CHAPTER -4

Evaluation of N-acetylcystiene effect on peripheral clock related circadian rhythm

4. Introduction

Multiple cellular clocks found in many organs and tissues make up the mammalian circadian system, which controls these clocks' regulation in a hierarchical fashion during the course of the day (Honma, 2018). The suprachiasmatic nuclei (SCN), which are at the top of the hierarchy, synchronizes the peripheral clocks, such as organ and tissue clocks, via electrical, endocrine, and metabolic signaling pathways that have an influence on cellular clocks' molecular processes (Herzog et al., 2017). Systemic signals such as body temperature, hormone metabolites, and feeding/fasting cycles have an impact on the most significant peripheral circadian clocks, such as the liver. Although the SCN acts as the system's main synchronizer, food consumption can have a direct impact on the liver's peripheral clocks. Additionally, modifications in central clock due to untimed light exposure may leads to alter in feeding schedule (Plano et al., 2017). Additionally, both the central and peripheral tissue clocks may be trained to respond to particular time cues (zeitgebers), such as light (a photic cue for the SCN), food (a non-photic cue for the liver), and physical activity (a non-photic cue for the skeletal muscle). Untimed light exposure can either directly or indirectly affect the phase connection between the liver's clocks and the SCN's central clock (Damiola et al., 2000).

According to several researches, prolonged exposure to low light alters eating habits and can interfere with the peripheral circadian clock, which then affects metabolism. Furthermore, prolonged low light might cause stress and may impact the

SCN, which may directly or indirectly affect the one of our key organs, liver (peripheral clock). These studies suggest that chronic dim light exposure changes feeding pattern, which can disrupt the peripheral circadian system causing alteration in metabolism. Further, chronic dim light can act as a stressor directly to the SCN and indirectly to the liver. Our previous report shows that chronic dim light increases the CORT level which causes increase in the stress. Such stress may further lead to oxidative stress and increase the free radical load that altered rhythmic function of mitochondrial bioenergetics, mitochondrial DNA and decreases the total ATP content, indicating that the mitochondrial functions are hampered by chronic artificial dim LL.

Although, the liver plays a central role in maintaining energy homeostasis while coping with large temporal variations of energy generation, storage, and utilization over the diurnal cycle, together feeding/fasting is a more potent zeitgeber for the liver clock than systemic cues controlled by the SCN (Damiola et al., 2000; Saini et al., 2013). Disturbances in the communication between the peripheral body clocks can desynchronize the circadian system, which is believed to contribute to the development of several diseases such as obesity, metabolic disorders (Albrecht, 2012) and non-fatty liver disease (Simões et al., 2018) in which mitochondrial dysfunctions are reported. Mitochondrial dysfunction usually results in excessive production of reactive oxygen species (ROS). Hence, one of the promising therapies is to reduce the production of ROS and to maintain the normal function of mitochondria. A widely use antioxidant agent, N-acetylcysteine (NAC) and melatonin is usually considered as a potent ROS scavenger in several reports (Rushworth & Megson, 2014; Korkmaz et al., 2009) and has been found to be effective in treating diseases such as ischemia/reperfusion injury (Chaves Cayuela et al., 2020) and lung cancer (Sayin et

al., 2014).

However, in clinical work, NAC is usually used as a mucolytic agent (Tenório et al., 2021) and prototypical antidote administered after an acetaminophen overdose, and now little attention has been paid to its antioxidant pharmacological property (Rushworth & Megson, 2014). Recently, several limited clinical trials indicated that NAC may be effective in attenuating hepatic dysfunction and ameliorating non-alcoholic fatty liver disease (Khoshbaten et al., 2010). However, the exact mechanism underlying its clinical effect so far is uncovered. Also, exposure to chronic dim light can lead to disruptions in metabolic energy homeostasis in rodents and humans (Fleury, et al., 2020; Karlsson, 2003; Obayashi et al., 2013). However, how ambient illumination impacts metabolism and energy balance is yet unknown, Therefore, we studied the effects of continuous dim light exposure on mice body weight, food intake and liver mitochondrial function. The role of NAC in regulating the peripheral clock is unexplored. Therefore, we proposed to test effect of NAC against dim light induced circadian rhythm disruption and mitochondrial dysfunction in the key peripheral clock organ, the liver.

4.1 Materials and methods

4.1.1 Animals

Swiss albino adult male mice (25-30g, eight week-old) were procured from the central animal house facility (Institute of Medical Sciences, Banaras Hindu University). They were housed in groups of six in polypropylene cages (41 × 28.2 × 15.3 cm). All the cages were placed in a light and climate-controlled environment (25 ± 1^0C, relative humidity 55±5%, and under 12:12 h light/dark (LD) cycle) for

seven days before the start of the experiment. Lighting schedules within the experimental chambers were controlled with the help of an electronic timer (Havells, India). Lights were switched on at 06:00 h Zeitgeber time (ZT0) and were switched off at 18:00 h (ZT12). Light intensity at the cage floor level was approximately 150 lux (Testo 540) during the day (L) and 0 lux during the night (D). The mice were fed a standard rodent diet (Pashu-Aahar, Varanasi, India). Entry to the experimental chamber for cleaning and providing animal food occurred at random hours, once in 10 days. Food and tap water are provided *ad libitum*. All the experimental protocols were conducted following the principles of laboratory animal care (The Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA], India) guidelines, as well as law approved by the Institutional Animal Ethical Committee (IAEC), Banaras Hindu University (IMS-BHU, No. Dean/2019/IAEC/1254).

4.1.2 Drugs

N-acetylcysteine (NAC) and melatonin were acquired from Sigma-Aldrich (St Louis, MO), USA. All drugs were solubilised in saline (NaCl 0.9%).

4.1.3 Experimental design

Study design figure 1 illustrates all the animals were divided into two set after acclimatization. One set of animals housed in a light control environment in a 12:12 hour light/dark (LD 12:12 h) chamber to entrain them for 21 days. A second set of animals were housed in dim light 24 hour (LL 24:00 h) condition for 21 days. Mice were treated with NAC 20, 40, 80 mg/kg and melatonin 1 mg/kg orally from day 14 to 21 everyday one hour after light switch off. On the day 22nd animals were sacrificed by cervical dislocation, blood was collected by trunk route; liver tissue was isolated

and bioenergetics was performed immediately. Blood was processed for plasma separation.

