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Research Article

Augmentation of Gut Microbiome improves Melatonin concentration, Sperm Parameters and Testicular Steroidogenesis in Light-polluted Rat

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ABSTRACT

Altering the natural circadian rhythm by prolonged exposure to light is known to alter reproductive functions in many species including humans. However, it is not clear if augmentation of the gut microbiome can protect male reproductive function when exposed to light pollution. The current study was designed to investigate if the administration of probiotics (pro) will mitigate the disruptive effect of light pollution on melatonin concentration, semen parameters and testicular steroidogenesis in the rat. Thirty male Sprague-Dawley rats were randomized into six groups (n=5) consisting of the no-Pro/12hrs group that had 12:12 hrs light:dark cycle only; the Pro/12hrs group that had 12:12 hrs light:dark cycle plus probiotic supplementation; the no-Pro/16hrs that had 16:8 hrs light:dark cycle; the Pro/16hrs that had 12:12 hr light:dark cycle in addition to probiotic supplementation; the no-Pro/24hrs that had 24:0 hrs light:dark cycle; and the Pro/24hrs that had 24:0 hrs light:dark cycle plus probiotic supplementation. Each animal in the probiotic groups was fed a 10×106 colony-forming unit of lactobacillus acidophilus and Bifidobacterium bifidum every other day. Experiment lasted for 21 consecutive days. Probiotic administration significantly ($p<0.05$) increased circulating levels of melatonin, testosterone, and estradiol compared with control and no-pro 16hrs and 24hrs light-polluted groups. Malondialdehyde (MDA) and corticosterone levels were significantly ($p<0.05$) reduced while antioxidants activities significantly ($p<0.05$) increased in the testis and epididymis. Sperm motility and concentration significantly ($p<0.05$) increased in the pro groups compared with control and no-pro groups. Augmentation of the gut microbiome might play a significant role in improving male reproductive function during light pollution

Keywords: *Semen, gut microbiome, probiotics, melatonin, light pollution, oxidative stress*

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INTRODUCTION

Decades ago, the alternation that occurred between the natural dark and light cycle was a predictable environmental fluctuation that organisms use to schedule their activities (Ouyang *et al*., 2018). However, since the invention of artificial lighting, the predictable day and night cycle has been interrupted (Ouyang *et al*., 2018) and allows humans to be more active at night (Swaddle *et al*., 2015; Gaston *et al*., 2017). The increasing use of artificial light comes with obvious benefits to the society, such as extending the length of the productive day (Chepesiuk, 2009), but we are also starting to see its dark side (Navara and Nelson, 2007) because when artificial lightning becomes inefficient and unnecessary, it is called light pollution (Chepesiuk, 2009).

Light pollution is the mutation of usual light levels in the surroundings produced by the outline of artificial light (Falchi *et al*.,2016). In the last decade, light pollution emitted through human-induced sources including streetlights, outdoor artificial lights at night (Levin *et al*., 2020), and the use of electronic devices with light-emitting screens has increased exponentially (Green *et al*., 2017). As a result, humans are perpetually susceptible to unintentional artificial light (Green *et al*., 2017).

It has been observed that the high amount of light use is causing pollution which is a growing problem, and it can have lasting adverse effects on both human and wildlife health (Sankhla *et al*., 2019). The health effects of over-illuminating light pollution may cause headache occurrence, worker fatigue, medically defined as stress, reduction in sexual function, and increase in anxiety (Burks, 1994). Also, it has been demonstrated that evening light exposure to computer screens may disrupt human sleep, attention abilities, and biological rhythms (Lavie and Tzischinsky, 1997). Light pollution has been demonstrated to interfere with sexual hormone secretions by disrupting the positive correlation between testosterone and estradiol levels (LeTallec *et al*.,

2015). However, as hormones mediate individual feedbacks to changing environment, endocrine systems might be one of the first systems affected to improve any negative health impacts (Ouyang *et al*., 2018).

