulates wide variety of functions throughout life such as neuronal development, synapse formation and plasticity. Reduction of *Bdnf* expression leads to spectrum of neurobehavioral phenotypes such as cognitive deficits, learning difficulties, and impaired memory (Uutela *et al*., 2012). BDNF-TrkB pathway has been reported to induce changes in brain functioning, however the role of BDNF-TrkB pathway key genes during circadian desynchrony and high fat high fructose diet in neurobehavioral phenotypes remains unknown.

Objectives

1. Establishing the basis of hepatic-hippocampal crosstalk and role of bile acids in NASH.
2. Diurnal and nocturnal studies of behavioural anomalies in chronodisruption (CD) and NASH and corrective role of exogenous melatonin.
3. Drawing correlation between behavioral anomalies and alteration in neural BDNF-TrkB pathway in CD and NASH.

Objective 1: Establishing the basis of hepatic-hippocampal crosstalk and role of bile acids in NASH.

Parameters assessed: Food, water and body weight monitoring thrice a week. Body circumference, glucose tolerance test, body weight, liver weight and liver histology were performed at the end of the experiment. Liver functional test, lipid profiles and thyroid hormones were determined from serum. mRNA of circadian clock genes *Clock*, *Bmal1*, *Per1*, *Per2*, and *Cry2* at 5 different time points (0, 6, 12, 18 and 24) has been performed. Also the key genes of bile acid metabolism such as *Cyp7a1*, *Cyp27a1*, *Cyp8b1* (BA synthesis), *Abcb11*, *Mdr2*, *Mdr3*, *Mrp2*, *Mrp3*, *Ost-β* (BA metabolism), *Fxr and Tgr5* (BA receptor) has been performed.

Observations: C57BL/6J mice were subjected to photoperiodic manipulation induced chronodisruption (CD), high fat high fructose diet and/or combination of both during the experimental regime of 18 weeks. The high fat high fructose (H) diet and combination of both i.e. chronodisruption and high fat-high fructose diet (HCD) significantly increased the body weight and body circumference of H and HCD was found while the CD group showed no change as compared to control. Melatonin treatment to (H) and (HCD) groups showed significant decrease in body weight. The high fat high fructose (H) diet group and HCD showed significant increment in the circulating titers of serum AST and ALT but melatonin treatment accounted for the decrease in titers of the said enzymes. A significant effect of high fat high fructose diet and or chronodisruption was found on circulating lipid profile markers. There was an increase in the levels of total cholesterols, triglycerides, total lipids, LDL cholesterol and VLDL cholesterol levels in H, HCD groups and the same was decreased in the melatonin treated groups. A significant increase in the T4 and decrement in T3 levels

were observed in H, CD and HCD groups but the same was reversed in melatonin treatment groups.

