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Melatonin Alleviates Biochemical, Hormonal and Histoarchitectural Alterations in a PCOS Rat Model Exposed to Constant Light by Enhancing Antioxidative Enzymes

Adyasha Purohit[1], Younis Ahmad Hajam[2] and Seema Rai[1*]

[1]Department of Zoology, Guru Ghasidas Vishwavidayalaya, Bilaspur – 495001, Chhattisgarh, India; drseemakamlesh@gmail.com

[2]Department of Life Sciences and Allied Health Sciences, Sant Baba Bhag Singh University, Jalandhar - 144030, Punjab, India

Abstract

The study evaluated the impact of pineal hormone (melatonin) on reproductive impairment induced by constant light/photoperiodic stress in female *Rattus norvegicus*. The experimental design consisted of 24 female rats weighing 170 ± 10 gm. The 24 rats were divided into two major groups comprising 12 rats each. Rats in one group were kept under constant light (600 lux) for 60 days to induce PCOS. After confirmation of PCOS by regular observation of vaginal smear, rats in each group were further divided into 2 groups containing 6 rats in each. 12 rats with no treatment were divided as control (0.9% saline) and melatonin (200µg/100gm bw). Rats exposed to the constant light were divided into LL (PCOS) and PCOS+MEL with six rats in each group. After 21 days, the rats were sacrificed, and their ovaries and oviducts were harvested. Blood samples were collected for corticosterone and melatonin, androgen and estradiol analysis, and harvested tissues were stored at -80^{0}c for further assessment. The results showed that polycystic ovary rats exhibited increased body weight and anti-oxidative enzyme levels. However, the polycystic ovary rats, that were treated with melatonin showed recovery with melatonin, corticosterone, body and tissue weight, and biochemical parameters. The study suggests that continuous light exposure can induce reproductive disorder by disrupting GnRH rhythmicity, but melatonin treatment restores reproductive homeostasis. The study provides insights into polycystic ovary syndrome pathogenesis and the potential therapeutic benefits of melatonin.

Keywords: Melatonin, Photoperiodism, Polycystic Ovary Syndrome, Oxidative Stress

1. Introduction

Photoperiodism is essential for physiological and developmental processes, as it affects hormonal regulation. The length of the light/dark phase influences mammalian reproduction, which relies on endogenous rhythm[1]. Exposure to constant short- or long-day photoperiods can cause photoperiodic stress, which is correlated to the intensity of light per unit area. In mammals, the pineal gland converts photoperiodic information into a melatonin secretory cycle via the SCN, resulting in high levels of melatonin at night and low levels during the day[2]. Melatonin acts in certain regions of the hypothalamus, probably via melatonin receptors, and its fate is determined by the length of the day and night[3]. Exposure to constant light or darkness can cause photoperiodic stress, which can interfere with photoperiodism and affect melatonin secretion timing and circulation levels. Due to the stress caused by an unbalanced photoperiod, melatonin, which also controls gonadotropin secretion, may lose its functional ability[4].

The pathophysiology of several illnesses revolves around stress, and photoperiodic disruption can

*Author for correspondence

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act as a stressor. In today's lifestyle, photoperiodic disruption is unavoidable, and factors such as night shift employment and increased screen time during the night often affect adults and enhance the rate of photoperiodic stress. The disruption of photoperiodism can make people more vulnerable to endocrine and metabolic conditions linked to diabetes and obesity[5]. Both circadian and metabolic imbalances can affect reproductive activity, and these two disturbances frequently occur simultaneously[6]. Ultimately, these disturbances can lead to hyperandrogenism and polycystic ovary syndrome[7].

Polycystic condition of the ovary is a complex reproductive impairment that deteriorates the fertility of females[8]. The diagnosis of PCOS has been historically challenging due to the absence of a clear cause or pathophysiological mechanism that could aid in the understanding of the Rotterdam criteria or NIH criterion, and the wide range of clinical symptoms associated with the condition[9,10]. Further, it is evidenced that PCOS patients often experience a combination of metabolic disorders that affect the total physiology of females[11]. The pathogenesis of PCOS may be influenced by genetic and developmental factors, as well as environmental factors, which generate reactive oxygen species, insulin resistance, low-grade inflammation, and dysregulation of the hypothalamus-pituitary-ovary axis[12]. Considering its ability to scavenge free radicals and influence gonadal hormone secretion, melatonin holds great promise as a means of combating the negative effects of constant light exposure[13]. Studies underscore the importance of melatonin as a valuable substance for protecting against oxidative stress and promoting overall health. Further, there is scanty research evidence reported on the photoperiodic effect on the reproductive cycle, circulatory level of hormones, and oxidative stress in non-seasonal breeders. We have tried to elucidate the involvement of melatonin in the disrupted sleep-wake cycle and reproductive health of women. The impact of competition in various domains of life as well as the fascination of the human being towards modern lifestyle (gaming, night shift, increased screen timing due to smartphones and laptops) cannot be ignored. It may consequently reduce sleeping hours causing various reproductive impairments such as PCOS. However, melatonin is a questioned molecule but a well-established strong free radical scavenger/ antioxidant. Therefore, we hypothesize that using melatonin may be a novel intervention for such reproductive disorders.

2. Material and Method

2.1 Chemical

Melatonin (Mel), Sodium citrate Thiobarbituric acid (TBA), Phenazine methosulfate (PMS), Nicotine Adenine Dinucleotide Phosphate (NADPH), Tris-hydrochloride acid (Tris-HCL), Glacial acetic acid, Glutathione reduced (GSH), Phosphoric acid, Nitroblue tetrazolium salt (NBT), Dithio-bis 2-nitrobenzoic acid (DTNB) were purchased from Himedia and Sigma.

2.2 Animal Maintenance

Female Wistar albino rats were procured with a range of 170±10 body weight from the Defense Research and Development Establishment (DRDE), Gwalior. Before experiments, the rats were given two weeks to acclimate. During this time, they were housed in standard humidity and temperature conditions and had access to specific feed pellets and water. All the experimental procedures were authorized by care guidelines established by the Institutional Animal Committee (IAEC) at Guru Ghasidas Vishwavidyalaya, India, with the usage of laboratory animals being approved under protocol number 994/G0/Re/S/06/CPCSEA.

2.2.1 Induction of Polycystic Ovary Syndrome

The experimental groups in this study were subjected to constant photoperiod conditions, achieved by exposing the rats to fluorescent light at 600 lux for 60 days. In contrast, control groups were kept in an environment of 12 hours of light and 12 hours of dark phases. Vaginal smears were collected from both groups at specific intervals (i.e., 30 days, 45 days, and the last 10 consecutive days before the end of constant photoperiodic exposure) to monitor the constant photoperiodic effect on the reproductive cycles of female rats.

Table 1. Experimental design

S.N	Group Name	Doses and condition	No
1.	Control (CON)	0.9 % alc. saline	6
2.	Constant Light-induced PCOS (LL(PCOS))	600 lux for 60 days	6
3.	LL (PCOS)+Melatonin (LL(PCOS)+MEL)	LL (600 lux) +200µg/100gm body wt.	6
4.	Melatonin (MEL)	200µg/100gm body wt.	6

After a 60-day constant light exposure period, body weights were recorded for both control and experimental groups. The animals were euthanized using anaesthetic ether at 6:30 pm under exposure to artificial red light. Blood samples were collected to analyze androgen, estradiol, melatonin, and corticosterone levels, with serum being separated by centrifugation at 3000 rpm for 15 minutes in a Remi, India (MAC-C-24BL) centrifuge. The weight of ovaries and uterine tubes measured and documented for comparative analysis between the groups. Biochemical parameters were measured to assess the level of oxidative stress in the ovaries and uterine ducts of both the control and experimental groups. All these procedures were performed following established scientific protocols and ethical guidelines to ensure both the safety and welfare of the animals involved and the scientific integrity of the study.

2.2.2 Preparation of Melatonin and Dose

The exogenous melatonin (200µg/100gm bw) was administered (0.5 ml) to the rats for 21 days with successful induction of PCOS. To execute this protocol the average weight of the rat was calculated. Based on average weight, 300µl of methanol was used to solubilize the melatonin, and the volume was made up using 0.9% saline.

2.3 Parameter Analyzed

2.3.1 Examination of Estrous Cycle

Disruption in regularities of the estrous cycle is the main marker of PCOS. To examine this, a vaginal smear was obtained from each group of rats for 10 days before the completion of the experiment. On the 60th day before sacrifice, a vaginal smear was collected. 70% alcohol was used to fix the smear on the slide and then stained in 0.1% crystal violet followed by DPX mounting. The presence or absence of leukocytes, nucleated epithelial cells, and cornified cells was under a fluorescence microscope (Axioscope-5, Carl-Zeiss, Germany) and captured for further quantitative study between the groups[14].

