Materials and Methods

Chemicals and Reagents

TRIzol, and SYBR select master mix were procured from Invitrogen (Thermo Fisher Scientific, USA). ORO stain (Cat. TC256) and ethanol (Cat. MB106) were purchased from Hi-media laboratories (Mumbai, India). BioRad Protein Assay Dye reagent concentrate, Polyvinylidene fluoride (PVDF) membrane, Precision Plus protein ladder, iScript cDNA synthesis kit and Clarity Western ECL substrate were procured from Bio-Rad Laboratories (CA, USA). Antibodies against BDNF (E-AB-18244), ERK1/2 (E-AB-12397), and SYN-1 (E-AB-33003) were purchased from Everon Life Sciences. Antibodies against β-actin (4970S) and anti-rabbit secondary antibody (7074P2) were purchased from Cell Signalling Technology (MA, USA). Antibody against TrkB (MAB397-SP) was purchased from Biotechne (MN, USA). RNA Later stabilizing solution was purchased from Ambion Inc. (Thermo Fisher Scientific, USA). ELISA kit for TNF-α, IL-6, IL-10, IL-12 and IL-17 were procured from Krishgen Biosystem, India and Peprotech, USA. Melatonin-ELISA (193596) kit was procured from MP Biomedical (CA, USA). Thyroid ELISA kit (T3, T4 and TSH) were procured from XEMA-MEDICA Co., Ltd. (Russia). Melatonin, Haematoxylin, eosin, and Protease inhibitor cocktail (PIC) were purchased from Sigma Aldrich (MO, USA). All other chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany).

In-vivo Experimental Design

Animal studies and experimental protocols

Male C57BL/6J mice (Total: 210, 6–7 weeks of age) were purchased from ACTREC Mumbai and maintained as per CPCSEA standard guidelines (23±2˚C, LD 12:12, laboratory chow and water ad libitum) followed by a week-long acclimatization. Protocol was approved by Institutional Animal Ethical Committee (IAEC) (Approval no. MSU/Z/IAEC03/01-2019 & MSU/Z/IAEC/05-2017) and experiments were conducted in CPCSEA approved (827/GO/Re/S/04/CPCSEA) animal house facility of Department of Zoology, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India.

Photoperiodic manipulation induced chronodisruption (CD)

The photoperiodic manipulation induced chronodisruption (CD) was performed as per the protocol published by Kettner et al. (2015) with minor modifications (Joshi et al., 2021; Kettner et al., 2016). Briefly, mice were housed in tandem in two separate rooms viz. Room 1 and Room 2 respectively and CD was achieved by transferring them between the said rooms. Room 1 comprised of 7:00 h to 19:00 h (light period)/19:00 h to 7:00 h (dark period) and Room 2 of 11:00 h to 23:00 h (dark period)/23:00 h to 11:00 h (light period). Thus, shifting mice from Room 1 to Room 2 on Monday resulted in phase advance of 8 h (lights off at ZT4) and transferring them back to Room 1 on Thursday resulted in a phase delay of 8 h (lights off at ZT20) (Fig. M1). All the transfer of mice between the two rooms was carried out at 10:55 h.