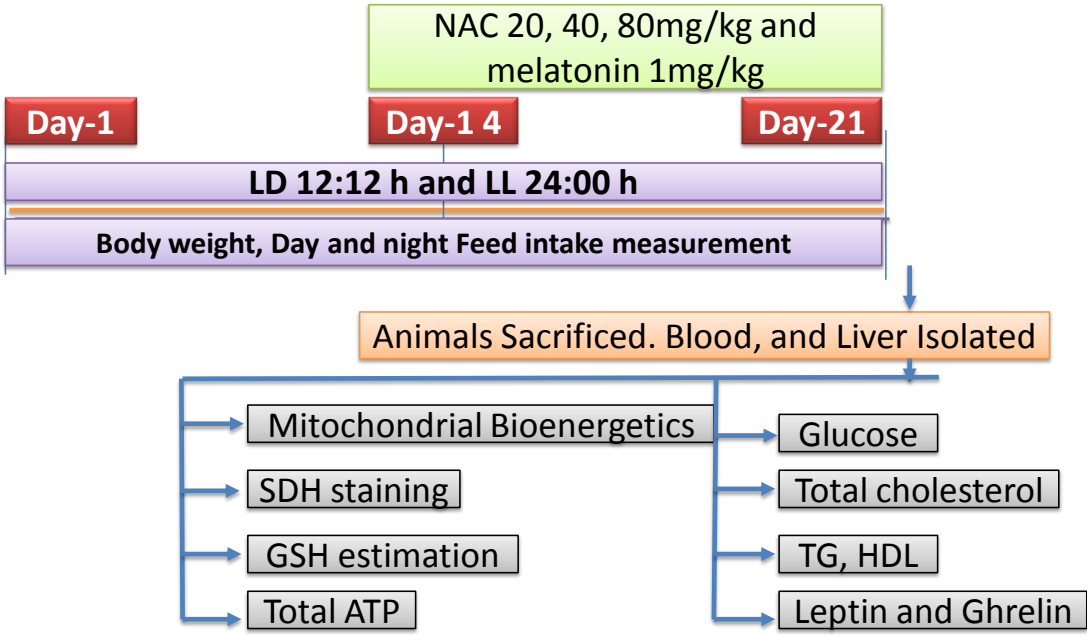


Figure 4.1 Study design

4.1.4 Determination of body weight and diurnal feed intake

At the beginning of the study all the mice were selected of same body weight i.e. 25±3 g. During the study period body weight was measured twice a week. Diurnal feed intake was calculated by determining the amount consumed during the light (06.00 am to 18.00 pm) or dark (18.00 pm to 06.00 am) phase of the light-dark cycle on a per cage basis. Feed was weighed Monday to Friday at 06.00 am and 17.45 pm. To determine what was consumed during the light phase, the amount of food that was weighed at 17.45 pm was subtracted from the food that was weighed at 06.00 am for the same day. To determine what was consumed during the dark phase, food that was weighed at the next day's 06.00 am time point was subtracted from the previous day's 17.45 pm measure. To correct for varying numbers of animals per cage, intake was divided by the number of animals in each cage and expressed as grams consumed per

mouse per cage.

4.1.5 Isolation of mitochondria from the liver

Isolation of mitochondria from the liver was done by differential centrifugal method (Berman & Hastings, 1999) with some slight modifications (Rajput & Krishnamurthy, 2022). Briefly, the right lobe of the liver was dissected from mice. 500 mg of liver tissue were homogenized with 20 strokes in isolation buffer (215 mM mannitol, 75 mM sucrose, 0.1 %w/v bovine serum albumin, 20 mM HEPES buffer and 1 mM of EGTA in 100 ml of distilled water and pH adjusted 7.2 with KOH) using a glass Teflon tissue homogenizer (Thomas Scientific; USA). The homogenate was centrifuged at 1,300 X g for 5 min at 4 °C. The supernatant was transferred to another tube and centrifuged at 14,000 X g for 10 min at 4 °C to pellet mitochondria. The supernatant was discarded, and mitochondria were washed by resuspending the pellets in an isolation buffer without EGTA and centrifuging at 14,000 X g for 10min. Mitochondria were resuspended in 1 ml of respiration buffer, and protein concentration was determined using the Lowry assay (Lowry et al., 1951) on a microplate reader (Biotek; Gen5, USA).

4.1.6 Measurement of mitochondrial respiration

Mitochondrial respiration was assessed as previously described by (Samaiya and Krishnamurthy 2015; Rajput and Krishnamurthy, 2022) with a miniature Clark-type electrode in a sealed, thermostatically controlled chamber at 37 ºC. Briefly, the mitochondria were added to the chamber and respiratory states were evaluated with suitable substrates and inhibitors. Purified mitochondrial protein was suspended in respiration buffer in a final volume of 250 µL. State II respiration was initiated by addition of pyruvate/malate; (P/M), represents a basal rate of respiration. State III

respiration was initiated by the addition of adenosine diphosphate; (ADP), the high level of oxygen utilization indicates that ADP is getting converted into ATP. State IV was measured by addition of oligomycin. State V was measured by addition of FCCP. This causing uncoupling of the ETC to ATP synthesis and represents the maximum rate of respiration. Rotenone was then added to shut down complex I mediated respiration. State V was determined by the addition of succinate. This is the maximum rate of respiration via complex II since FCCP is present in the system. The respiratory control rate (RCR) was calculated by dividing state III respiration (presence of ADP) to state IV respiration (absence of ADP), a rate limiting step of oxidative phosphorylation.

4.1.7 Staining of succinate dehydrogenase

OCT blocks of liver tissue were cryosectioned between -15 to -20 ºC on the Leica CM1860 Cryostat (Leica Microsystems Inc., Buffalo Grove, IL, USA) Histology Research Laboratory, and 5 µm tissue slices were placed on glass slides. Sections were incubated in a solution of 4 mg/ml nitro blue tetrazolium chloride, 0.2 M Tris, 0.05 M $MgCl_2$, and 0.83 M sodium succinate (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at 37 °C. The sections were then transferred to 15% formol saline containing 0.9% w/v NaCl and 15% w/v paraformaldehyde and incubated for 15 min at room temperature. Sections were washed in distilled water for 3 min, dehydrated in 93%, 95%, and 100% alcohol for 3 min each. The sections were rinsed in xylene, mounted with 2 drops of DPX mounting medium (Sigma Aldrich), and cover slipped.