2.3.2 Morphological and Gravimetric Analysis

The Morphological appearance of ovaries and uterine tubes was observed to note any changes in ovary and uterine morphology. The weight of ovaries, uterine tubes, and body weight were also recorded.

2.3.3 Histological Study

Histology was performed after the removal of ovaries and oviduct and fixed in Bouin's solution. After 10 hours the tissues were kept in running water and cleared in 70% alcohol. Samples were embedded in paraffin wax and sectioning at a thicknesses of 6-7 um was performed. All sections were processed for staining with hematoxylin and eosin. The dehydration process was completed gradual change of alcohol followed by and clearance in xylene. Sections were observed and captured under a fluorescence microscope (Axioscope-5, Carl-Zeiss, Germany)[15].

2.3.4 Biochemical Assay

The level of oxidative stress and effect of melatonin was documented by performing several biochemical assays such as Lipid peroxidation (LPO), Catalase (CAT), Reduced glutathione (GSH) and the enzymes responsible for its oxidation and reduction, Glucose 6-phosphate dehydrogenase (G6PDH), Glutathione reductase (GR), Glutathione peroxidase (GPX), Glutathione-s-transferase (GST), Superoxide dismutase (SOD), Catalase (CAT), and total protein content.

2.3.5 Lipid Peroxidation (LPO)/ Thiobarbituric Acid-Reactive Substances (TBARS) Assay

The photoperiodic oxidative stress on reproductive tissues was analyzed using this parameter. The ovary tissue was weighed and homogenized in 10% Tris Hydrochloride buffer with a pH of 7.5. The homogenates were centrifuged at 3000 xg for 15min at 4°C, to determine the TBARS activity. To conduct the assay, the supernatant was combined with SDS, acetic acid, and TBA in a required ratio and kept in a hot water bath for 1 hour at 95°C. The reaction mixture was rapidly shaken with a pyridine reagent (15:1) and n-butanol. It was cooled under running water and rotated at 1500 xg for 10 minutes. The absorbance was read in a spectrophotometer at 534 nm. The standard curve was calibrated using 10nm of TEP as a reference and the LPO was expressed as TBARS nmol/g tissue weight[16].

2.3.6 Glutathione Reduced (GSH) Assay

Glutathione-reduced (GSH) content was determined following the Sedlak and Lindsay method, with some modifications. Ovary and uterine tissues were

homogenized in Tris buffer supplemented with sucrose (10X). To the reaction mixture, 0.01M of DNTB was further mixed, and subsequently, the volume was maintained by the addition of 10 ml of methanol. The entire reaction was incubated at room temperature for 30 minutes, followed by centrifugation at 3000 rpm for 15 minutes. The formation of a yellow-coloured chromogenic complex indicates the presence of GSH content, and the absorbance was documented at 420 nm[17].

2.3.7 Catalase (CAT) Assay

The homogenates were taken in a test tube and phosphate buffer was added to it. The solution was kept at 25°C for half an hour. Hydrogen peroxide was added to the mixture to initiate the reaction and absorbance was read at 240 nm after 3 minutes[18].

2.3.8 Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was measured using the protocol developed by Kakkar *et al*., (1983). A solution was prepared that contained sodium pyrophosphate (pH 7.0, 0.052 Mm), NBT (300 M), and Phenazine methosulfate (185 µl). NADH was added to the reaction to start and incubated at 30°C. Glacial acetic acid was added to halt the reaction. 4 ml of n-butanol was then added to the mixture, mixed, and allowed to stand for 10 minutes. The value of absorbance at 560 nm was determined following the solution's rotation to extract the butanol layer containing the chromogen[19].

2.3.9 GSH Cycle Indices

2.3.9.1 Glucose 6-Phosphate Dehydrogenase (G6PDH)

The anti-oxidative enzyme G6PDH was analyzed using the protocol given by Ellis and Kirkman (1961) with some modifications. In this experiment, a total reaction mixture was prepared which was composed of NADP+, Tris buffer, phenazine methosulphate, magnesium chloride, dye solution and homogenate. The process of oxidation of chemicals was allowed after the incubation of the reaction mixture for 10 minutes at 37°C. Glucose 6-phosphate was added to initiate the reaction. A blank solution was prepared using all the chemicals and distilled water instead of tissues. The optical density was obtained in one minute for 3.5 minutes at a wavelength of 640 nm. The molar extinction coefficient of reduced enzyme activity and the temperature correction factor were denoted as a change in OD/min. It was multiplied by the factor 6/17.6 to analyze the fate of the enzyme. The enzymatic assay of tissue was recorded as mIU/mg of protein. This protocol was carefully executed to ensure scientific accuracy and correctness[20].

2.3.9.2 Glutathione Peroxidase (GPx) Activity

The effect of oxidative stress on the level of Glutathione peroxidase was observed by following the modified method of Pagalia and Valentine (1967). The supernatant was obtained from tissue extract, and a reaction mixture was prepared by adding EDTA, GSH, H_2O_2 and phosphate buffer, 0.2 ml of EDTA, 0.1 ml of 2.5 mM H_2O_2, 0.2 ml of GSH, and 0.4 ml of 0.4 M phosphate buffer. The solution was kept at room temperature for 10 minutes and then the addition of 10% TCA halted the reaction. The supernatant was extracted after centrifugation of this mixture followed by the inclusion of disodium hydrogen phosphate and DTNB. Optical density was obtained at 420 nm. The activity of GPX was denoted as nano-moles of glutathione oxidized per minute per milligram of protein (µmol of GSH/min/g protein). The calculated results were noted as Unit/min/mg protein[21].

2.3.9.3 Glutathione Reductase (GR)

The fate of Glutathione reductase was observed using the protocol given by Goldberg and Spooner (1983), with few modifications. As per the experimental protocol, a reaction mixture was prepared with the addition of NADPH, phosphate buffer, and oxidized glutathione, to a test tube containing 0.2 ml of tissue homogenate. Absorbance was measured at the wavelength of 340 nm, with a decrease in optical density (OD) measured against the blank at 340 nm. The enzymatic activity was measured in terms of µm/min/L = U/ml of NADPH oxidized/min/mg protein. The results were noted as unit/min/mg protein[22].

2.3.9.4 Glutathone S-Transferase (GST)

Glutathione S-transferase (GST) activity was evaluated by following the method of Habig *et al*., (1974) with slight modifications. A reaction mixture of 2.9 ml was prepared using 0.1 ml of CDNB, and 0.1 ml of GSH and phosphate buffer. The reaction was initiated with the addition of 100 µl homogenate and the optical density was obtained in the wavelength of 340 nanometers. As it was an enzymatic parameter the result was noted down in 15 seconds for three minutes using a spectrophotometer (Perkin Elmer). A blank solution was prepared as distilled water instead

of tissue homogenates to examine the nonspecific binding of the substrates. Changes in absorbance at 340 nm were measured. The results were recorded in a scientifically correct manner[23].

2.3.10 Total Protein Content in Tissues

The total protein content was determined using a modified method developed by Lowry *et al.* (1951). To begin, 0.2 ml of homogenate was placed into test tubes, to which 0.2 ml of 10% TCA was added to precipitate the proteins. Five different concentrations of BSA were prepared ranging from 0.2 ml to 1 ml. Reagent C was freshly prepared in a 50:1 ratio of reagents A and B and supplemented to the homogenates including the BSA followed by 10 minutes of incubation. Follin's reagent was added to the mixture and kept in the dark for half an hour. The optical density of all the samples of the experimental group was noted in an absorbance of 625 nm and the outcome was stated as mg of protein per 100 mg of sample[24].

2.3.11 Hormonal Assay

The blood was collected from the heart at an anaesthetic condition, using a 2ml syringe followed by centrifugation at 3000 rpm for 15 minutes. The serum was pipetted out from the tube. Serum corticosterone, melatonin, and estradiol levels in all experimental groups were determined by following the instructions provided in the commercial kit. CSB – E05112r, 12-MNTL-96-U, 55-ESTRT-E01, 11-ANRHU-E01respectively.