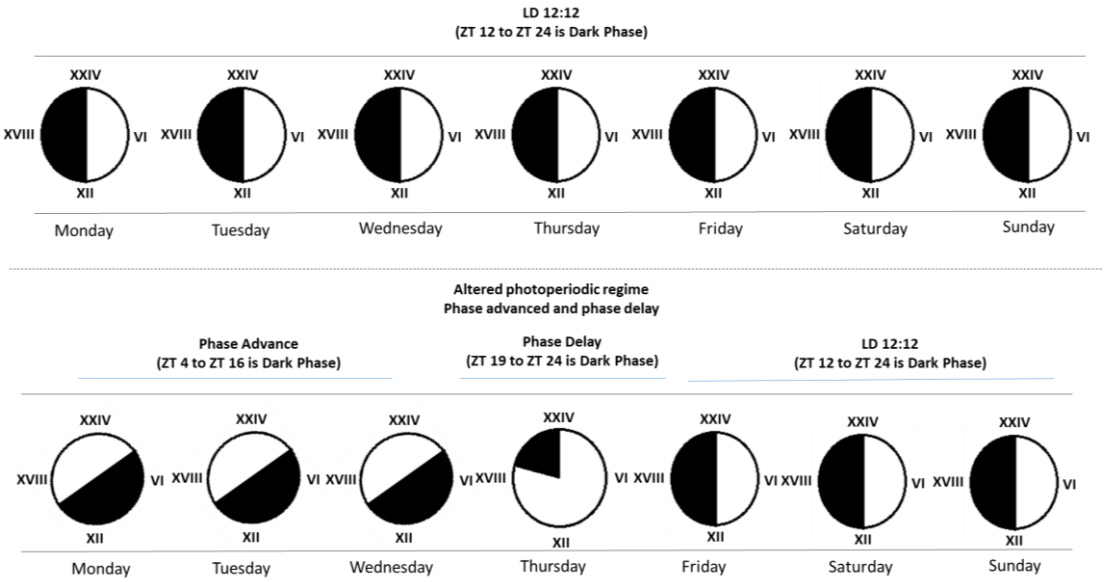

Figure M1: Schematic representation of normal and altered photoperiodic regime followed in the study. C57BL/6J mice of control and high fat-high fructose diet (H) group were maintained with L:D 12:12 photoperiodic regime; CD, HCD, CDM and HCDM were subjected to 8 hours phase advance on Monday and 8 hours phase delay on Thursday for 18 weeks.

Mice were randomly divided into seven groups with 30 animals per group. The entire period of experiment was of 18 weeks.

• Group I (C) was fed with standard chow diet.

• Group II (CD) was subjected to photoperiodic manipulation induced chronodisruption for 18 weeks.

• Group III (H) was fed with high fat diet + 20% Fructose in water (H diet) (Love et al., 2017).

• Group IV (HCD) was fed with high fat diet + 20% Fructose in water (H diet) and subjected to photoperiodic manipulation induced chronodisruption for 18 weeks.

• Group V (CDM) was subjected to chronodisruption (CD) and Melatonin (M) was injected (i.p.: 10 mg/Kg B.W.) from 10^{th} to 18^{th} weeks.

• Group VI (HM) was fed with (H) diet and Melatonin (M) was injected (i.p.: 10 mg/Kg B.W.) from 10^{th} to 18^{th} weeks.

• Group VII (HCDM) was fed with (H) diet and subjected to chronodisruption (CD) for 18 weeks and also Melatonin (M) was injected (i.p.: 10 mg/Kg B.W.) from 10^{th} to 18^{th} weeks.

Food intake, water intake and body weights were recorded thrice every week throughout the period of study (Fig.1).

Table 1: Composition of high fat diet (Joshi et al., 2021).

Macronutrient Composition	
Protein % of Energy	20
Carbohydrate % of Energy	20
Fat % of Energy	60
Energy MJ/kg	21.8
Ingredient, g/kg	
Casein	258.4
L-Cystine	3.9
Corn Starch	0
Maltodextrin	161.5
Sucrose	88.9
Cellulose	64.6
Lard	316.6
Soybean oil	32.3
Mineral Mix (a)	12.9
Vitamin Mix (b)	12.9
Choline Bitartrate	2.6

(a) Mineral mix adds the following components (per g mineral mix): sodium chloride, 259 mg; magnesium oxide, 41.9 mg; magnesium sulphate, 257.6 mg; chromium K sulphate, 1.925 mg; cupric carbonate, 1.05 mg; sodium fluoride, 0.2 mg; potassium iodate, 0.035 mg; ferric citrate, 21 mg; manganous carbonate, 12.25 mg; ammonium molybdate, 0.3 mg; sodium selenite, 0.035 mg; zinc carbonate, 5.6 mg.

(b) Vitamin mix adds the following components (per g vitamin mix): retinyl acetate, 0.8 mg; cholecalciferol, 1.0 mg; DL-a-tocopheryl acetate, 10.0 mg; menadione sodium bisulfite, 0.05 mg; biotin, 0.02 mg; cyanocobalamin, 1 µg; folic acid, 0.2 mg; nicotinic acid 3 mg; calcium pantothenate, 1.6 mg; pyridoxine–HCl, 0.7 mg; riboflavin, 0.6 mg; thiamin HCl, 0.6 mg.

Melatonin administration

Melatonin (Sigma, M-5250; St. Louis, MO, USA) was freshly dissolved in minimum volume of ethanol (less than 1%) and saline solution (0.9% NaCl) was added so as to achieve a dose concentration of 10 mg/kg body weight. Care was taken that the tubes containing melatonin solution are not induced to light-induced degradation and were covered with aluminium foils. Daily evening intraperitoneal injection (i.p.: 10 mg/Kg B.W.) of melatonin was dosed from 10^{th} to 18^{th} weeks of the experiment.