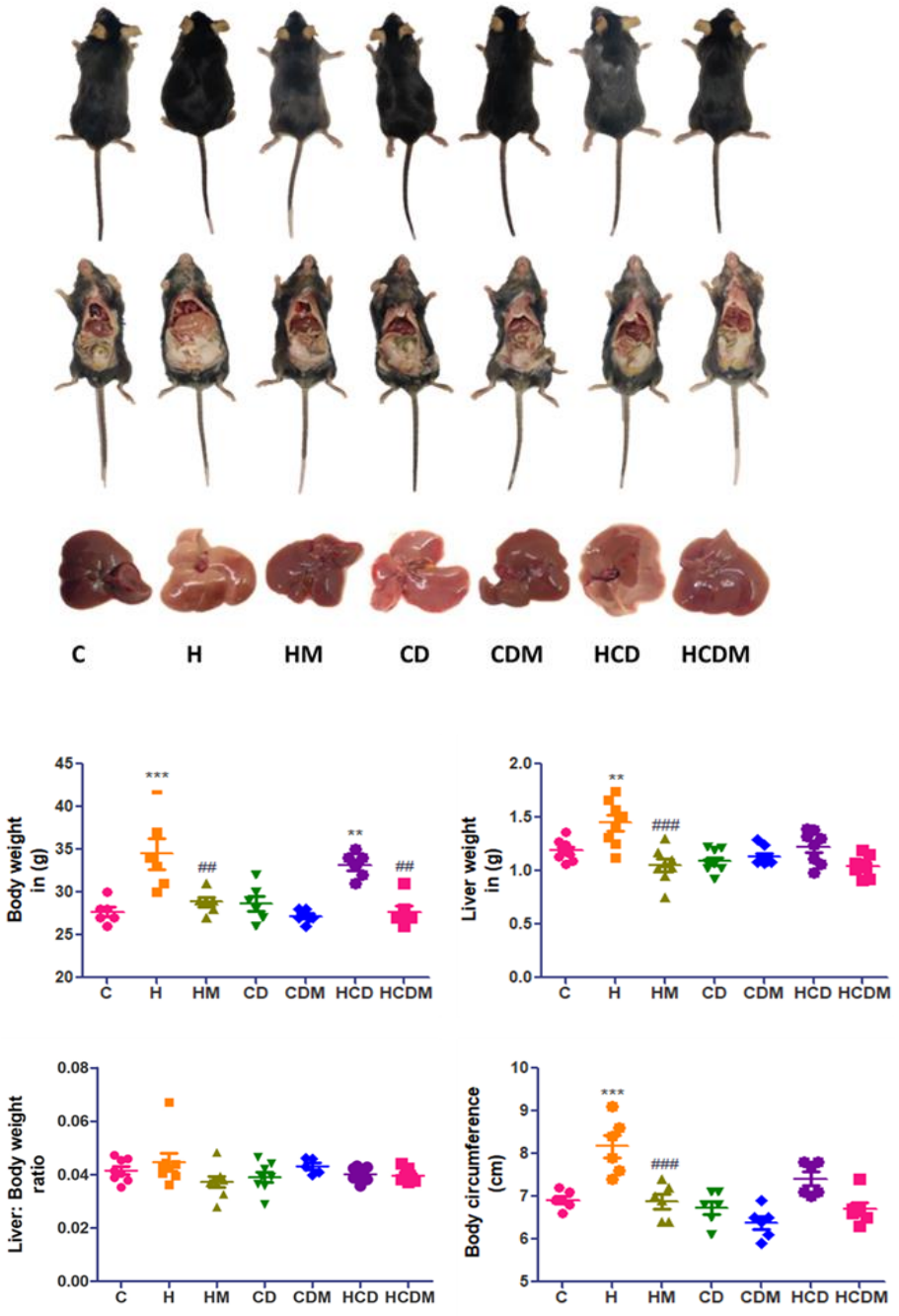

Figure 1. Changes in whole body weight, liver weight and body circumference of C57BL/6J mice fed with high fat high fructose diet and/or subjected to chronodisruption and an improvement following melatonin treatment. Data represented as mean ± SD *P<0.05, **P<0.01, ***P<0.001 vs control, #P<0.05, ##P<0.01, ###P<0.001 vs H, CD and HCD. n=6