2.3.12 Statistical Analysis

The results were expressed as mean ± SE. Data were analyzed using ANOVA in SPSS (version 26.0), followed by post-hoc analysis. Statistical significance was set at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

3. Results

3.1 Effect of Constant Light (LL) and Melatonin Treatment on Estrous Cycle

The vaginal smear of the control group showed evidence of all four phases with leukocyte cells, cornified cells, and nucleated epithelial cells. These findings indicated that the estrous cycle in this group was normal. However, examination of a smear of the last 10 consecutive days in the PCOS group, before the end of constant photoperiodic exposure of the experimental group, showed complete disruption of the estrous cycle. This disruption was determined by the presence of only leukocytes in the vaginal smear under the microscope, indicating the seizing of the diestrus phase (Figure 1a). The smear before the sacrifice of PCOS rats was observed to be at the diestrus phase. Treatment with melatonin re-established a normal reproductive cycle, where only the melatonin-treated group showed no changes in the estrous cycle.

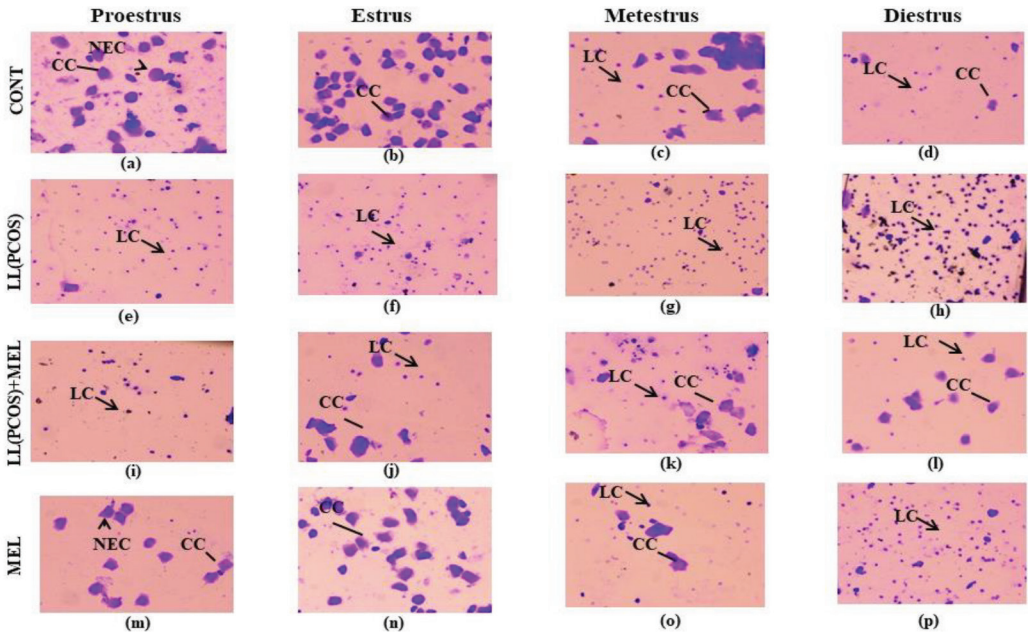
A quantitative analysis was made by counting the different marker cells of the estrous cycle of all stages. It was observed that the distribution of cornified, nucleated epithelial, and leukocyte cells across all four phases of the estrous cycle was similar in experimental groups (CON, LL(PCOS), and LL(PCOS)+MEL). However, the group that was exposed to constant light (LL) showed an irregular distribution of these biomarker cells. However, as shown in Figure 1(b), the distribution of these biomarker cells was restored in the rats given melatonin treatment (LL(PCOS)+MEL). These findings imply that, in rats exposed to continuous light, melatonin treatment may impact the regulation of the estrous cycle.

3.2 Effect of Constant Light Exposure (LL) and Melatonin Treatment on Morphology of Ovary and Uterine Duct

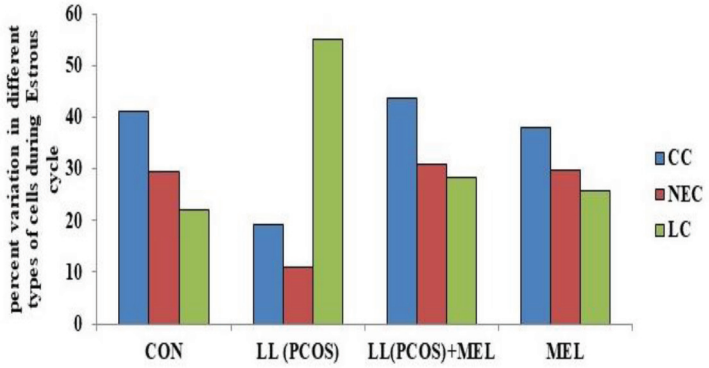
Morphological observations in both the control and melatonin-treated groups did not reveal any significant changes. In contrast, the group of rats with exposure to constant light for 60 consecutive days exhibited swelling in the ovaries and shrinkage in the length and width of the oviducts. Nevertheless, as shown in Figure 2, treating these rats with melatonin treatment restored the structural impairments. Based on these results, melatonin may be inferred to shield reproductive tissues from the damaging effects of prolonged exposure to light.

3.3 Effect of Constant Light Exposure (LL) and Melatonin Treatment on Body, Ovary and Uterine Duct Weight

An interesting result was found in the weight analysis due to the obesity and tissue-specific oxidative stress. An increment in the body weight was found in the rats



(a)



(b)

Figure 1. **(a)** The microscopic observation (10X10) of the vaginal smear depicts the effect of constant light and melatonin in all experimental groups, the proestrus phase (a, e, i, m), estrus phase (b, f, j, n), metestrus phase (c, g, k, o), and diestrus phase (d, h, i, p). In the LL (PCOS) group, all four phases (e, f, g, h) showed the presence of only Leukocytes (LC) confirming the disruption of the reproductive cycle. The effect of melatonin was determined by the presence of Cornified cells (CC), Nucleated epithelial cells (NEC), and Leukocytes (LC). In the LL(PCOS)+MEL group, (i, j, k, l) all the cells neutralized towards the control group. The only melatonin-treated groups (m, n, o, p) represented a healthy estrus cycle similar to the control group (a, b, c, d). CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin. **(b)** Effect of constant light (LL) and melatonin (MEL) on the estrous cycle of a female rat model. Histogram represents N = 6; CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+Melatonin; MEL = Melatonin, CC = Cornified cell; NEC = Nucleated epithelial cell; LC = Leukocyte.

with PCOS as compared to the control whereas tissue weight was noted to be decreased. A trend of recovery was found after the administration of exogenous melatonin as seen in Figures 3 a,b. There were no notable differences between the control and the rats treated only with melatonin. These results simply that melatonin therapy might be advantageous for PCOS-affected rat's reproductive systems (Figures 3a,b).

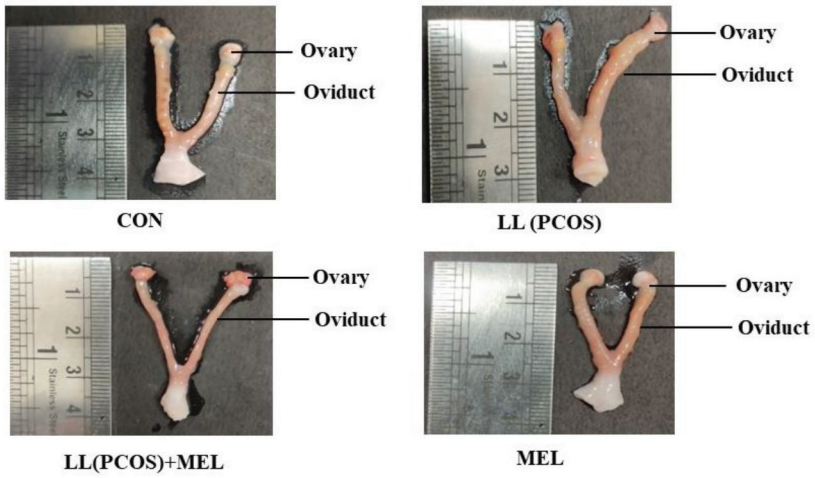


Figure 2. Effect of constant light and melatonin treatment on the morphology of ovary and oviduct showing swollen ovary and decreased uterine length in PCOS group. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin.

3.4 Effect of Constant Light (LL) and Melatonin Treatment in the Histology of Ovary Tissue

The rat having 12L: 12D showed follicles surrounded by theca cells and granulosa cells in the ovary. The morphometric observation showed the presence of more than 2-3 cysts in ovary sections of the PCOS group followed by constant light exposure for 60 days. The treatment of melatonin helped in rebuilding histological architecture by regeneration of follicular cells. The group treated with only melatonin showed no alteration in the histological architecture of the ovary.