Each SDH stained liver slide had four images captured on a Nikon Eclipse Ci with a camera attached (Nikon Inc., Melville, NY, USA; Evolution MP, Media

Cybernetics Inc.) at 40X. Image J was utilized to measure percent area of staining by converting each image to an 8-bit threshold that resulted in black (stained area) or white (absence of stain) images.

4.1.8 Measurement of GSH

GSH was fluorometrically measured using ELISA assay Kit (ab65322, Abcam). Briefly, all the materials equilibrated to room temperature and prepared reagents prior to use. All the standards, controls and samples were measured in triplicate. 100 µl of standardand 50 µl of samples were added to respective wells. Sample wells were made up with lysis buffer. After that 2 µl of GST reagent was added followed by 2 µl of MCB into each sample and standard wells and plate was shaken and incubated at 37°C for 1 hour. Fluorescence was measured in a fluorescence plate reader at Ex./Em. = 360±20 nm/460±20 nm.

4.1.9 Measurement of total ATP content

Total ATP content was measured using fluorometric ATP Assay Kit (ab83355, Abcam). Briefly, approximately 10 mg of tissue was washed in cold PBS, and then homogenized in 100 µl ice cold 2N perchloric acid (PCA) with a Dounce homogenizer (Thomas Scientific) using 10-15 passes. Samples were incubated on ice for 30-45 min, and then centrifuged at 13,000 g for 2 min at 4°C. Supernatant was transferred to a fresh tube, and volume was brought to 500 µl by adding ATP assay buffer.

PCA was precipitated by adding 100 µl of ice-cold 2M KOH and sample was vortexed. A 5 µL aliquot was used for testing using pH paper, and pH was adjusted to 6.5-8 by addition of 0.1 M KOH or PCA. Samples were centrifuged at 13,000 g for 15

min at 4°C and supernatant was collected and measured fluorometrically at Ex/Em = 535/587 nm (Spark 10M, and Plate: Thermo Fisher Scientific-Nunclon 96 Flat Black with transparent bottom, NUN96fb). To calculate the dilution factor introduced by the deproteinization step (DDF), following formula was applied:

$$DDF = Sample\ volume\ (PCA + Assay\ Buffer) + volumeKOH / initialsamplevolumeinPCA$$

Concentration of ATP (nmol/µL or µmol/mL or mM) in the test samples was calculated using the formula-ATP concentration = (BV× D) ×DDF

Where:

B = amount of ATP in the sample well calculated from standard curve (nmol or mM).

V = sample volume added in the sample wells (µL).

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up)

4.1.10 Measurement of leptin and ghrelin

Leptin and ghrelin were measured in plasma sample collected at ZT 15 in each group using ELISA kit (KLM0652) and (KLM1133) respectively according to the krishgen bioscience manufacturer guideline. Briefly 50 µl prepared standards were added to respective standard wells and 40 µl samples to respective sample wells. Then 10 µl biotinylated leptin antibodies were added to respective sample wells. After that 50 µl streptavidin: HRP conjugate was added to all wells and mixed well. Plate was covered with the plate sealer and incubated for 60 minutes at 37°C. Plate content was aspirated and washed the plate 4 times with diluted wash buffer (1X) and residual

buffer was removed by firmly tapping plate upside down on absorbent paper. The plate was wiped of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. Then 100 µl TMB Substrate was added to all wells and again incubated the plate at 37 °C for 10 minutes. After incubation 100 µl of stop solution was added to all wells. The wells were turned from blue to yellow in colour. Then absorbance read at 450 nm with a microplate within 10-15 minutes after addition of stop solution.

4.1.11 Estimation of plasma glucose

On 22nd day of the experimental protocol, 1 ml of blood was collected through retro-orbital puncture and centrifuged at 4000 ×g for 5 min at 4°C to obtain plasma for measuring the glucose. The plasma glucose was determined spectrophotometrically (BioTek Instruments Inc., Epoch, USA) in triplicate using commercially available kits.

4.1.12 Estimation of lipid profile

Plasma concentrations of total cholesterol, high-density lipoprotein (HDL), and triglyceride were enzymatically determined using standard validated commercial available kits method.

4.1.13 Statistical analyses

To determine significant changes in mitochondrial bioenergetics RCR, CORT, GSH, SDH, leptin, ghrelin, and total ATP, one-way analysis of variance (ANOVA) were performed. When the data were statistically significant ($p \leq 0.05$), a post-hoc Tukey test for multiple comparisons was performed to evaluate the statistical

difference between the different groups. Data analyses and figures were drawn by using Graph Pad Prism 5.0 (La Jolla, CA) and Microsoft Excel 2007 software. Data are reported as mean ± SD throughout the text. All experiments were performed as per existing institutional guidelines.

4.2 Results

4.2.1 NAC recovered the body weight elevated by LL exposure

The significant elevation in the body weight was observed in the LL exposed mice ($p < 0.001$) on 2nd week of the study when compared to the LD group. Treatment with NAC 20 and 40 in the LL exposed mice significantly reduced the elevated body weight ($p < 0.05$) on third week when compare to LL group but the NAC 80 has profound effect on the body weight ($p<0.0001$) on third week (Fig 4.2). Two way ANOVA reveals that the difference in treatment factor ($F_{5,144} = 11.75$, $p < 0.05$), time factor ($F_{2,144} = 42.54$, $p < 0.05$), and interaction between treatment and time ($F_{2, 144} = 2.034$, $p < 0.05$).