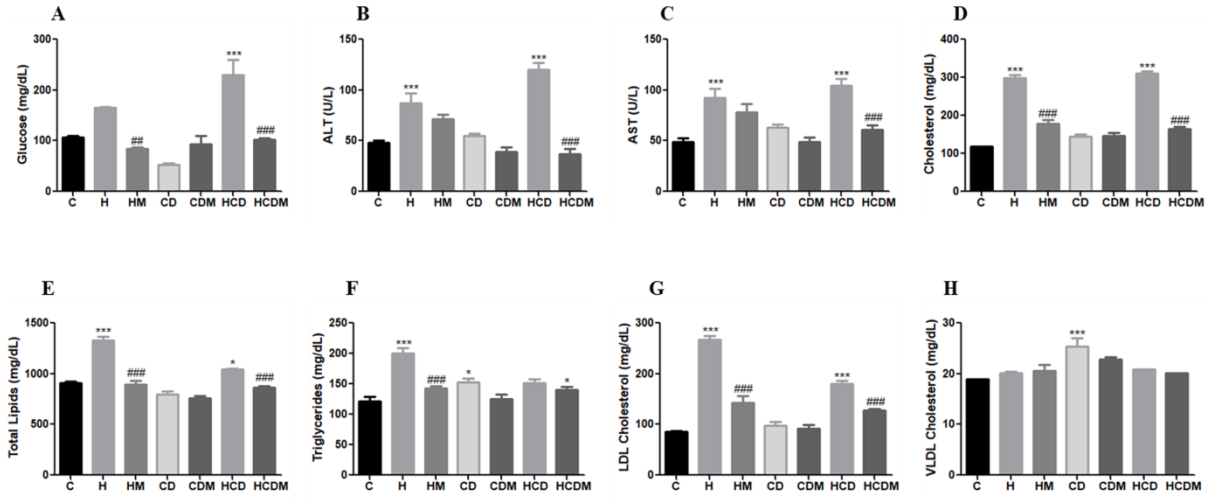

Figure 2. Serum blood glucose, markers of liver function and lipid profile. Note the therapeutic potential of Melatonin against C57BL/6J mice high fat high fructose diet fed and /or subjected to chronodisruption. Data represented as mean ± SD *P<0.05, **P<0.01, ***P<0.001 vs control, #P<0.05, ##P<0.01, ###P<0.001 vs H, CD and HCD. (n=6)

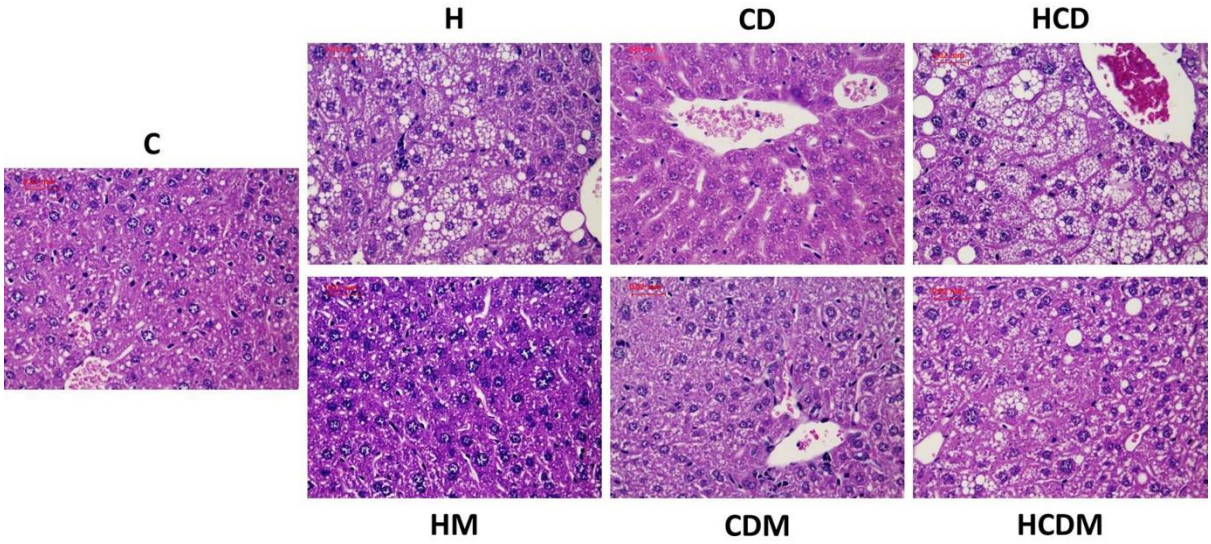

Figure 3. Histological features by hematoxylin and eosin staining. Histopathological images from H and HCD groups displayed ballooning hepatocytes and steatosis.