3.5 Effect of Constant Light (LL) and Melatonin Treatment in Different Biochemical Parameters

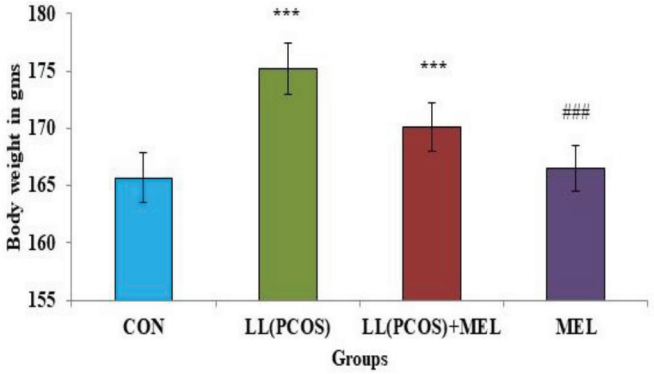
In response to photoperiodic stress in the ovary-uterine system, PCOS rats exhibited a significant increase in the value of TBARS (Figure 5(a)) concomitant with a decrement of reduced glutathione (GSH) content (Figure 5(b)) and an activity of anti-oxidative enzymes (SOD, CAT) (Figures 5(c, d)). This decrease also corresponded with reduced protein content. However, exogenous melatonin administration lowered the TBARS value with an increment in GSH content, catalase, and superoxide dismutase in the ovary-uterine system of the PCOS group. The treatment of melatonin recovered the total cellular protein content (Figure 5(e)) which was decreased in the reproductive tissues of the PCOS group. Interestingly, melatonin alone did not show any negative effects on the activities of antioxidative enzymes. After receiving melatonin treatment, PCOS rats showed an increase in all biochemical parameters related to the ovary-uterine system in contrast to the control group. These findings imply that melatonin, probably because of its anti-oxidative qualities, may have a curative effect on PCOS caused by oxidative stress.

3.6 Effect of Constant Light (LL) and Melatonin Treatment in G-Cycle

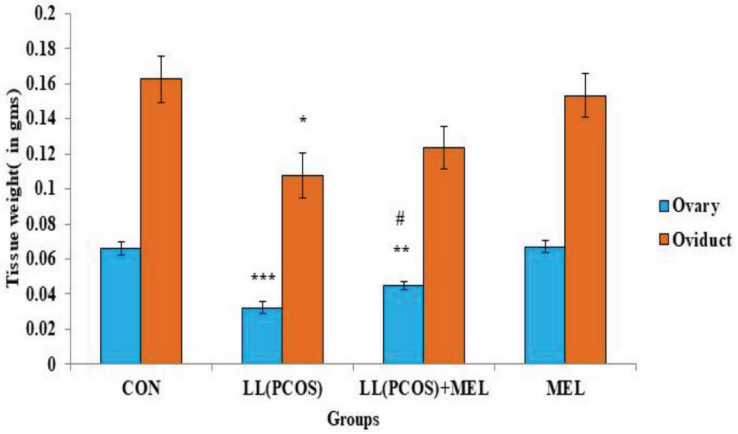
The enzymes responsible for oxidation and reduction of glutathione (Glutathione-s-transferase, Glutathione peroxidase, Glucose-6-phosphate dehydrogenase, Glutathione reductase, Glutathione reductase) were found to be lower in the reproductive tissues of PCOS rats as compared to control model (Figures 6(a,b,c,d)). Exogenous melatonin was recorded as a beneficial agent as the level of all the anti-oxidative enzymes regulating the estrous cycle was found to be recovered. This finding also synchronized with the level of reduced glutathione in all experimental groups of rats. Melatonin alone did not show any negative effects on the activities of antioxidative enzymes suggesting the nontoxic effect of this hormone.

3.7 Effect of Constant Light (LL) and Melatonin Treatment on Serum Androgen Level

The study showed an elevation of serum androgen level in LL (PCOS) during the diestrus phase of the estrous



(a)



(b)

Figure 3. **(a)** Effect of light and melatonin (MEL) on the body weight of constant light-induced Polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6; ***P≤0.001: CON vs LL (PCOS); CON vs LL+MEL $^{###}$P≤0.001: LL (PCOS) vs LL+ MEL; CON= Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin. **(b)** Effect of light and melatonin (MEL) on the ovary and uterine duct weight of constant light-induced Polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean± SE; N = 6; ***P≤0.001: CON vs LL (PCOS); **P≤0.01: CON vs LL+MEL; $^{\#}$P≤ 0.05: LL (PCOS) vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin.

cycle, compared to the control. LL (PCOS) group treated with exogenous melatonin neutralized the circulatory level of androgen towards the control group. The group of animals administered only with melatonin showed a very less significant difference than the control group.

3.8 Effect of Constant Light (LL) and Melatonin Treatment on Serum Estradiol Level

The study showed a decrease in the serum estradiol level in the LL (PCOS) group during the diestrus phase of the estrous cycle, compared to the control. LL (PCOS) group treated with exogenous melatonin neutralized the circulatory level of the reproductive hormone towards the control group. The group of animals administered only with melatonin showed a less significant difference than the control group.

3.9 Effect of Constant Light (LL) and Melatonin Treatment on Serum Corticosterone Level

The study showed elevated serum corticosterone levels in the LL (PCOS) group compared to the control. LL (PCOS) group treated with exogenous melatonin neutralized the

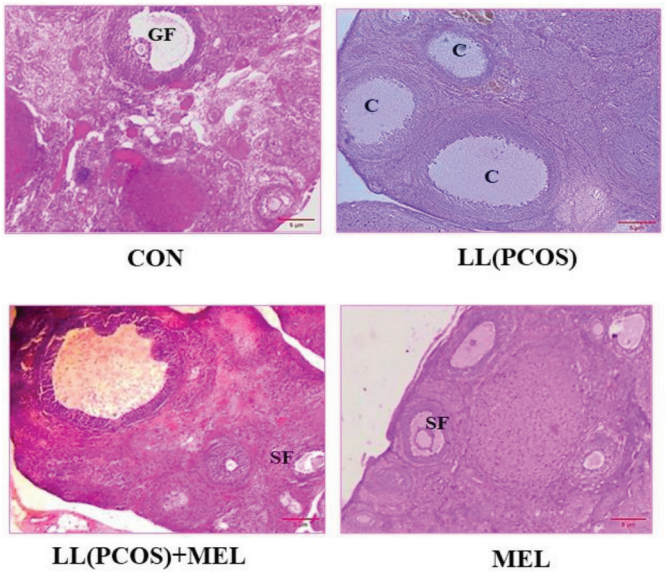


Figure 4. Effect of light and melatonin in Histo-architecture of ovary tissue (5 µm;10X10). Control ovary showing normal growth of the ovary follicle. Constant light-induced histo-micrograph appeared with more than two cysts, as well and there was the absence of normal follicle. Histo-architecture of ovary tissue of light-induced PCOS rats who received melatonin that appeared with many developing follicles including secondary follicles. Melatonin injection shows similarities with the control group. Graafian Follicle (GF), Cyst (C), Secondary follicle (SF). CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin.

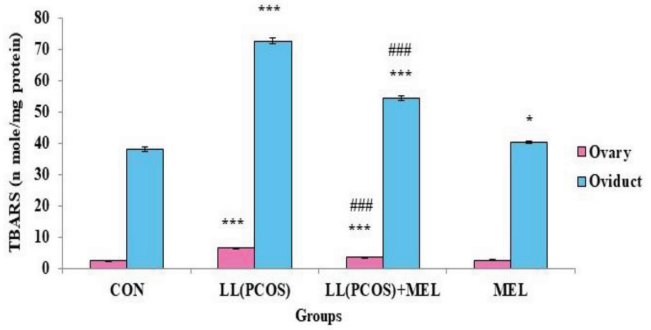
circulatory level of corticosterone towards the control group. The group of animals administered only with melatonin showed a very less significant difference compared to the control group.

3.10 Effect of Constant Light (LL) and Melatonin Treatment on Serum Melatonin Level

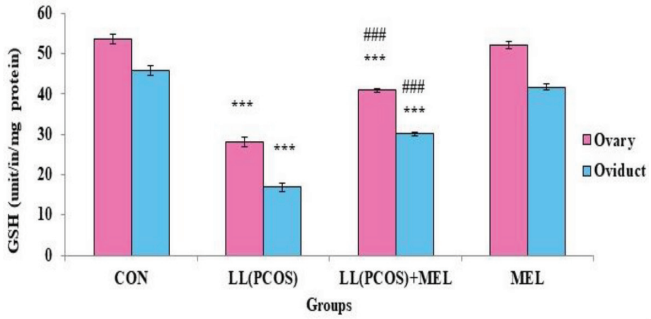
The study found that the serum melatonin of the PCOS group was significantly lower than that of the control group. However, the LL group treated with exogenous melatonin showed a trend toward recovery in melatonin levels. The circulatory level of melatonin in the group treated with only melatonin was found to be higher than the control group with a less significant difference. These findings suggest that pineal gland function and melatonin levels may play a role in the pathophysiology of PCOS under constant light exposure, and that exogenous melatonin may have a potential therapeutic effect in this context.