Blood and Tissue collection

At the end of the experimental protocol, mice were fasted overnight and blood was collected via retro-orbital sinus under mild isoflurane anaesthesia. Blood was allowed to clot at room temperature (RT) for 30 min and serum was isolated by centrifuging at 3000 rpm at 4°C for 10 min. Serum was separated and stored at -80°C for all the subsequent analysis. Later, mice were euthanized and liver and in brain (hippocampus) were carefully removed and stored for histochemical analysis. For histology, the samples were fixed in 4% PFA, dehydrated in a graded series of ethanol and embedded in paraffin using standard protocol. For immunoblotting, collected tissue were directly snap frozen in liquid nitrogen. Gene expression studies involved collection of tissues in RNA later, snap frozen in liquid nitrogen and stored at -80°C until further use.

Serum biochemical parameters

Serum samples (stored at −80 °C) were thawed and were used for performing liver function test (AST, ALT and ALP). Further, serum total cholesterol (TC), total lipids (TL), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and high-density lipoprotein cholesterol (HDL-C) were estimated using readymade kits made by Reckon Diagnostic Ltd., India.

Corticosterone immunoassay and procedure

Serum corticosterone (CORT) was measured using a single antibody competitive enzyme immunoassay incorporating a polyclonal CORT antibody as per (Narayan et al., 2013). The CORT antibody was diluted to 1:20,000, HRP (Horseradish peroxidase)-conjugated CORT 1:100,000 and CORT standards were used as 1000–1.9 pg/well. The

corticosterone antiserum (CJM006) was found to cross-react with corticosterone 100%, desoxycorticosterone 14.25%, progesterone 2.65%, tetrahydrocorticosterone 0.90%, testosterone 0.64%, cortisol 0.23%, prednisolone 0.07%, 11-desoxycortisol 0.03%, prednisone < 001%, cortisone < 001% and estradiol < 001% (Narayan et al., 2013). The corticosterone enzyme immunoassay was performed as described previously (Kumar et al., 2019, 2021). CORT antibody was diluted in coating buffer (0.05 M sodium bicarbonate buffer, pH 9.6) before coating a 96-well plate (Nunc-Immuno maxisorp, Thermo Fisher Scientific, Roskilde, Denmark), and incubated overnight at 4 ◦C and washed four times with washing buffer (0.15 M NaCl and 0.05% Tween 20, Sigma, India). CORT (50 µL) standards were diluted in assay buffer (0.1 M PBS, pH 7, containing 0.1% BSA) were added to the wells immediately followed by 50 µL of HRP (Horseradish peroxidase) conjugated CORT. After 2 h of incubation at room temperature, the plates were washed and 50 µL of TMB/H2O2 (Genei, Bangalore, India) and substrate was added and allowed to stand in dark for 10–15 min. Termination of the reaction was achieved by addition of 50 µL of 1N HCL and absorbance read at 450 nm in the ELISA reader (Thermo Multiskan Spectrum Plate Reader, version 2.4.2, Thermo Scientific, Vantaa, Finland). The CORT antibody sensitivity was calculated to be 1.95 pg/well at 90% binding.

Melatonin ELISA

Serum melatonin levels were assessed by an ELISA kit (MP Biomedical Inc., Irvin CA) as per the manufacturer's instructions. Briefly, 50 µl of samples, controls, and standards were pipetted in precoated ELISA plate wells, followed by melatonin-biotin, antiserum mixes (50 µl each) and incubated overnight at 4 ºC. Next day, contents were washed thrice with buffer (provided in the kit) and re-incubated with 150 µl conjugate for 2 hours at room temperature. Again, the wells were washed thrice with buffer and incubated with 200 µl PNPP substance for 20-40 minutes at room temperature. Reaction was terminated by adding stop solution (100 µl). Readings were recorded at 405 nm using Bio-Tek Multimode Reader, (HTX, Synergy, USA). A standard curve was made and the readings of samples were calculated using their average optical densities.

Metagenomic study of fecal samples

Fresh fecal samples were collected from the control and experimental groups of mice and transferred to the lab for metagenomic studies. DNA were isolated separately using a commercially available kit according to the manufacturer's instructions (QIAGEN Stool kit; QIAGEN, CA) and metagenomic DNA was used as a template in PCR to amplify 16S r DNA.