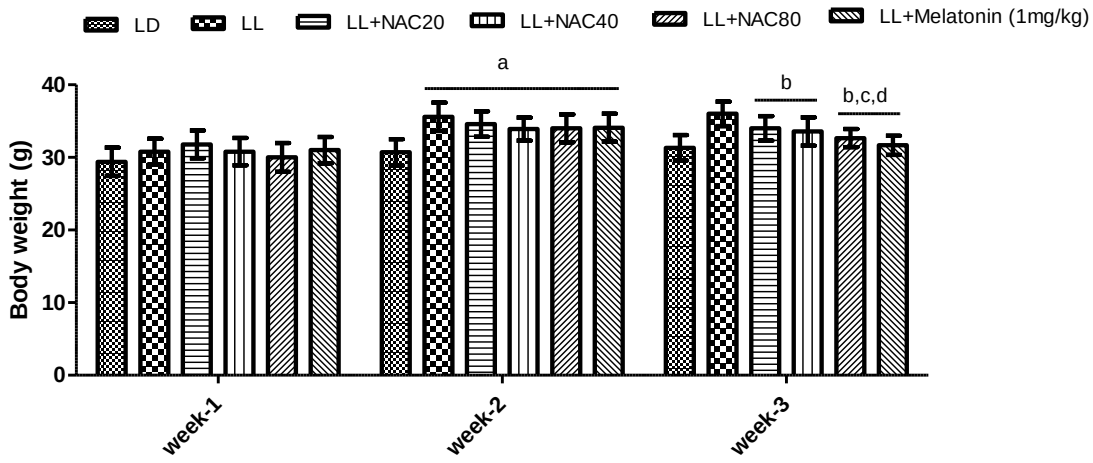


Figure 4.2. Body weight of mice. Bars represent groups mean ± SD (n=12). [a]$p<0.05$ compared to LD [b]$p<0.05$ compared to LL, [c]$p<0.05$ compared to LL+NAC20 and [d]$p<0.05$ compared to LL+NAC40. Two-way ANOVA followed by Bonferroni post hoc test.

4.2.2 NAC restored the altered diurnal feed intake in LL exposed mice

The LD group mice were consumed more food during the night and less during the day throughout the study ($p \geq 0.05$; Fig 4.3a). Whereas in LL group, the mice consumed more food during the night and less during the day on first week of the study but on week two, LL exposed mice shifted to consume more grams during the day and less during the night ($p \leq 0.01$; Fig 4.3b). Treatment with NAC at different doses showed reversal of food intake in LL mice in week three. Two way ANOVA reveals that the difference in treatment factor ($F\ 2, 99) = 1656$, $p < 0.05$), time factor ($F\ 2, 99) = 42.82$, $p < 0.05$), and interaction between treatment and time ($F\ 4, 99) = 482.8$, $p < 0.05$).