4. Discussion

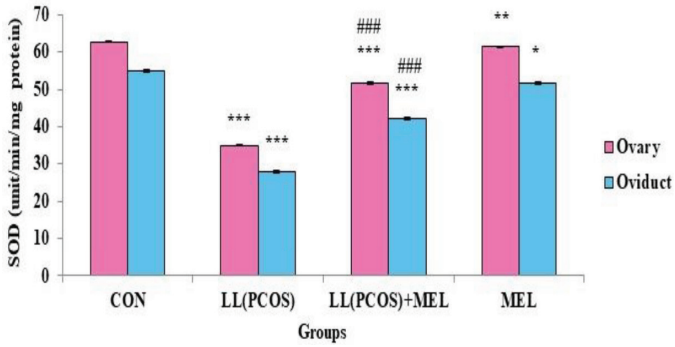
The treatment for the polycystic ovary condition is difficult due to its complicated etiology of pathophysiology with polymorphic clinical symptoms. The susceptibility to artificial light (600 lux) for 60 days was found to be the reason behind reduced endogenous melatonin and anovulatory condition. During the last 10 days of light exposure, all vaginal smears of rats were observed with very low concentrations of nucleated epithelial cells. On the 60th day of the experiment or the 10th day of the smear examination, a vast number of leukocytes was also noted which indicated the seized diestrus phase. The hormonal analysis of PCOS rats suggested that during the diestrus phase, there was an elevation in the androgen level presenting a hyperandrogenism condition. During this phase, the circulatory level of estrogen was found to be abated. The PCOS model treated with melatonin showed a recovery in the disrupted estrous cycle. Increased level of estradiol was found in the serum analysis as the rats were in the metestrus phase of the reproductive cycle. Restoration of the androgen level was evidenced in the metestrus phase of LL(PCOS)+MEL. The group treated with 0.9% saline showed no alteration in the reproductive cycle. As it was under normal rhythmic exposure all four phases of the healthy reproductive cycle were evaluated microscopically. All the assumption was evidenced by a quantitative study of the vaginal smear of all the phases with the condition of hyperandrogenism. It indicates how


TBARS (n mole/mg protein)
80
70
60
50
40
30
20
10
0
***
***
###
***
###
***
*
Ovary
Oviduct
CON
LL(PCOS)
LL(PCOS)+MEL
MEL
Groups

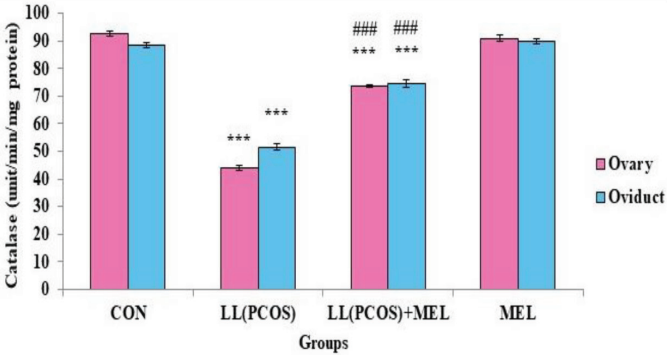
(a)


GSH (unit/in/mg protein)
60
50
40
30
20
10
0
***
***
###
***
###
***
Ovary
Oviduct
CON
LL(PCOS)
LL(PCOS)+MEL
MEL
Groups

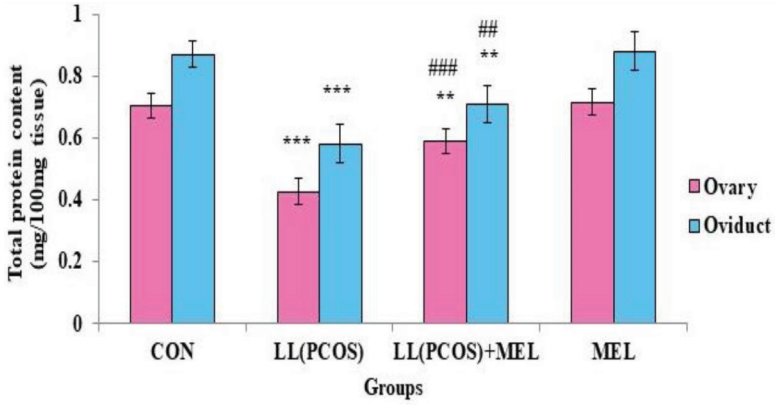
(b)


SOD (unit/min/mg protein)
70
60
50
40
30
20
10
0
***
***
###
***
###
***
**
*
Ovary
Oviduct
CON
LL(PCOS)
LL(PCOS)+MEL
MEL
Groups

(c)


Catalase (unit/min/mg protein)
100
90
80
70
60
50
40
30
20
10
0
***
***
###
***
###
***
Ovary
Oviduct
CON
LL(PCOS)
LL(PCOS)+MEL
MEL
Groups

(d)



(e)

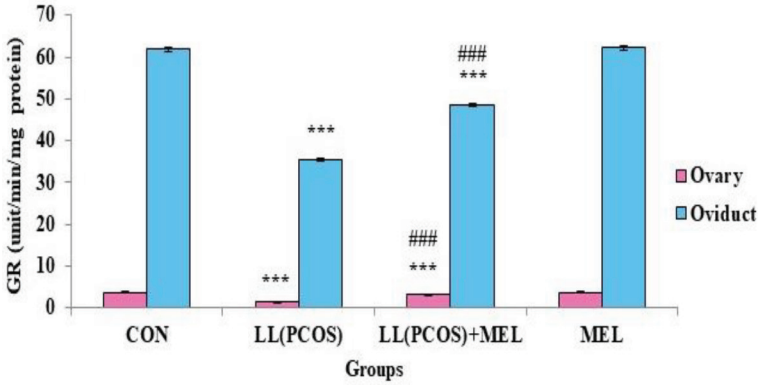
Figure 5. **(a)** Effect of light and melatonin (MEL) on TBARS value in ovary and oviduct of constant light-induced Polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6; $^{*/\#}$P≤0.05. $^{**/\#\#}$P≤0.01, $^{***/\#\#\#}$P≤0.001; * represents CON vs LL (PCOS); CON vs LL+MEL; CON vs MEL: $^{\#}$ represents LL (PCOS) vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS + Melatonin; MEL= Melatonin. **(b)** Effect of light and melatonin (MEL) on reduced glutathione (GSH) in ovary and oviduct of constant light-induced Polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean± SE; N = 6; $^{*/\#}$P≤0.05,$^{**/\#\#}$P≤0.01, $^{***/\#\#\#}$P≤0.001; *represents CON vs LL (PCOS); CON vs LL+MEL; CON vs MEL: $^{\#}$ represent LL vs LL+MEL.CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin. **(c)** Effect of light and melatonin (MEL) on Superoxide dismutase (SOD) in ovary and oviduct of constant light-induced Polycystic ovary syndrome (PCOS) rat model.Histogram represents Mean ± SE; N = 6; $^{*/\#}$P≤0.05, $^{**/\#\#}$P≤0.01, $^{***/\#\#\#}$P≤0.001; * represents CON vs LL(PCOS); CON vs LL+MEL; CON vs MEL: $^{\#}$ represents LL (PCOS) vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin. **(d)** Effect of light and melatonin (MEL) on Catalase (CAT) in ovary and oviduct of constant light-induced Pol cystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6; $^{*/\#}$P≤0.05, $^{**/\#\#}$P≤0.01, $^{***/\#\#\#}$P≤0.001;* represents CON vs LL (PCOS); CON vs LL+MEL; CON vs MEL: $^{\#}$represents LL (PCOS) vs LL+MEL.CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin. **(e)** Effect of light and melatonin (MEL) on total protein content in ovary and oviduct of constant light-induced Polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N=6;$^{*/\#}$P≤0.05, $^{**/\#\#}$P≤0.01, $^{***/\#\#\#}$P≤0.001; *represents CON vs LL (PCOS); CON vs LL+MEL; CON vs MEL:$^{\#}$ represents LL (PCOS) vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin.

photoperiodism influences endogenous rhythm and is associated with all physiological activity.