Probiotics are living microorganisms, which, when consumed or administered in an adequate amount, provide health benefits to the host (Fuller, 1989). They can be consumed in several forms of fermented or non-fermented food products (Ouwehand *et al*., 2002). Probiotics have specific properties and are natural residents of the human intestinal tract (Salminen *et al*., 2005). The most common aim of using probiotics is to improve the composition of intestinal microbiota that would be beneficial to the host (Fujimura *et al*., 2010). However, it has been observed that feeding of probiotics to aged male animals increased their subcuticular folliculogenesis and this yields luxuriant fur only in probiotics-fed mice (Levkovich *et al*., 2013). These observations led to the hypothesis that probiotics may play a role in causing the glow of health which is commonly seen in much younger animals (Levkovich *et al*., 2013).

Meanwhile, reports have demonstrated the beneficial effects of probiotics in human health conditions such as diarrhea (Chouragui *et al*., 2008), colorectal cancer (Lenoir *et al*., 2016), and also male sexual health (Poutahidos *et al*., 2014). Probiotics have shown evidence for a positive role in reversing fertility disorders and for hormonal imbalances (Garcia-Velasco *et al*., 2017). Also, a study observed that probiotic-fed male mice had elevated levels of androgen hormones (Poutahidos *et al*., 2014). This study was, therefore, designed to investigate the effects of light pollution on male reproductive function while testing the hypothesis that such effects are amendable to gut microbiome supplementation.

MATERIALS AND METHODS

Animals: Thirty (30) adult male Sprague-Dawley rats aged 12 weeks, weighing between 200 – 220g were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were kept at room temperature (28°C – 30°C) in the Animal House of the Department of Human Anatomy College of Medicine, University of Lagos. The animals were kept in standard rat cages and had access to feed and water ad libitum through the experiment. They were allowed to acclimatized for a period of 2 week under natural light/dark cycle. Animal handling and procedure was in accordance to United States National Institute of Health Guide for the Care and Use of Laboratory Animals (1996).

Study Design: Animals were randomized into six groups (n=5). These consist of Group I: the no-Pro/12hrs group that had 12:12 hrs light:dark cycle; Group II: the Pro/12hrs group that had 12:12 hours (hrs) light:dark cycle plus probiotic supplementation; Group III: the no-Pro/16hrs that had 16:8 hrs light:dark cycle; Group IV: the Pro/16hrs that had 12:12 hrs light:dark cycle in addition to probiotic supplementation; Group V: the no-Pro/24hrs that had 24:0 hrs light:dark cycle; and Group VI: the Pro/24hrs that had 24:0 hrs light:dark cycle plus probiotic supplementation. This experiment lasted for 21 consecutive days.

Probiotics administration / Induction of Light Pollution: Vita Tree probiotics supplement manufactured by Vita Tree Nutritionals, Canada was used for the study. The supplement is a sweet tasting powder in a small capsule. Each capsule contained Lactobacillus Acidophilus, A-136 (3.33 billion colony-forming units) and Bifidobacterium bifidum, A-020 (1.67 billion colony-forming units). Aliquots providing 10×106 colony- forming unit (CFU) per rat were prepared. This was dissolved in normal saline which served as the vehicle and administration was done orally every other day (Dardmeh *et al*., 2017). Light pollution was achieved by exposing the animal to 5 lux of white light at night either for 4 or 12 hours (Bedrosian *et al*., 2013).

Collection of Samples: At the end of day 21, animals were euthanized and blood samples were collected through left ventricular puncture, centrifuged at 3000rpm for 15 minutes, and the supernatant was collected for hormonal assay. The testes and caudal epididymides were also harvested for analysis. The left testis was homogenized in 10% cold phosphate buffer and centrifuged to obtain supernatant for oxidative stress studies while the left epididymis was minced in 5 mL of normal saline to obtain epididymal fluid into which spermatozoa have swum.