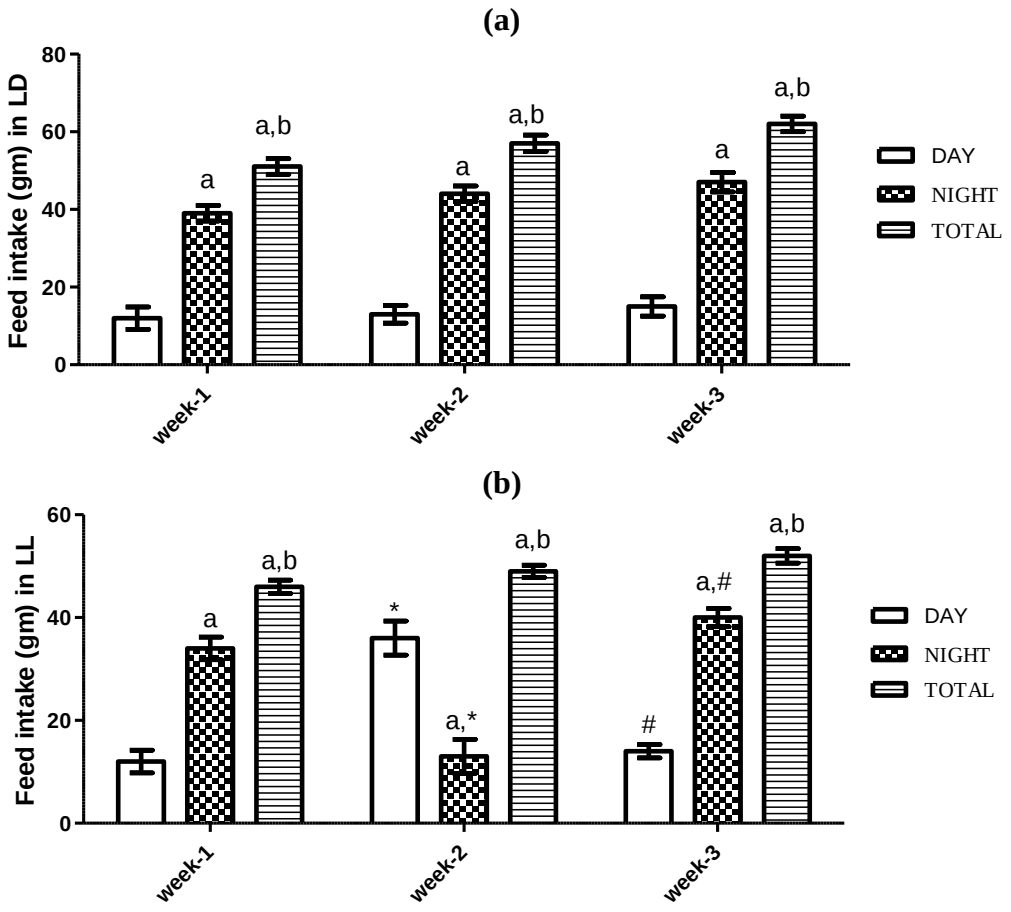


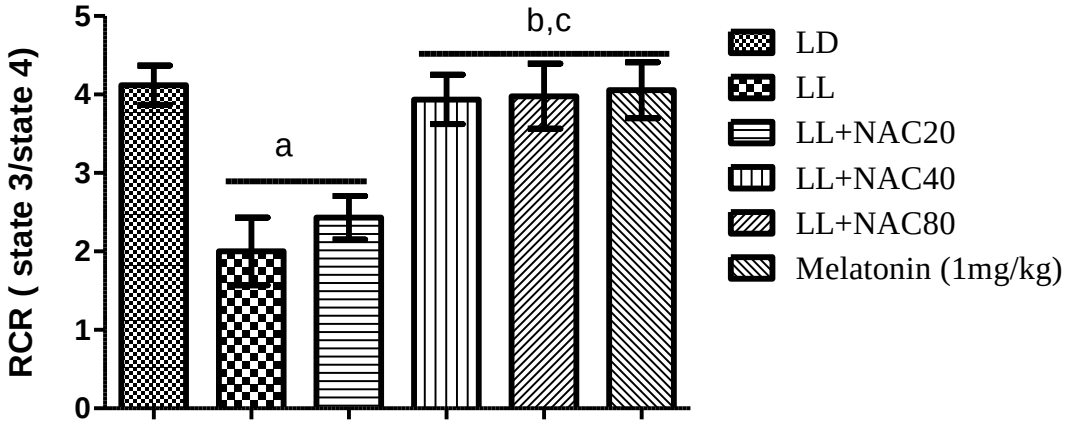
Figure 4.3. Feed intake of mice/week during day, night and total in LD (a) and LL (b) condition. Bars represent groups mean ± SD (n = 12). [a]$p<0.05$ compared to food consumed during day, [b]$p<0.05$ compared to food consumed during night, [*]$p<0.05$

compared to food consumed in week-1. #p<0.05 compared to food consumed in week-2 of respective groups. Two-way ANOVA followed by Bonferroni post hoc test.

4.2.3 NAC improved the mitochondrial RCR and states in the Liver

The effect of various doses of NAC on LL exposed mice, RCR (state 3/state 4 respirations) and changes in oxygen consumption in different states of mitochondrial respiration in the liver are shown in Figure 4.4 a and b, respectively. Mitochondrial bioenergetics was hampered in LL exposed mice. One-way ANOVA revealed that NAC 40 mg and 80 mg/kg was significantly improved RCR ($F_{5, 23} = 29.67$, $p < 0.05$, Fig 2a), and different states of mitochondrial respiration, namely, state 2 ($F_{5, 23} = 26.46$, $p < 0.05$), state 3 ($F_{5,23} = 45.76$, $p < 0.05$), state 4 ($F_{5, 23} = 38.68$, $p < 0.05$), state 5 complex I ($F_{5, 23} = 18.38$, $p < 0.05$), and state 5 complex II respiration ($F_{5, 23} = 15.61$, $p < 0.05$, Fig 5b), But 20 mg/ kg was not effective to restore the mitochondrial function.

(a)



(b)

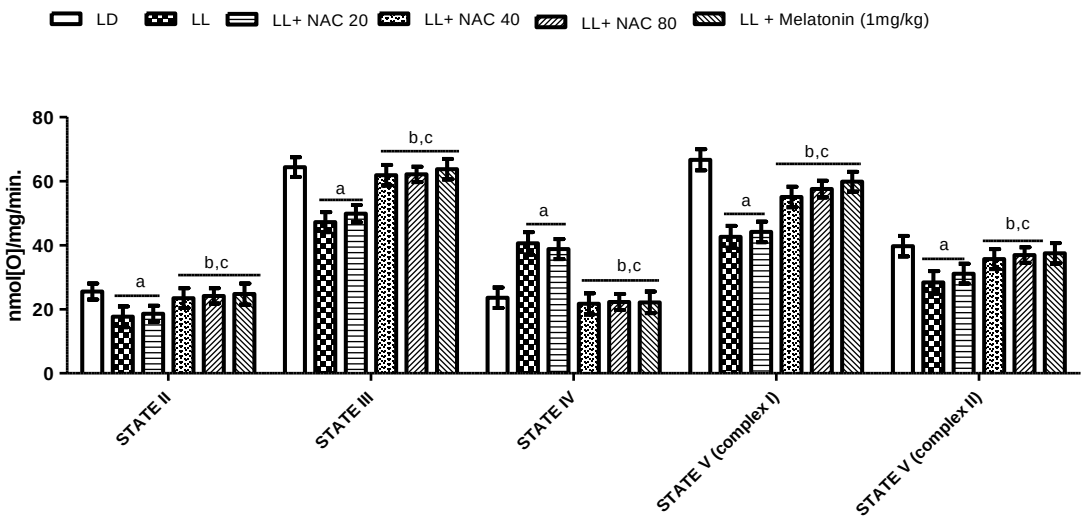
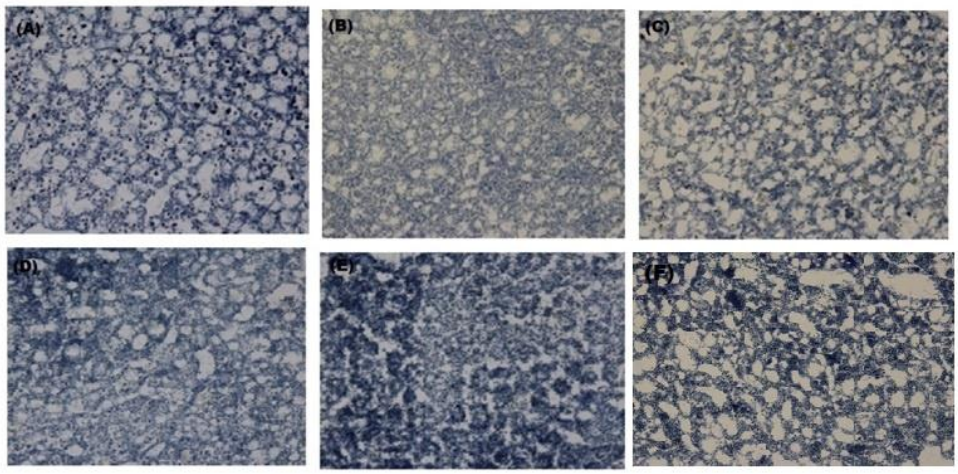


Figure 4.4. Respiratory control ratio (RCR) (a) and States (b) in liver mitochondria. Bars represent groups mean ± SD (n=4). $^{a}p \leq 0.05$ compared to LD, $^{b}p \leq 0.05$ compared to LL, $^{c}p<0.05$ compared to LL+NAC20. One-way ANOVA followed by Tukey post hoc test.

4.2.4 NAC improved succinate dehydrogenase (SDH) activity in liver

The percentage area of SDH represented in the Figure 4.5 a and b. One-way ANOVA revealed that there was significantly low SDH in the LL group when compared to LD group. Treatment significantly improved SDH dose dependently ($F_{5, 23} = 12.22$, $p \leq 0.05$, Fig 4.5) at all the three doses of NAC.