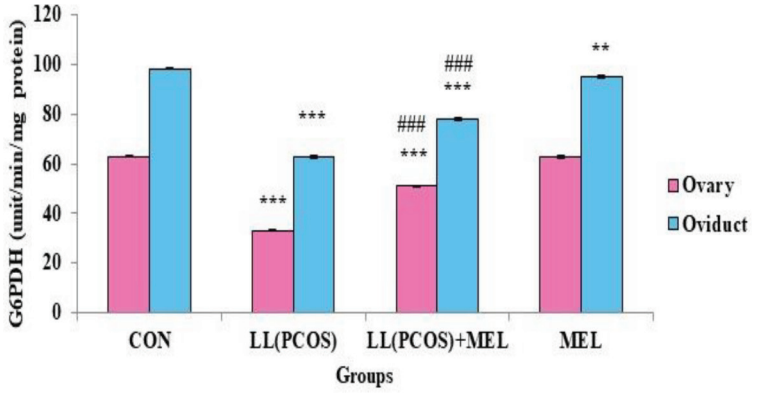
The management of hormonal disruption is a successful strategy for treating the ovary cycle which was observed in melatonin treated group[25]. Melatonin treatment for PCOS has shown its mechanism of action. The length of the sleeping period was completely reduced as rats were exposed to light for 24 hours which also hampered their feeding pattern. An interesting result was observed as the body weight of PCOS rats was found to increase due to high androgen levels which caused muscular growth. A change in behavior and feeding pattern was observed in PCOS which was confirmed with the serum corticosterone level. The LL group having higher corticosterone levels depicts the possibilities of Alteration of CRH, ACTH, and ultimately the circulatory level of corticosterone. It was also discovered that a physiological pinealectomy hampered the rhythmic secretion of endogenous melatonin. The altered circulatory level of this pineal hormone affects the secretion of the gonadotropic hormone (estradiol) regulated by the HPO axis. It may lead to the deteriorated secretion of reproductive hormones[26]. This histo-architecture of ovary tissue suggests that the estradiol level disruption may execute the irregular reproductive cycle in LL (PCOS). The Formation of more than two cysts may be due to the altered androgen and estradiol levels. The recovery trend of corticosterone, estradiol, androgen and endogenous melatonin hormone in the LL(PCOS)+MEL group indicates that there might be interference with melatonin which acts on the HPA and HPO axis. The

hyperandrogenism condition was found restored after administration of melatonin and hence balances of estradiol level also. The recovery of all the hormones was observed as exogenous melatonin administration creates an environmental condition of a regular sleep-wake cycle. The sleeping length of the LL(PCOS)+MEL group was found normalized towards the control group. The group treated only with melatonin had very less significant difference with the control group suggesting that there is no harmful effect of exogenous melatonin.

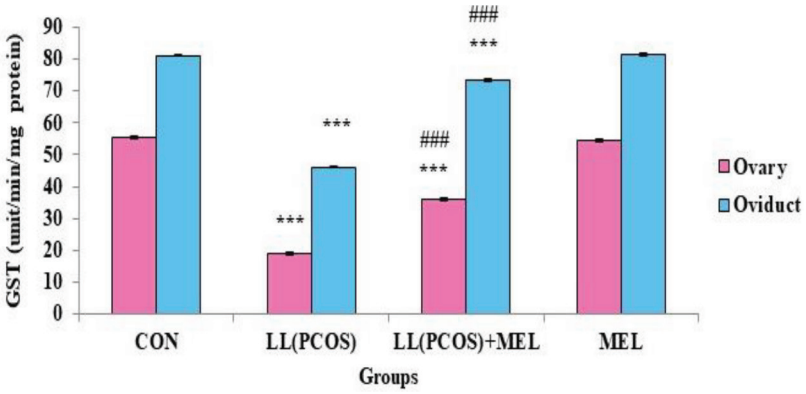
The assessment of free radicals was measured by Lipid peroxidation assay. The Breakdown product of ROS was notably high in the experimental model treated with light. It was evidenced that hypersynthesis of free



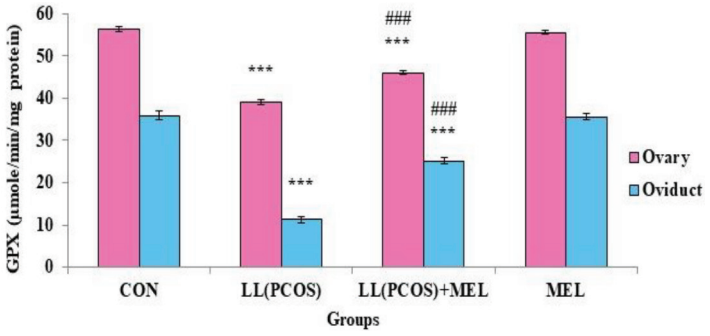
(a)



(b)



(c)



(d)

Figure 6. **(a)** Effect of light and melatonin (MEL) on Glutathione reductase (GR) in ovary and oviduct of constant light-induced polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6; */#P≤0.05, **/##P≤0.01, ***/###P≤0.001; * represents CON vs LL(PCOS); CON vs LL+MEL; CON vs MEL: # represents LL(PCOS) vs LL+MEL CON= Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant-light induced PCOS+ Melatonin; MEL = Melatonin. **(b)** Effect of light and melatonin (MEL) on Glucose-6-phosphate dehydrogenase (G6PDH) in ovary and oviduct of constant light-induced polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6; */#P≤0.05, **/##P≤0.01, ***/###P≤0.001; * represents CON vs LL(PCOS); CON vs LL+MEL; CON vs MEL: # represents LL(PCOS) vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin. **(c)** Effect of light and melatonin (MEL) on Glutathione-s-transferase (GST) in ovary and oviduct of constant light-induced polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6; */#P≤0.05, **/##P≤0.01, ***/###P≤0.001; * represents CON vs LL(PCOS); CON vs LL+MEL; CON vs MEL: # represents LL(PCOS) vs LL+MEL. CON= Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin. **(d)** Effect of light and melatonin (MEL) on Glutathione peroxidase (GPX) in ovary and oviduct of constant light-induced polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean±SE; N = 6; */#P≤0.05, **/##P≤0.01, ***/###P≤0.001; * represents CON vs LL(PCOS); CON vs LL+MEL; CON vs MEL: # represents LL(PCOS) vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin.

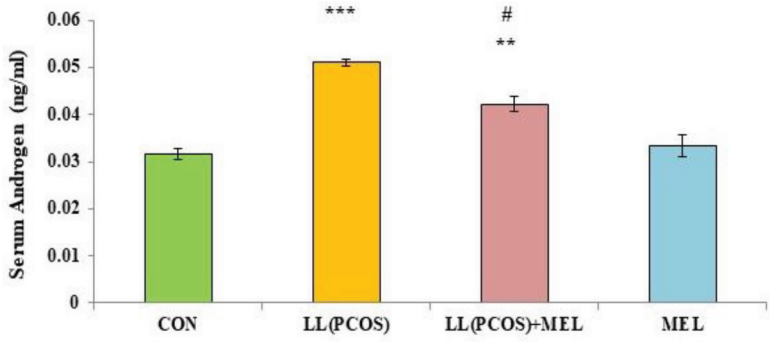


Figure 7. Effect of light and exogenous melatonin (MEL) in androgen level of blood serum of constant light-induced Polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6; **/##P≤0.01, ***/###P≤0.001; * represents CON vs LL (PCOS); CON vs LL+MEL; CON vs MEL: # represents LL (PCOS) vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS + Melatonin; MEL = Melatonin.

radicals and excessive oxidative stress levels can lead to physiological impairment in tissue-specific ways. The anti-oxidative enzyme present in mammals (superoxide dismutase, catalase, reduced glutathione) was affected as the plasma membrane and structural arrangement of cells was ruptured. A downregulation in GPX, GR, G-6-PDH, and GST levels was documented which is the explanation for the reduction in the ratio of reduced and oxidized glutathione[27-29]. Overloaded free radical generation due to photoperiodic oxidative stress may be the best

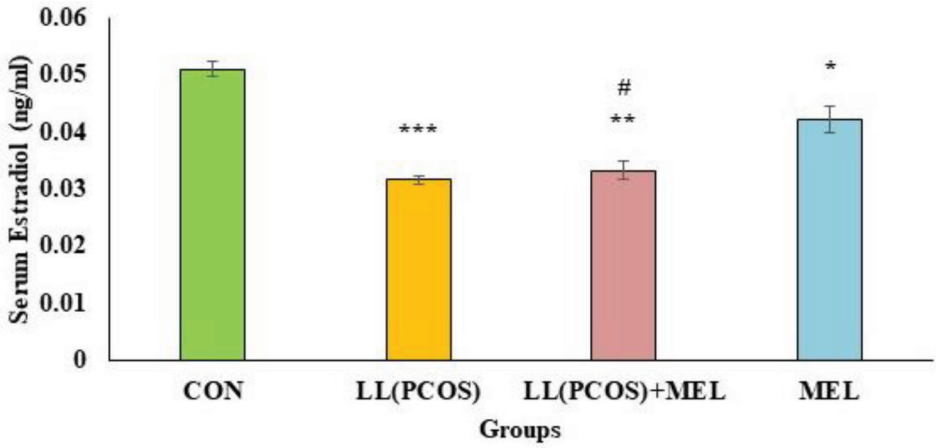


Figure 8. Effect of light and exogenous melatonin (MEL) in androgen level of blood serum of constant light-induced Polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6; **/##P≤0.01, ***/###P≤0.001; * represents CON vs LL (PCOS); CON vs LL+MEL; CON vs MEL: # represents LL (PCOS) vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin.