The hepatic core clock genes (*Clock*, *Bmal1*, *Per1*, *Per2*, and *Cry2*) in mice subjected to H, CD and HCD were examined. We observed that the CD group showed significant disruption of all the core clock genes but the high fat high fructose (H) group showed an arrhythmic

expression of positive core clock genes (*Clock* and *Bmal1*). But the shallow expression of the circadian clock transcripts was reversed in the melatonin treatment group. The key genes of bile acid metabolism such as *Fxr and Tgr5* (BA receptor), *Cyp7a1, Cyp27a1, Cyp8b1* (BA synthesis), *Abcb11, Mdr2, Mdr3, Mrp2, Mrp3, Ost-β* (BA metabolism) were also studied. Significant alterations were found in the bile acid receptors (*Fxr and Tgr5*), bile acid synthesis (*Cyp7a1, Cyp27a1, Cyp8b1*) and metabolism genes (*Mrp2, Mrp3, Ost-β*) in H, CD and HCD groups as compared to control.

Objective 2: Diurnal and nocturnal studies of behavioural anomalies in chronodisruption (CD) and NASH and corrective role of exogenous melatonin.

Parameters assessed: C57BL/6J mice were subjected to photoperiodic manipulation induced chronodisruption (CD), high fat high fructose diet (H) and/or combination of both during the experimental regime of 18 weeks. Behavioural anomalies such as depression, anxiety, spatial learning and memory, spontaneous locomotor activity, and cognition was tested using various behavioral test such as force swim test (FST) and tail suspension test (TST) for depression, Social interaction test for social recognition and behaviour, marble burying test (MBT) for repetitive and anxiety-related behavior, novel object recognition test (NOR) to evaluate recognition memory, elevated plus maze test (EPM) and hole-board test for anxiety and spontaneous locomotor activity test using infrared actimeter, barnes maze test and morris water maze test for spatial learning and memory. A core symptom of depression in humans is inability to experience pleasure from enjoyable activities and rewarding the same anhedonia behaviour was recorded in experimental mice by sucrose preference test. The immobility time, swimming path and tracking of the mice was analysed using ANY-maze software.

Observations: C57BL/6J mice were subjected to a high fat high fructose diet (H), chronodisruption (CD) or combination of both (HCD) for 18 weeks. After 16 weeks, behavioral paradigms of depression, anxiety, spatial learning and memory, possible locomotor activity and cognition were performed.

In the present study the effect of (H), (CD) or combination of both (HCD) on the immediate spatial memory was assessed using barnes maze and morris water maze test. In Morris water maze the frequency of crossing the platform zone was significantly less in CD and HCD groups and the same was reversed with melatonin treatment in all the groups. The depression-like behavior was evaluated by force swim and tail suspension test. In force swim test there was increase in the immobility time in H, CD and HCD groups but interestingly there was only decrement in the CDM group and not in other two melatonin groups (i.e. HM and HCDM). But in tail suspension test there was significant increment in the immobility time of all three disease control groups i.e. H, CD and HCD groups. Treatment with melatonin decreases the time spent immobile in the said groups. Thus high fat high fructose diet, chronodisruption and combination of both i.e. HCD increases the immobility time in both force swim and tail suspension test.

The repetitive and anxiety-like behavior was measured using marble burying, elevated plus maze and hole-board test. In the marble burying test, H and HCD mice exhibited more signs of anxiety-like behavior and buried more marbles in a 30 min session as compared to control but decreased anxiogenic behavior was observed following melatonin treatment. Anxiety was determined using the elevated plus maze (EPM) test, which uses the time spent in the open arms as an indicator of the level of anxiety. The group fed with a high fat high fructose diet and/or subjected to chronodisruption or combination of both spent less time in the open arm and also had fewer entries in the open as compared to the control group. These results suggest that mice fed with a high fat high fructose diet and/or subjected to CD are more anxious. But the same was reversed in the melatonin treated groups.