(i)



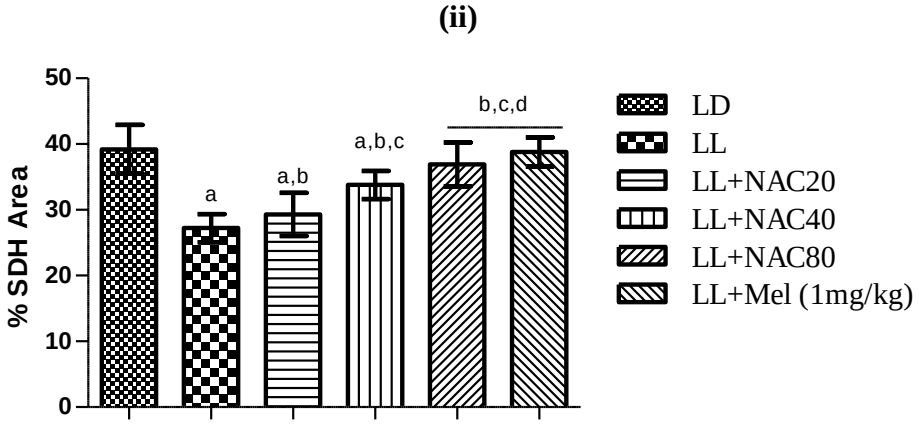


Figure 4.5 SDH (i) Staining images (A) LD, (B) LL, (C) LL+NAC20, (D) LL+NAC40, (E) LL+NAC80, (F) LL+ Melatonin 1mg/kg (ii) Bar graph in liver. Bars represent groups mean ± SD (n=4). [a]p<0.05 compared to LD, [b]p<0.05 compared to LL, [c]p<0.05 compared to LL+NAC20. One-way ANOVA followed by Tukey post hoc test.

4.2.5 NAC dose dependently improved the GSH in the liver

We measured the amount of GSH in the liver under LD, LL and NAC treatment regimen to determine the anti-oxidants level. One-way ANOVA showed significant decrease ($p \leq 0.05$) of GSH level in LL group as compare to LD group. NAC dose-dependently increases the GSH level ($F_{5, 23} = 173.5$, $p \leq 0.05$, Figure 4.6) in the entire treatment group.

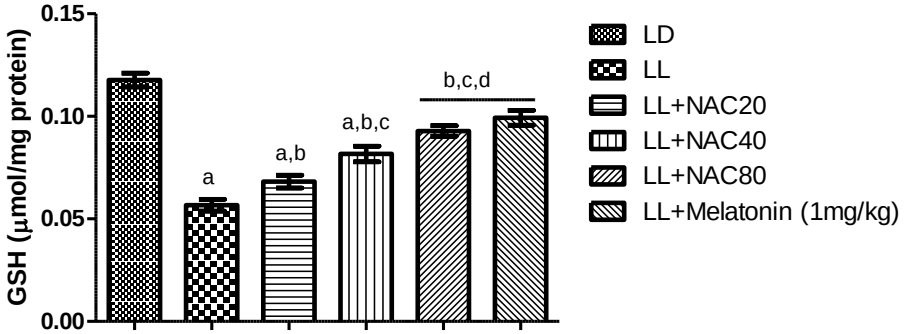


Figure 4.6. GSH level in the liver. Bars represent groups mean ± SD (n = 4). [a]p<0.05 compared to LD, [b]p<0.05 compared to LL, [c]p<0.05 compared to LL+NAC20 and [d]p<0.05 compared to LL+NAC40. One-way ANOVA followed by Tukey post hoc test.

4.2.6 NAC dose dependently improved ATP level in the liver

Total ATP was fluorimetrically measured in the liver homogenate. One-way ANOVA shows continuous light exposed mice were found to be compromised in their ATP level. Post-test revealed there were significant dose dependent improvement in ATP level (F 5, 23= 64.08, $p \leq 0.05$, Fig 4.7) with all the three doses of NAC.

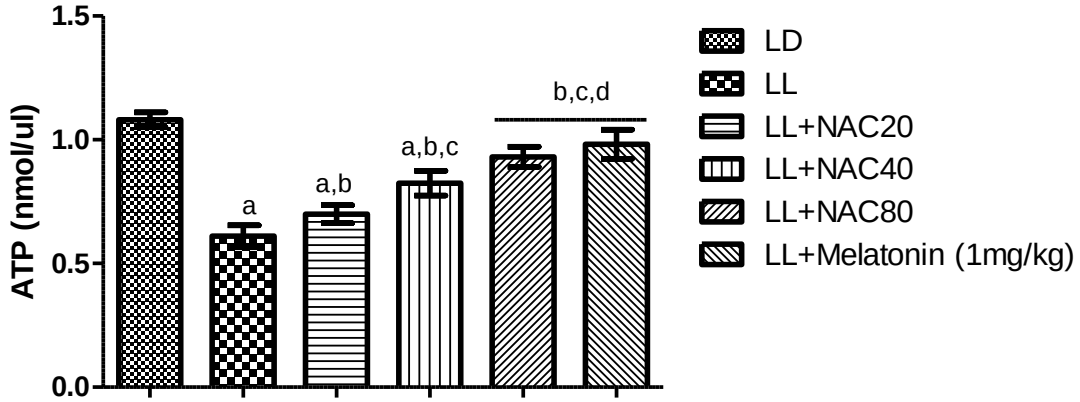


Figure 4.7. ATP level in the liver. Bars represent groups mean ± SD (n = 4). [a]p<0.05 compared to LD, [b]p<0.05 compared to LL, [c]p<0.05 compared to LL+NAC20 and [d]p<0.05 compared to LL+NAC40. One-way ANOVA followed by Tukey post hoc test

4.2.7 NAC decreases the blood glucose level, total cholesterol, triglyceride and increases HDL in LL exposed mice

Plasma concentrations of glucose, total cholesterol, triglyceride and HDL shown in (Figure 4.8 a,b,c,d). One way ANOVA showed there was significant increase in the blood glucose ($p \leq 001$), total cholesterol ($p \leq 01$), triglyceride ($p \leq 001$), and decrease the level of HDL ($p \leq 01$) in LL exposed mice. Post-test revealed that treatment with NAC at different doses significantly decreases the blood glucose ($F\ 5, 47 = 23.06$, $p \leq 0.05$), total cholesterol ($F\ 5, 47= 29.85$, $p \leq 0.05$), TGA ($F\ 5, 47= 5.356$, $p \leq 0.05$) and increases the HDL ($F\ 5, 47 = 11.65$, $p \leq 0.05$), compared to the

LL mice. 20 mg/kg was less effective ($p \leq 0.05$) in total cholesterol and not effective ($p \geq 0.05$) in TGA and dose dependent effect was not observed in these parameters.