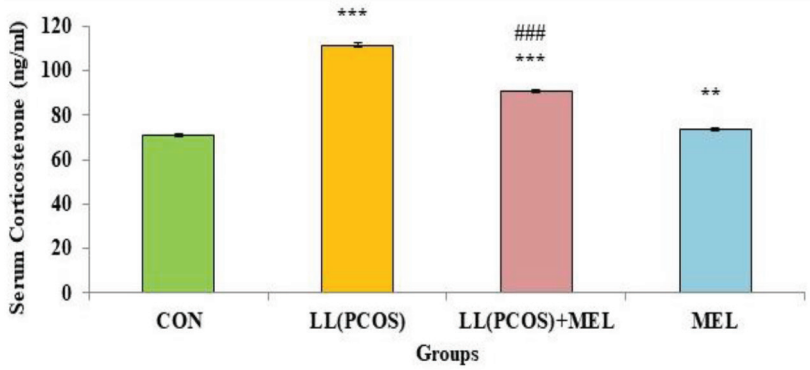


Figure 9. Effect of light and exogenous melatonin (MEL) in corticosterone of blood serum of constant light-induced Polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6; **/##P≤0.01, ***/###P≤0.001; * represents CON vs LL (PCOS); CON vs LL+MEL; CON vs MEL: # represents LL (PCOS) vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS + Melatonin; MEL = Melatonin.

explanation for the impairment of these endogenous anti-oxidant defence systems. It might prevent the ovary cells from carrying out reproductive processes like follicle maturation and ovulation[30].

The level of free radicals was noted remarkably high in the light-induced PCOS group which was seen to be recovered after treatment with melatonin. It suggests the presence of anti-oxidant properties in this neuro-hormone which targets the damaged cells and scavenges the free radical. The already existing free radical scavenger (superoxide dismutase, catalase, reduced glutathione) helps repel oxidative stress up to a mark[31]. But when the free radical exceeds the limit, the level of anti-oxidative enzyme starts deteriorating and that is the case of the PCOS group. An increase in free radical load might have damaged the tissue. The ultimate result could be shrinkage of tissue and a decrement in ovary weight. A reduction in the level of free radicals was noted after the administration of melatonin, as it is crucial for PCOS patients and may cause tissue dysfunction and other problems. These dysfunctions cause the proteins and lipids that complicate reproduction to oxidize. Melatonin acts as a free radical scavenger, helping to counteract oxidative stress, through both direct and indirect antioxidative actions, and can help limit oxidative stress of the cells, thereby protecting DNA damage[32,33]. Notably the hydroxyl radical can be

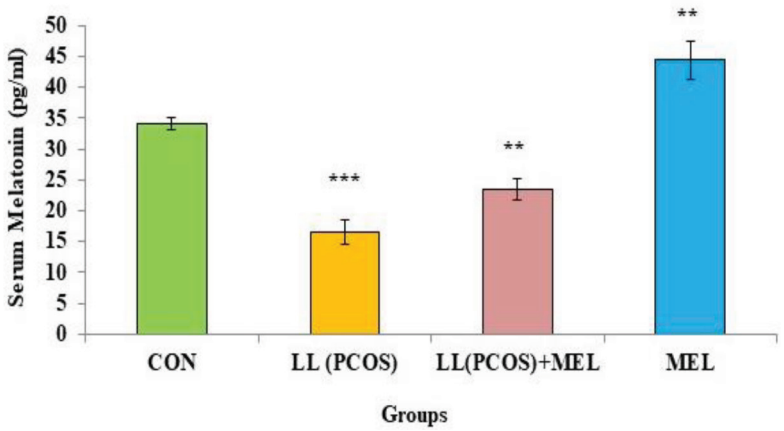


Figure 10. Effect of light and exogenous melatonin (MEL) in Melatonin of blood serum of constant light-induced Polycystic ovary syndrome (PCOS) rat model. Histogram represents Mean ± SE; N = 6;**P≤0.01; ***P≤0.001; * represents CON vs LL (PCOS); CON vs LL+MEL. CON = Control; LL (PCOS) = Constant light-induced PCOS; LL(PCOS)+MEL = Constant light-induced PCOS+ Melatonin; MEL = Melatonin.

dealt with the help of melatonin[34]. This neurohormone scavenges reactive oxygen species by forming adducts with intermediate products and might be helped to improve and up-regulate the anti-oxidative enzymes (SOD and CAT)[35]. Substrate: enzyme ratio of GSH and its associated anti-oxidative enzyme (GR, GST, GPX, G-6-PDH) has shown to increase concomitantly. The replenishment of estrous-cycle-associated anti-oxidative enzymes in the treated group might have been accomplished after the involvement of melatonin. Consequently, the generation of free radicals is simultaneously reduced[36,37]. Melatonin may therefore be an effective treatment to repair the disruption brought on by prolonged exposure to light.

In this study, the experiment's innovative practical method was a physiological pinealectomy, to evaluate the impact of an exogenous melatonin supplement. According to studies, the emergence of ovary cysts might be caused by a decrease in melatonin levels or vice versa. In addition, a tissue-specific response where the oviduct has been noted as more susceptible to constant light stress than ovary tissue is a novel finding of the present research. The higher androgen level and strong antioxidant defence mechanism of ovary tissues have significantly better adaptive mechanisms against oxidative stress than the oviduct tissues. Furthermore, it has been already established by various researchers that there is less prostaglandin (PG) F2-alpha and macrophages and more circulatory blood found in the ovary[38]. It could be the reason for increased antioxidant levels in the ovary. It is also suggested that the impact of stress of higher androgen levels due to constant light might have affected the sleeping pattern of the female rats resulting in lower circulatory levels of melatonin as noted in the present study. Further, melatonin restored all the impairments of reproductive tissues by neutralizing the impact of free radical stress acting as a buffering molecule and restoring the damages up to the control level. Studies on animals and people show that melatonin directly affects ovary function. Due to its low toxicity, melatonin medication is safe even when given in high dosages. When used to prevent and cure reproductive problems, it appears to have satisfactory results. Thus, it was evidenced that the alterations in reproductive physiology as a consequence of constant light exposure can be restored by melatonin.

5. Conclusion

The current study demonstrates that continued exposure to light can create an environment of oxidative stress which can impair ovary function and lead to PCOS-like symptoms in rats. However, exogenous melatonin administration can effectively scavenge these free radicals and restore normal ovary follicle function. These results showed a significant ramification for creating a new way to manage PCOS. Furthermore, this study clarifies the intricate neuroendocrine processes that contribute to polycystic conditions in the ovary and could advance our knowledge of the pathophysiology of this disorder. The avoidance and management of PCOS in females will be significantly impacted by these results.