The V3–V4 region of the 16S r-RNA gene was PCR amplified using four pair of primers with metagenomic DNA template and Master mix (Fermentas, UK). A total of 25 µl of reaction mixture consisting of 10 pmol of each primer, 30–75 ng of template DNA, 12.5 µl of Master mix (Fermentas, UK). The PCR amplification was performed by Thermal Cycler (Applied Biosystems, USA) and PCR conditions were adjusted in laboratory. The desired products generated from all 4 sets of primers were purified using Qiagen DNA Gel Extraction Kits (QIAGEN, CA) in accordance with the directions of the manufacturer. After quantification by nanodrop, the equimolar PCR products from four pairs of primes were pooled for each low and high FCR groups.

Metagenomic DNA was isolated from 160 mg fecal sample having solid particles within it. In the first step, samples were shaken vigorously to dissociate bacteria attached to solid particles followed by centrifugation at 250 rpm. The supernatant was collected carefully and subjected to genomic DNA extraction using the QIAamp DNA Stool Mini Kit (QIAGEN GmbH, Germany) as per the manufacturer's instructions with minor modifications as suggested by (Zened et al. 2013). The DNA was eluted in 50 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA) and stored at − 80 °C for further use. The quantitative and qualitative evaluation of DNA was done using Nanodrop-1000 spectrophotometer (Thermo Fisher Scientific, MA). The same procedure was repeated separately for the rest of the samples. Fecal samples (07) of respective experimental groups were pooled in equimolar concentration to minimise bias of sampling procedure. The primer pair covering V3–V4 regions of 16 s rRNA gene were used for amplification. The PCR reaction mixture, conditions and PCR purification were done using procedure described by Rey et al. (2014) in 25 µl reaction mixture with 10 ng of metagenomic DNA. PCR products were subjected to 2.0% agarose gel electrophoresis. Bands of expected size were excised and purified using MinElute PCR Purification Kit (Qiagen

GmbH, Germany). Further, libraries were prepared using Nextera XT DNA Library Preparation Kit (Illumina Inc., CA) for sequencing as described by the manufacturer's library preparation protocols. The final purified libraries were diluted to 4 nm, denatured and mixed with PhiX (about 30% of final DNA amount) and sequenced on the MiSeq desktop sequencer (Illumina) (2 × 300 run) at the Centre of Excellence in Biotechnology, AAU, Anand, Gujarat, India.

Metagenomic (fecal sample) data analysis

Illumina-generated sequence reads were analysed through open source online server Galaxy Workflow for analysis of 16s metagenomics data (GALAXY). Briefly, low-quality regions were trimmed by the software and quality control was performed. The data was filtered using the work flow itself and Vsearch tool was used to compare the sequences with SILVA database (Quast et al., 2012). The data was clustered and the chimera was removed to prepare the consensus sequences and OTUs were determined with reference to SILVA database. A range of alpha diversity parameters were estimated using workflow itself. We calculated alpha-diversity (observed species, Chao1 estimator of richness and Shannon's diversity index) matrices and the rarefaction curve was generated from a number of reads for each sample against the observed species. Shannon's index (depicting microbial richness) was estimated along with Chao1 and ACE indices, to determine the taxonomic diversification. Comparative analysis was plotted through BIOME software for phylum, genera and species in order to determine the variation between taxonomic profiles among different samples.

Neuro-behavioral test for locomotion, anxiety, depression and spatial learning and memory

Male C57BL/6J mice were subjected to high fat high fructose diet fed and /or photoperiod manipulations induced chronodisruption and were used for behavioral tests (26 weeks age). Tests were performed at both diurnal ZT3 to ZT9 (i.e. 10-00 a.m. to 4-00 p.m.) and nocturnal ZT15 to ZT21 (i.e. 10-00 p.m. to 4-00 a.m.) timings. After each experiment and/or trial the apparatus was cleaned with 70% ethyl alcohol, and blotted dry with a sterile paper towel. Uniform conditions were maintained across the animal

training and probe trials. Mice were initially habituated to the testing room for at least 1h prior to the behavioural tests and care was taken to ensure that the locations of spatial clues were kept identical for all the experimental groups. Testing rooms had background white noise of >60dB (no vibrations) and no artificial aroma that could possibly distract mice. During the observation periods, videos were recorded using a digital web camera (Logitech Webcam C270; 1280 x 720 pixels) connected to Dell Inspiron Laptop to ensure un-interrupted recording. Analysis of the various behavioral tests were done using ANYMaze software (Stoelting Co., Wood Dale, IL). Spontaneous locomotor test-for locomotory deficits, Hole board, Marble burying and Elevated plus maze test- for anxiety, Morris water maze test- for spatial learning & memory and Force swim, tail suspension and Sucrose preference test- for depression-like behavior were performed for control and experimental groups.