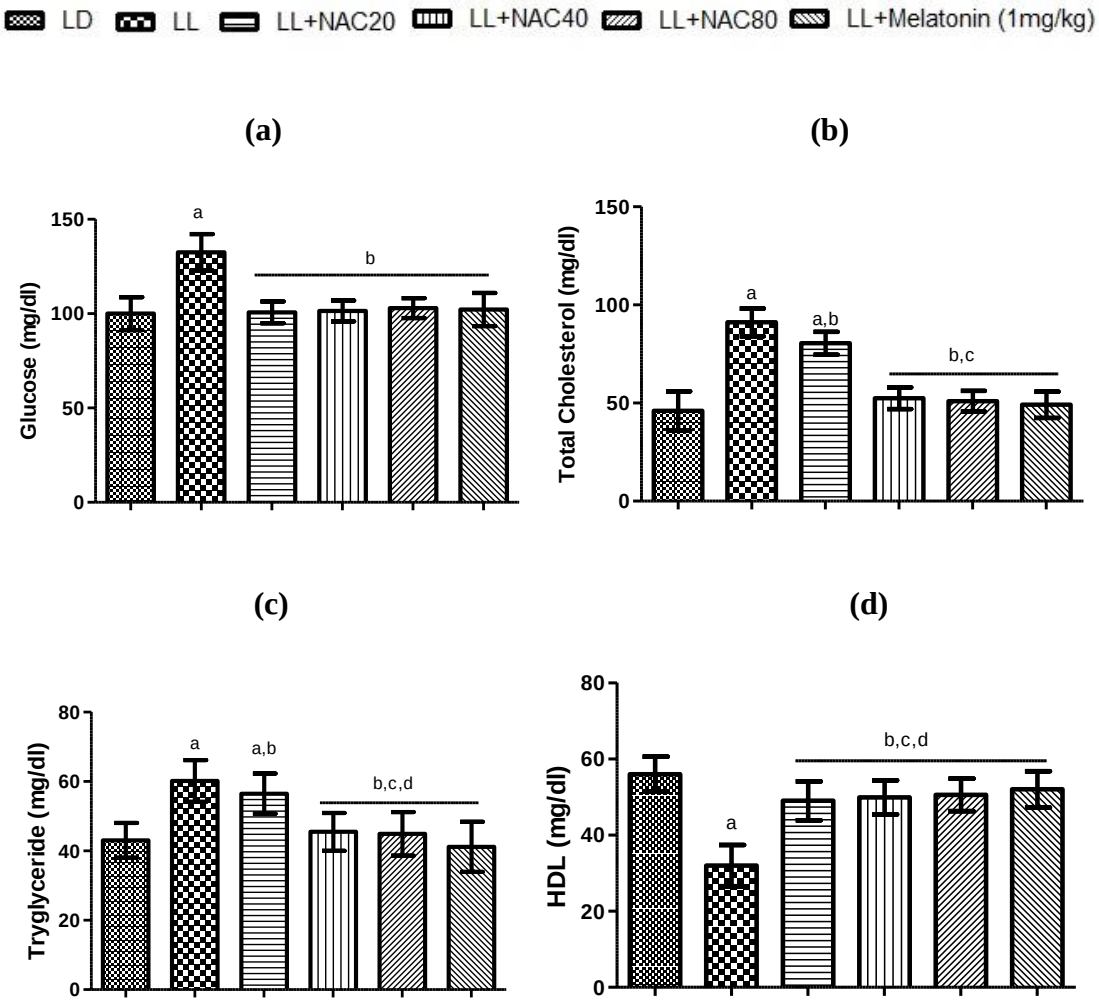


Figure 4.8. Blood plasma glucose level (a), triglyceride (b), total cholesterol (c), and HDL (d). Bars represent groups mean±SD (n=8). $^{a}p<0.05$ compared to LD, $^{b}p<0.05$ compared to LL, $^{c}p<0.05$ compared to LL+NAC20. One-way ANOVA followed by Tukey post hoc test.

4.2.8 Leptin and ghrelin

We measured the level of leptin and ghrelin hormone under LD, LL and NAC treatment regimen. One-way ANOVA showed significant dose dependent increases in leptin ($F_{5, 23} = 37.92$, $p \leq 0.05$, Figure 4.9 a) and significant dose dependent

reduction in ghrelin (F 5, 23 = 273.3, $p \leq 0.05$, Figure 4.9 b) with all the three doses of NAC.

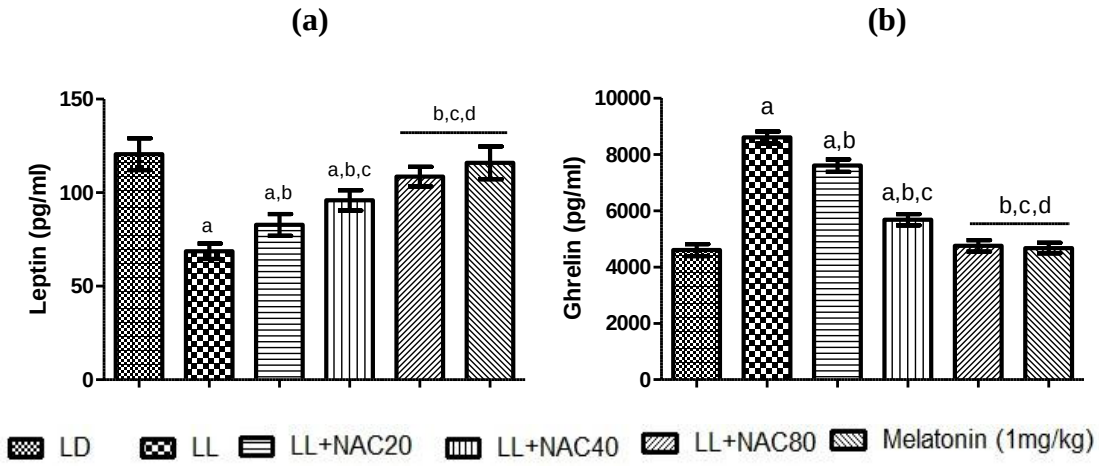


Figure 4.9. Plasma Leptin (a) and Ghrelin (b) level. Bars represent groups mean±SD (n=4). [a]p<0.05 compared to LD, [b]p<0.05 compared to LL, [c]p<0.05 compared to LL+ NAC20 and [d]p<0.05 compared to LL+ NAC40 One-way ANOVA followed by Tukey post hoc test.