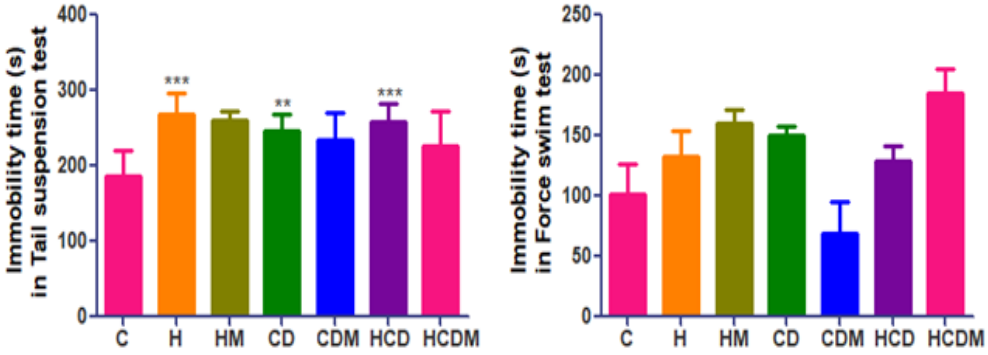

Figure 4. Behavioural response to tail suspension and force swim test. Increased immobility in the H, CD and HCD groups as compared to the control group (n=6). An increase in immobility time by H, CD and HCD groups demonstrates depression-like behavior. Results show mean ± SEM values.