Spontaneous Locomotor Activity (locomotory deficits)

The spontaneous locomotor activity was assessed using infrared actimeter (Model: ACT-01, Orchid Scientific & Innovative India Pvt. Ltd., India) with clear square Plexiglas arena (50 cm × 50 cm) and 32-infrared sensors. The frame is equipped with 32 IR cells, out of which 16 cells are on X-axis and 16 cells are on Y-axis in infrared actimeter. The mouse was placed in the centre of the arena and the locomotor activity was measured for a duration of 10 min during which the instrument control panel displayed the number of beam brakes on all axis in IR actimeter mode. The data were expressed as the total light beam interruptions (locomotor counts).

Hole-board test (HBT; for anxiety)

Hole-Board test is also based on a disinhibition of natural exploratory tendencies by stress/anxiolytic treatments. In this test, the numbers of head dips have been recorded in infrared actimeter using a hole board plate (16 holes) comprising of an open field of the same size as mentioned above but made of clear Plexiglas walls. The floor was made of opaque Plexiglas with evenly distributed 16 holes (each 3.8 cm diameter and 10 cm deep). Total count for the head-dips were recorded from the control panel of the actimeter.

Marble-burying test (MBT; for anxiety)

This test was performed as described (Gaikwad et al., 2010) with some minor modifications. Briefly, mice were single-caged in experimental cages (4–5 cm-thick rice husk) for 10 min (habituation phase) followed by a 40-min home cage resting period. Later, mice were re-introduced into the experimental cages (for 30 min), comprising of twenty sterilized glass marbles arranged in a 4x5 matrix (marble diameter~10 mm; overlaid on the bedding). Photographs of the experimental cages were recorded at 0, 10, 20 and at 30 min during the testing phase and the number of marbles buried were counted (Gaikwad et al., 2010).

Elevated plus maze test (EPM; for anxiety)

The elevated plus maze consisted of two closed arms (5x30 cm, with a clear 15-cm-high perspex walls) and two open arms (5x30 cm long) placed 40 cm above the floor. During a single 5-min trial, the mice were placed in the centre of the maze and the numbers of entries in each arm were counted and the time spent was recorded (with digital camera). The data was analysed using Anymaze Video Tracking System (Stoelting, Wood Dale, IL, USA).

Morris water maze test (MWM; for spatial learning and memory)

MWM tests was conducted as described by Vorhees & Williams, 2006. The test comprised of 03 phases viz. pre-training, training and probe-test. Mice were pre-trained (without platform) in water-maze pool (diameter 150 cm, depth 50 cm, temperature 22 ± 1°C) for 01 day for acclimatization. During training trials for 04 days, mice had to find a hidden, submerged platform wherein a different starting locations was employed each time. After 24 h of training, probe-tests were performed (without a platform) to assess the time spend in the target quadrant (where the platform was placed) for a duration of 60 sec. Tracking videos were analysed with ANY-maze software (Stoelting Co., Wood Dale, IL). The swimming distance during training trials and the time spent in target quadrant during probe trials were used to evaluate the spatial learning and memory of the individual mice.

Force swim test (FST; for depression)

Immobility time in mice was evaluated inducing forced swimming in open cylindrical container (dia. 10 cm, ht. 25 cm), containing 19 cm water (25 ± 1 °C). Each mice was video graphed and the calculations of mobile time (desperate swimming and an effort to come out of the container) or immobile time (when mice ceased struggling and remained floating motionless on water surface) was noted. High indices of immobility time was considered as an indication of depression-like activity (Porsolt et al., 1977).