4.3 Discussion

Here, we demonstrate the effect of chronic dim light on metabolism e.g., body weight, food intake and mitochondrial bioenergetics. Changes in eating behavior and weight gain are all caused by exposure to ecologically realistic amounts of low light. These findings suggest that continuous low light exposure, a frequent and seemingly harmless environmental modifications that can affect the metabolism and mitochondrial function and display quick and persistent increases in body mass in mice exposed to low light. Although, in this study total daily food intake was altered in mice exposed to chronic dim light. LL exposed mice consumed more food during the light period and less during the dark period which was opposite that of mice, that were housed in 12: 12 h LD condition. Such disorganization in the feeding rhythm may contribute to increased body weight (Arble, et al., 2009; Hatori et al., 2012;

Sherman et al., 2012). These findings are significant because they provide evidence for how mild changes in environmental lighting can alter circadian and metabolic function. Exposure to light at night is pervasive in modern society and typically considered a harmless environmental perturbation; however, our results demonstrate that chronic dim light exposure alters homeostatic functions in liver measured at ZT 15 where the mitochondrial respiration was maximum. Detailed analysis of temporal changes induced by untimed light exposure may provide insight into the onset and progression of obesity and metabolic syndrome and other disorders involving sleep and circadian disruption.

Further, chronic dim light exposure increased the blood glucose level and altered the lipid profile. Our results are in line of previous published research describing that increases in night time light exposure is associated with increased body mass, waist circumference and triglyceride levels, and poor cholesterol balance (Obayashi et al., 2013). Further, all these disturbances caused by dim light exposure are associated with high level of ROS and reduced glutathione level in the liver. We have observed in our previous chapter (chapter 3) that NAC at different doses restore the GSH level and other mitochondrial functions. Hence, we have tested NAC at different doses against altered blood glucose and lipid profile as well as mitochondrial bioenergetics and function in liver tissue. The protective effect of NAC related to its restoration of mitochondrial function may be because it has ability to directly scavenge free radicals (Ezeriņa., 2018). Also, NAC acts as a precursor of the principal thiol present in the cell, the tripeptide glutathione (GSH) and it has been demonstrated that GSH is very important in maintaining protein thiol groups, membrane integrity and ATP synthesis (Meister., 1995; Ballatori., 2009; Miquel et al., 1995). In our

present study, we have measured the mitochondrial bioenergetics, mitochondrial enzyme SDH, and GSH, total ATP and blood glucose as well as lipid profile that were altered after chronic dim LL exposure in mice, which were improved by different dose of NAC treatment. Our results are in line with a published report showing that NAC maintains hepatic GSH during ischemia in rat (Fukuzawa et al., 1995). It has also been shown that NAC is able to rescue mitochondrial GSH *in vivo* and restore important mitochondrial functions (Traber J. et al., 1992). These results support our findings that NAC treatment not only significantly restored mitochondrial GSH levels but also provided beneficial effects on mitochondrial function (RCR, ATP etc.). A second potential mechanism of the NAC effect is that it can directly scavenge free radicals. NAC has been shown to scavenge hydroxyl radicals and hypochlorous radicals (Aruoma et al., 1989). Reports show that it protects endothelial cells against oxidative stress either by directly scavenging free radicals or by elevating cellular GSH level (Cotgreave et al., 1991). Studies have shown that even brief exposure to altered lighting conditions and food schedules can result in adverse metabolic and cardiovascular consequences (Scheer et al., 2009; Morris et al., 2012). These statements are supported by our current data showing that mice chronically exposed to dimly illuminated room as opposed to being held in the dark had elevated body mass and changes in diurnal feed intake. This gets further support by another study in rodent, describing deleterious effects of artificially dim light on metabolism (Fonken et al., 2010). Mice exposed to dim light, shift the timing of food intake toward the light phase rather than dark phase leading to body mass gain. However, the mechanism by which light at night induces these changes is not fully understood.

In our previous study, we have found that chronic dim light alters the rhythmic

pattern of mitochondrial function and corticosterone which may have been as a result of increase in the free radical load in the SCN due to chronic light exposure. We to used one of the most widely known antioxidant and glutathione precursors, N-acetylcysteine to scavenge the free radical which was found to improved mitochondrial function as well as CORT, melatonin and the clock gene level in the SCN. Similarly, NAC in liver ameliorates the adverse effects of chronic dim LL on the peripheral clocks in the liver. Liver, on other hand is directly and indirectly is regulated by the principal clock (SCN). Any disturbances to the SCN by untimed light exposure may indirectly affect the metabolism (hunger hormone leptin, and ghrelin), physiology (sleep-wake cycle and fasting-feeding cycle) as well as behavior (locomotor activity rhythm) of organisms. Our notions are in agreements with the idea that the energy production, utilization, storage and homeostasis are associated with the main body organ liver which is directly regulated by the central circadian clock (SCN) (Barclay., et al 2012).

4.4. Summary

In conclusion, chronic dim light exposure influenced the liver mitochondrial function; decreased the mitochondrial succinate dehydrogenase and GSH level, as well as other metabolic parameters for example blood glucose, lipid profile, and leptin, ghrelin hormone. NAC at different doses improved the GSH, total ATP and leptin, ghrelin level but 20 mg/kg was not able to restore mitochondrial bioenergetics. NAC improved glucose and lipid profile in similar manner at all the three doses. On body weight and SDH, NAC 20 and 40 mg have the same effect while 80 mg/kg reduced more when compare to other treatment group.