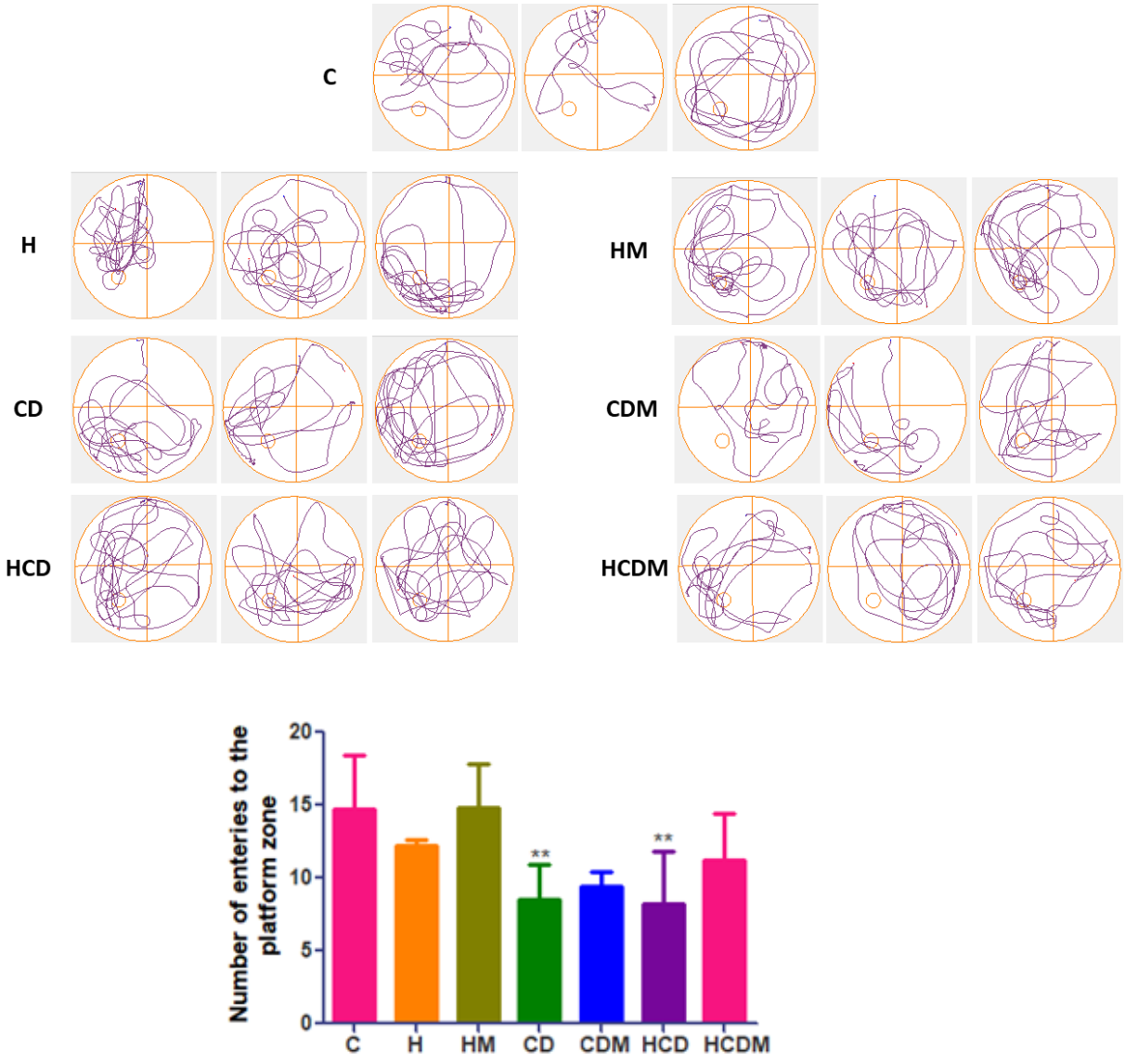

Figure 5. Spatial learning and memory was assessed in Morris water maze test. A representative image of swim paths of day 4 (probe day), showing that control animals were able to find the hidden platform more easily than H, CD and HCD groups. The values are mean ± SEM (n=6 animals per group).