Biochemical Assays: Using serum obtained from blood, corticosterone, melatonin, testosterone (TT) assays were done using an ELISA kit obtained from Biovision Inc (USA). The procedures for the assays were strictly done according to the manufacturer's instructions. Oxidative stress markers were further assayed using supernatant obtained from the testicular homogenate. MDA level, as a proxyl index of lipid peroxidation, was determined by the reaction between thiobarbituric acid and MDA (Mihara and Uchiyama, 1978). Superoxide dismutase (SOD) activity was determined by its ability to inhibit the auto-oxidation of epinephrine at absorbance maximum of 320nm. The reaction was carried out in 0.05N HCl according to Sun and Zigman (1978). Catalase (CAT) activity was determined by measuring the exponential disappearance of H2O2 by Aebi (1984). Glutathione (GSH) level was determined by the method of Ellman (1959) which is based on the reaction of Ellman's reagent 5,5' dithiobis (2-nitrobenzoic acid) DNTB) with the thiol group of GSH. Zinc (Zn) levels in the serum was determined colorimetricly according to the methods of Makino *et al*. (2000).

Sperm Analysis: The epididymal fluid ratio of 1:20 was prepared by adding 0.1ml of fluid to 1.9 ml of water. After mixing the dilution thoroughly, a Neubauer improved hemocytometer with the aid of a Leica D750 microscope was used to determine sperm concentration and quality. As previously described, spermatozoa within five of the red blood cell squares including those which lie across the outermost lines at the top and right sides were counted, while those at the bottom and left sides were left out. The number of spermatozoa counted was expressed in millions/ml (Sokol *et al*., 2000).

To assess motility, a drop of epididymal fluid was delivered onto a glass slide such that the spermatozoa were

evenly distributed, covered by a 22x22 mm coverslip, and examined under the light microscope at a magnification of x100 while several fields were evaluated. Sperm motility was classified as progressively motile, non-progressively motile, or immotile relying on the WHO (2010) classification of sperm motility. After assessing different microscopic fields, the relative percentage of motile spermatozoa was estimated and reported to the nearest 5% using the subjective determination of motility (Sokol *et al*., 2000).

Statistical Analysis: For statistical analysis, one-way ANOVA with Bonferroni's multiple comparison post-test was performed. Statistical significance was set at $P<0.05$, and where applicable, data were reported with the plus or minus standard error of mean (±SEM). All statistical analyses were performed with the GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA).

RESULTS

Prolong exposure to light reduces Sperm concentration: Figure 1A shows a significant decrease in sperm concentration in no-Pro/24hrs group compared with the other groups. While the sperm concentration of no-Pro/16hrs and Pro/24hrs are significantly higher than no-Pro/24hrs, they are significantly lower than no-Pro/12hrs, Pro/12hrs, and Pro/16hrs groups. No significant difference was observed in sperm concentrations in the 12hrs group. Figure 1B shows a significant decline in sperm progressive motility in no-Pro/24hrs and Pro/24hrs groups compared with the 12hrs and 24hrs groups. While there is a significant increase in the pro/24hrs group compared with the no-Pro/24hrs, there is no significant difference among the 12hrs and 16hrs groups at $P<0.05$. Figure 1C shows a significant decrease in the sperm non-progressive motility in no-Pro/16hrs group compared with any other groups. There are no significant differences among the 12hrs and 24hrs groups. In Figure 1D, there is no significant difference, at $P<0.05$, between the sperm immotility in no-Pro/12hrs and Pro/12hrs groups. While the sperm immotility in the Pro/16hrs group is significantly lower than no-Pro/16hrs, no-Pro/24hrs, and Pro/24hrs groups, it is significantly higher than no-Pro/12hrs and Pro/12hrs groups.