Tail suspension test (TST; for depression)

The tail suspension test (TST), shares a common theoretical basis and behavioral aspects with the FST (Steru et al., 1985). In TST, mice were suspended by their tails using adhesive tape to a horizontal bar for 6 minutes and videography for each mice was recorded. Classically, the suspended mice perform immediately escape-like behaviours, followed by an immobile posture. Engaging in escape like behaviour for longer period of time is read as an indices of good health whereas; higher duration of immobility was considered to be indices of depression-like behaviour. The video clips were visually analysed (by a volunteer blinded to the experimental groups) and the immobility time was recorded using a stopwatch.

Sucrose preference test (SPT; for depression)

This test uses the gustatory preference of sugar wherein; a decline in interest indicates depression symptoms and anhedonia (Serchov et al., 2016). Animals were allowed free access to 1% sucrose (w/v) and water solution in two bottles. To avoid bottle side preference, the positions of sucrose and water bottles were switched 04 times in 24h. The amount of sucrose /water consumed were recorded after 24 h and the sucrose preference was calculated according to the following formula:

$$\textbf{Sucrose Preference (\%)} = \frac{\textbf{sucrose intake } (g)}{\textbf{sucrose intake } (g) + \textbf{water intake } (g)} \times 100$$

Serum cytokine and thyroid hormones analysis by ELISA

For systemic inflammation analysis, serum cytokine profile of pro- and anti-inflammatory cytokines (TNFα, IL6, IL10, IL12, and IL17) and thyroid (T3, T4 and TSH) hormones was performed using ELISA kits by Krishgen Biosystem, India, Peprotech (USA) and XEMA-MEDICA Co., Ltd. (Russia) as per manufacturer's instructions.

RNA isolation and cDNA synthesis

Liver and hippocampal tissue samples were homogenized using Trizol reagent (Invitrogen, Thermo Scientific, USA) followed by lysis/homogenization and phase separation using chloroform. RNA was precipitated from aqueous phase using pre-chilled isopropanol and centrifuged to a pellet. The same was subsequently washed with pre-chilled 75% ethanol and dissolved in diethyl pyro carbonate (DEPC)-treated water. Purity of the RNA samples was assessed by measuring A260/A280 ratio in UV-Vis spectrometer (PerkinElmer, USA). Samples with A260/A280 within the range of 1.8-2.0 were used for gene expression studies. Samples were quantified using absorbance at 260 nm as follows:

Concentration (µg/ml) = A260 x 40 x dilution factor

Where, 40 = extinction coefficient (40 µg/ml of RNA= 1 absorbance)

1 µg of RNA was used to synthesize cDNA by reverse transcription using iScript cDNA synthesis kit (Bio-Rad Laboratories, USA) according to manufacturer's protocol and cDNA was used as template for quantitative RT-PCR.

Quantitative RT-PCR

Quantitative RT-PCR was performed using Power Up SYBR Green Master Mix (Thermo Fisher Scientific, USA) as per the manufacturer's protocol and the reaction was run on the QuantStudio-3 Real-Time PCR System (Applied Biosystem, Thermo Fisher Scientific, USA). mRNA expression of the genes of interest were analyzed using 2-ΔΔCT method using GAPDH as endogenous control. Primers used for this study are listed in Table 2.

Western blotting

For extracting total protein lysate, tissue was homogenized in RIPA buffer (50 mM tris (pH 8.0), 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% triton-X-100) containing PIC and 1 mM PMSF followed by incubation in ice-bath for 2 h. The tissue lysates were centrifuged at 10,000 rpm at 4˚C for 20 min and resultant supernatant was subjected to protein estimation using Bio-Rad protein assay dye reagent (Bio-Rad Laboratories, USA). Equal quantity of protein from each sample was denatured in 6X loading dye at 95°C-100°C for 5 min. 25 µg of each protein sample was separated by SDS-PAGE and subsequently transferred onto PVDF membrane using standard protocol of Trans-Blot Turbo Transfer System (Bio-Rad Laboratories, USA). Transfer of proteins was checked by staining membrane with 0.05% Poncaeu S. Membrane was destained in distilled water followed by blocking with 3% BSA in Tris buffered saline (TBS) for 1 h at RT. The membrane was incubated overnight in anti-BDNF (1:1000), anti-TrkB (1:1000), anti-ERK1/2 (1:1000) and anti-SYN-1 (1:1000) antibodies in 3% BSA. After washing thrice with TBS containing 0.1% Tween 20 (TBST), the membrane was probed with HRP-linked anti-rabbit secondary antibody (1:5000) for 1 h at RT. Blots were developed using Clarity western ECL reagent (Bio-Rad, CA, USA) and X-ray films. Anti-β-actin antibody (1:5000) was used to determine equivalent loading.