6. Acknowledgment

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7. References

1. Kennaway DJ. The role of circadian rhythmicity in reproduction. Hum Reprod Update. 2005; 11(1):91-101. https://doi.org/10.1093/humupd/dmh054 PMid:15569698
2. Jan JE, Reiter RJ, Wasdell MB, Bax M. The role of the thalamus in sleep, pineal melatonin production, and circadian rhythm sleep disorders. J Pineal Res. 2009; 46(1):1-7. https://doi.org/10.1111/j.1600-079X.2008.00628.x PMid:18761566
3. Malpaux B, Migaud M, Tricoire H, Chemineau P. Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. J Biol Rhythms. 2001; 16(4):336-47. https://doi.org/10.1177/074873001129002051 PMid:11506379
4. Shao S, Zhao H, Lu Z, Lu Z, Lei X, Zhang Y. Circadian rhythms within the female HPG axis: From physiology to aetiology. Endocrinology. 2021; 162(8). https://doi.org/10.1210/endocr/bqab117 PMid:34125877 PMCid:PMC8256628
5. Soliz-Rueda JR, López-Fernández-Sobrino R, Bravo FI, Aragones G, Suarez M, Muguerza B. Grape seed proanthocyanidins mitigate the disturbances caused by an abrupt photoperiod change in healthy and obese rats. Nutrients. 2022; 14(9). https://doi.org/10.3390/nu14091834 PMid:35565801 PMCid:PMC9100649
6. Kloss JD, Perlis ML, Zamzow JA, Culnan EJ, Gracia CR. Sleep, sleep disturbance, and fertility in women. Sleep Med. Rev. 2015; 22:78-87. https://doi.org/10.1016/j.smrv.2014.10.005 PMid:25458772 PMCid:PMC4402098
7. Mills J, Kuohung W. Impact of circadian rhythms on female reproduction and infertility treatment success. Curr Opin Endocrinol Diabetes Obes. 2019; 26(6):317-21. https://doi.org/10.1097/MED.0000000000000511 PMid:31644470
8. Basheer M, Rai S. Exogenous melatonin revives the letrozole-induced toxic variations in biochemical parameters in polycystic ovary syndrome in rats. Uttar Pradesh J Zoo. 2020; 113-23.
9. Khan MJ, Ullah A, Basit S. Genetic basis of Polycystic Ovary Syndrome (PCOS): Current perspectives. Appl Clin Genet. 2019; 12: 249-60. https://doi.org/10.2147/TACG.S200341 PMid:31920361 PMCid:PMC6935309
10. Rani R, Hajam YA, Kumar R, Bhat RA, Rai S, Rather MA. A landscape analysis of the potential role of polyphenols for the treatment of Polycystic Ovary Syndrome (PCOS). Phytomed Plus. 2022; 2(1). https://doi.org/10.1016/j.phyplu.2021.100161
11. Cena H, Chiovato L, Nappi RE. Obesity, polycystic ovary syndrome, and infertility: A new avenue for GLP-1 receptor agonists. J Clin Endocrinol Metab. 2020; 105(8): e2695-709. https://doi.org/10.1210/clinem/dgaa285 PMid:32442310 PMCid:PMC7457958
12. Uçkan K, Demir H, Turan K, Sarıkaya E, Demir C. Role of oxidative stress in obese and nonobese PCOS patients. Int J Clin Pract. 2022:1-9. https://doi.org/10.1155/2022/4579831 PMid:35685525 PMCid:PMC9159123
13. Genario R, Morello E, Bueno AA, Santos HO. The usefulness of melatonin in the field of obstetrics and gynecology. Pharmacol Res. 2019; 147. https://doi.org/10.1016/j.phrs.2019.104337 PMid:31276773
14. Begum N, Manipriya K, Begum R, *et al*. Simple and rapid method for rat estrous cycle identification using crystal violet-hormonal consideration. Int J Pharm Sci Res. 2020; 5(4):54-9. https://doi.org/10.21477/ijapsr.5.4.1
15. Rodrıguez-Burford C, Barnes MN, Berry W, Partridge EE, Grizzle WE. Immunohistochemical expression of molecular markers in an avian model: A potential model for preclinical evaluation of agents for ovary cancer chemoprevention. Gynecol Oncol. 2001; 81(3):373-9. https://doi.org/10.1006/gyno.2001.6191 PMid:11371125
16. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-8. https://doi.org/10.1016/0003-2697(79)90738-3 PMid:36810
17. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal biochem. 1968; 25:192-205. https://doi.org/10.1016/0003-2697(68)90092-4 PMid:4973948
18. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952; 195(1):133-40. https://doi.org/10.1016/S0021-9258(19)50881-X
19. Kakkar P, Das B, Vismanathan PN. A modified spectrophotometric assay of superoxide dismutase (sod). Indian J of BiochemBiophys. 1984; 21: 130-2.
20. Ellis HA, Kirkman HN. A colorimetric method for assay of erythrocyte glucose-6-phosphate dehydrogenase. Proc Soc Exp Biol Med. 1961; 106:607-9. https://doi.org/10.3181/00379727-106-26418 PMid:13726525
21. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70(1):158-69.
22. Goldberg DM, Spooner RJ. Assay of glutathione reductase. In: Bergmeyen HV (ed), Methods of enzymatic analysis, 3rd Edition, Vol. 3, Deerfiled Beach: Verlog Chemie; 1983. p. 258-65.

23. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249(22):7130-9. https://doi.org/10.1016/S0021-9258(19)42083-8 PMid:4436300
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193: 265-75. https://doi.org/10.1016/S0021-9258(19)52451-6 PMid:14907713
25. Wawrzkiewicz-Jałowiecka A, Kowalczyk K, Trybek P, Jarosz T, Radosz P, Setlak M. In search of new therapeutics - Molecular aspects of the PCOS pathophysiology: Genetics, hormones, metabolism and beyond. Int J Mol Sci. 2020;21(19). https://doi.org/10.3390/ijms21197054 PMid:32992734 PMCid:PMC7582580
26. Basheer M, Rai S, Ghosh H. Therapeutic efficacy of melatonin against Polycystic Ovary Syndrome (PCOS) induced by letrozole in Wistar Rat. Pak J Biol Sci. 2018; 21(7):340-7. https://doi.org/10.3923/pjbs.2018.340.347 PMid:30417994
27. Genario R, Morello E, Bueno AA, Santos HO. The usefulness of melatonin in the field of obstetrics and gynecology. Pharmacol Res. 2019; 147. https://doi.org/10.1016/j.phrs.2019.104337 PMid:31276773
28. Mohammed D, Mahmoud H, Ahmed O, Santos HO. Comparison between the effect of oral administration of alum and aqueous extract of moringa oleifera seeds on physiological liver parameters of male albino rats. Adv Anim Vet Sci. 2021; 9(10):1745-56. https://doi.org/10.17582/journal.aavs/2021/9.10.1745.1756
29. Hajam YA, Rai S, Pandi-Perumal SR, Brown GM, Reiter RJ, Cardinali DP. Coadministration of melatonin and insulin improves diabetes-induced impairment of rat kidney function. Neuroendocrinol. 2022; 112(8):807-22. https://doi.org/10.1159/000520280 PMid:34673653
30. Zimmerman S, Reiter RJ. Transient responses of melatonin to stress. Melatonin Research. 2022; 5(3):295-303. https://doi.org/10.32794/mr112500133
31. Lamtai M, Azirar S, Zghari O, Ouakki S, El Hessni A, Mesfioui A. Melatonin ameliorates cadmium-induced affective and cognitive impairments and hippocampal oxidative stress in rat. Biol Trace Element Res. 2021; 199:1445-55. https://doi.org/10.1007/s12011-020-02247-z PMid:32613486
32. Basheer M, Rai S. Melatonin vs. Phytomelatonin: Therapeutic uses with special reference to Polycystic Ovary Syndrome (PCOS). Cogent Biology. 2016; 2(1). https://doi.org/10.1080/23312025.2015.1136257
33. Johnson WM, Wilson-Delfosse AL, Mieyal JJ. Dysregulation of glutathione homeostasis in neurodegenerative diseases. Nutrients. 2012; 4:1399-440. https://doi.org/10.3390/nu4101399 PMid:23201762 PMCid:PMC3497002
34. Sreekumar PG, Ferrington DA, Kannan R. Glutathione metabolism and the novel role of mitochondrial GSH in retinal degeneration. Antioxidants. 2021; 10: 661. https://doi.org/10.3390/antiox10050661 PMid:33923192 PMCid:PMC8146950
35. Huang Y, Zhang X. Luteolin alleviates polycystic ovary syndrome in rats by resolving insulin resistance and oxidative stress. Am J Physiol Endocrinol. 2021; 320(6):E1085-92. https://doi.org/10.1152/ajpendo.00034.2021 PMid:33900851
36. Elmosalamy SH, Elleithy EM, Ahmed ZS, Rashad MM, Ali GE, Hassan NH. Dysregulation of intraovary redox status and steroidogenesis pathway in letrozole-induced PCOS rat model: A possible modulatory role of l-Carnitine. Beni-Suef Univ J Basic Appl Sci. 2022; 11(1). https://doi.org/10.1186/s43088-022-00329-6
37. Hong Y, Yin Y, Tan Y, Hong K, Jiang F, Wang Y. Effect of quercetin on biochemical parameters in letrozole-induced polycystic ovary syndrome in rats. Trop J Pharm Res. 2018; 17(9):1783-88. https://doi.org/10.4314/tjpr.v17i9.15
38. Jabbour HN, Sales KJ, Catalano RD, Norman JE. Inflammatory pathways in female reproductive health and disease. Reproduction. 2009; 138(6):903. https://doi.org/10.1530/REP-09-0247 PMid:19793840