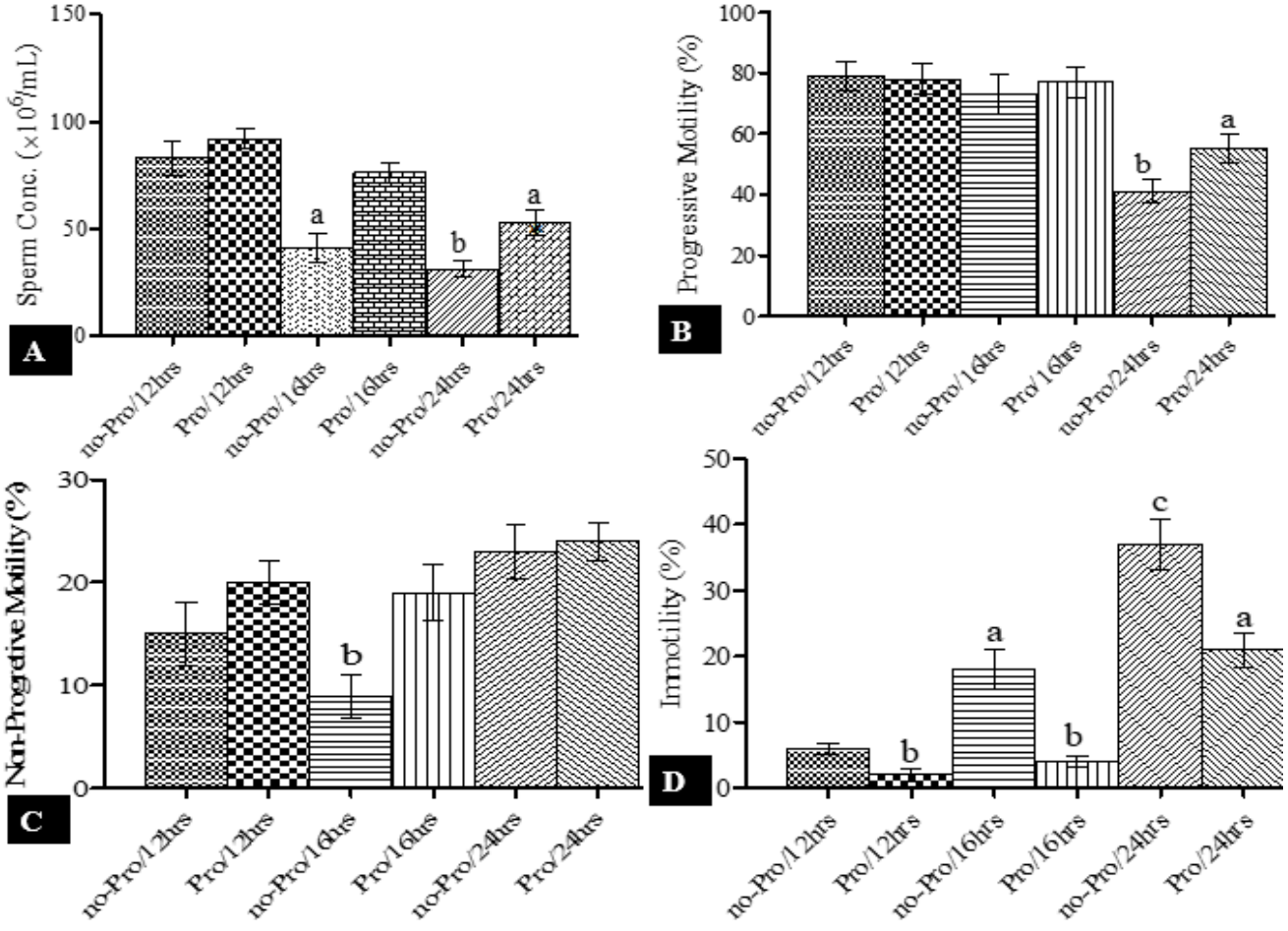

Figure 1:
Epididymal sperm parameters following exposure to light pollution and probiotics supplementation. In 1A, [a] indicates statistically significant difference from bars without asterisk while [b] is significantly different from [a] at $P<0.05$; In 1B, [a] indicates statistically significant difference from bars without asterisk while [b] is significantly different from [a] at $P<0.05$. In 1C, [a] indicates a statistically significant difference from bars without asterisk while [b] is significantly different from [a] at $P<0.05$. In 1D, [a] indicates a statistically significant difference from bars without asterisk while [b] is significantly different from [a] at $P<0.05$ and [c] indicates significance difference from all other groups