Statistical analysis

Data was expressed as mean ± SD. All the groups were compared with control and disease control groups by one-way analysis of variance (ANOVA) by Tukey's multiple comparison tests using Graph Pad Prism 5.0 (CA, USA). Rhythmic variations in clock gene expression were analysed two-way ANOVA. *P < 0.05, **P < 0.01 and ***P < 0.001 were considered to be significant as compared to control. #P < 0.05, ##P < 0.01 and ###P < 0.001 were considered to be significant in melatonin treated groups compared to its respective disease control groups.

Table 2: List of Primers for Real-time PCR

Gene Name	Forward primer	Reverse primer
CLOCK	CACTCTCACAGCCCCACTGTA	CCCCACAAGCTACAGGAGCAG
BMAL1	ACATAGGACACCTCGCAGAA	AACCATCGACTTCGTAGCGT
CPT-1	CGATCATCATGACTATGCGCTACT	GCCGTGCTCTGCAAACATC
PPARα	TGCAAACTTGGACTTGAACG	TGATGTCACAGAACGGCTTC
FAS	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
SREBP1C	GCAGCCACCATCTAGCCTG	CAGCAGTGAGTCTGCCTTGAT
CD36	TGAATGGTTGAGACCCCGTG	TAGAACAGCTTGCTTGCCCA
TNF-α	GTGGAACTGGCAGAAGAG	AATGAGAAGAGGCTGAGAC
IL-1β	TCTATACCTGTCCTGTGTAATG	GCTTGTGCTCTGCTTGTG
IL-4	GTAGGGCTTCCAAGGTGCTT	GGCATCGAAAAGCCCGAAAG
IL-6	TGGATGCTACCAAACTGGAT	TGGATGCTACCAAACTGGAT
IL-10	AAGGGTTACTTGGGTTGCCA	TTCAGCTTCTCACCCAGGGA
IL-12	ATTACTCCGGACGGTTCACG	ACGCCATTCCACATGTCACT
IL-17	ACCGCAATGAAGACCCTGAT	TCCCTCCGCATTGACACA
MCP-1	TGACCCCAAGAAGGAATGGG	GACCTTAGGGCAGATGCAGTT
Nf-κB	GAGGTCTCTGGGGGTACCAT	AAGGCTGCCTGGATCACTTC
CREB	GAAGAACAGGGAGGCAGCAA	AGTCCCATTCCTCACACCGT
IBA-1	TGGGTTTGTGTTCGTCAGGC	CATGGTGGGGACAGGAAGTAG
FXR	CAAAATGACTCAGGAGGAGTACG	GCCTCTCTGTCCTTGATGTATTG
TGR5	CCCACCGCCAGCTGTGTGAG	CCCCATGGCCACAGGCACAG
CYP7A1	GGGCAGGCTTGGGAATTTTG	AACGCTCAGCAGTCGTTACA
CYP8B1	TCAGACTCCAGGGATGTTGC	GGAAGTTCTGGGCTAAGGGA
CYP27A1	ACAGGAGGGCAAGTACCCAA	CCATTGCTCTCCTTGTGCGA
ABCC2	GGTGTTGGGCTTATGGTTC	GCAGCAAGTTCCTGAGTTC
ABCC4	GCAGCGAGAAACGGAACAG	GGTTGCTGATGCTGCCTAGTT
OST-β	CAGGAACTGCTGGAAGAAATGC	GCAGGTCTTCTGGTGTTTCTTTGT
BDNF	TCATCCCTCCCCGAGAGTTC	TGGGCTCAATGAAGCATCCG
TrkB	GTCAGCCCTCACGTCACTTC	CAACTGCGGTAGCAGGACA
SYN-1	ATCTCTGGTCCCACTCGTCA	ACATCCTGGCTGGGTTTCTG
PSD-95	GAAGACCTCTCAGGCCCCTA	AGGGGTAGGGTTATTGGGCT
NT3	CCGGTGGTAGCCAATAGAACC	GCTGAGGACTTGTCGGTCAC
NT-4	AGCCGGGGAGCAGAGAAG	ACCTCCTCACTCTGGGACTG
GAPDH	GTCGGTGTGAACGGATTTGG	TAGATGCCTGCTTCCCATTC