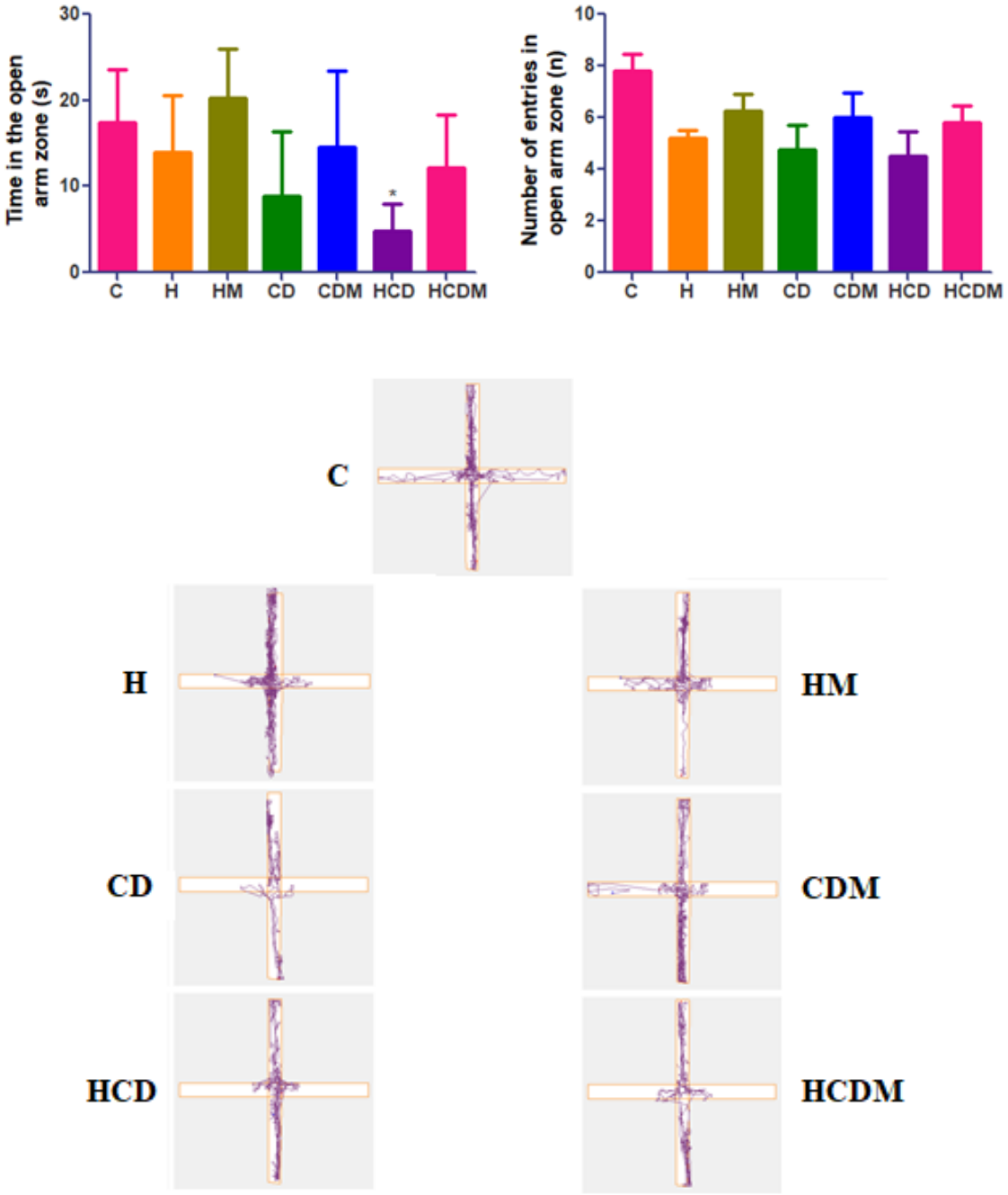

Figure 6. Effect of high fat high fructose and/or chronodisruption on the number of entries (n) and time spent (s) in the open arms zones evaluated in the elevated plus maze test along with the tracking images. The values are mean ± SEM (n=6 animals per group).

Objective 3: Drawing correlation between behavioral anomalies and alteration in neural BDNF-TrkB pathway in CD and NASH.

Parameters assessed: C57BL/6J mice were subjected to photoperiodic manipulation induced chronodisruption (CD), high fat high fructose diet (H) and/or combination of both during the experimental regime of 18 weeks. Levels of corticosterone were determined from serum. H&E, cresyl violet staining and immunohistochemistry of GFAP was used to detect the morphological changes of neurons in the hippocampus. mRNA of brain growth factors (*Bdnf, Gdnf, Ngf* and *Igf-1*), neurotrophins (*Nt3, Nt4*), synaptic plasticity markers (*Syn-1*, *Psd-95*) and inflammatory markers (*Tnf-α, Il-1β, Il-6* and *Tlr-4*) in the hippocampus has been performed. Also the blot analysis in the hippocampus of BDNF, TrkB, Synapsin-1 and ERK1/2 has been performed.

Observations: The work is currently in progress and likely to be completed in two months.

Key findings:

- High fat high fructose diet (H), Chronodisruption (CD) or a combination of the two (HCD) treatments in C57BL/6J mice had accounted for significant changes in the bile acid receptors (*Fxr and Tgr5*), bile acid synthesis (*Cyp7a1, Cyp27a1, Cyp8b1*) and metabolism genes (*Mrp2, Mrp3, Ost-β*) suggesting that experimentally induced lifestyle disorder impacts the hepatic bile acid metabolism by providing an anabolic impedes.
- The resultant behavioural anomalies such as depression-like behaviour, impaired learning and cognitive deficits coupled with anxiety-like behaviour in C57BL/6J mice were observed in all the three disease groups (H, CD and HCD). Exogenous melatonin could restore the behavioural patterns in varying degrees.
- Melatonin also improved the behavioural paradigm like counts in locomotor activity, head dipping's in hole-board test and, spontaneous learning and memory.
- Hippocampal region of H, CD and HCD mice showed an improvement in brain derived growth factors (*Bdnf* and *Ngf*), neurotrophins (*Nt3* and *Nt4*) and synaptic markers (*Psd95* and *Syn-1*) following melatonin treatment that correlates with corrective changes in the said behavioural patterns.

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