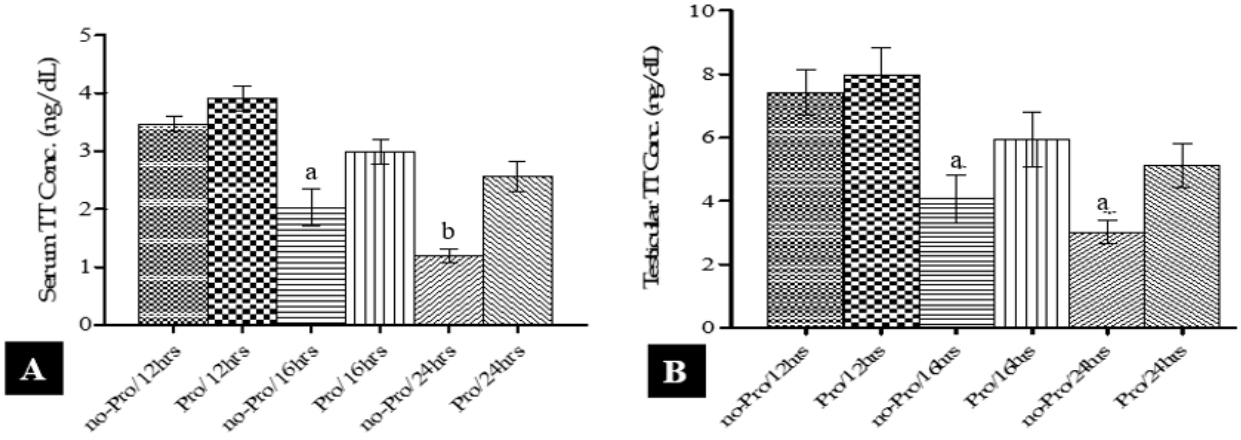

Figure 2:
Serum and Testicular TT concentrations following probiotic administration and exposure to light pollution. In 2A, [a] indicates a statistically significant difference from bars without an asterisk, while [b] is significantly different from a In 2B, [a] indicates a statistically significant difference from bars without an asterisk.

Light pollution attenuates both testicular and circulating levels of Testosterone: Figure 2A shows that serum testicular testosterone concentration in no-Pro/16hrs and no-Pro/24hrs groups is significantly lower compared with any other group. However, no significant difference exists among the 12hrs groups, Pro/16hrs, and Pro/24hrs groups. Figure 2B shows that testicular testosterone concentration in no-Pro/16hrs and no-Pro/24hrs groups is significantly lower compared with any other group. There is no significant difference among the 12hrs groups, Pro/16hrs, and Pro/24hrs groups.

Probiotic mitigates the negative impact of light pollution on melatonin level: Serum melatonin level in no-Pro/16hrs and no- Pro/24hrs groups is significantly lower compared with any other group (Figure 3). There is no significant difference between the no-pro/12hrs, pro/12hrs, Pro/16hrs, and Pro/24hrs groups. However, melatonin level is significantly lower in the no- pro/24hrs group compared with no-pro/16hrs.

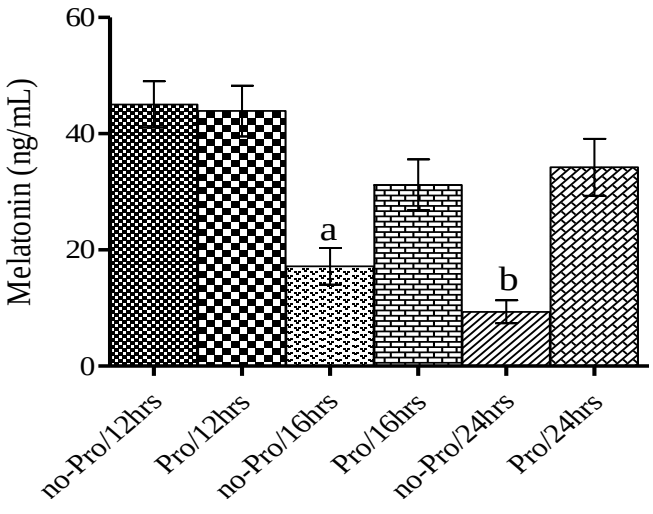

Figure 3:
Circulating melatonin levels following exposure to light pollution and administration of probiotics.[a] shows significantly (P<0.05) lower melatonin level compared with all other groups except no-Pro/24hrs, [b] which is significantly lower compared with no-Pro/16hrs.

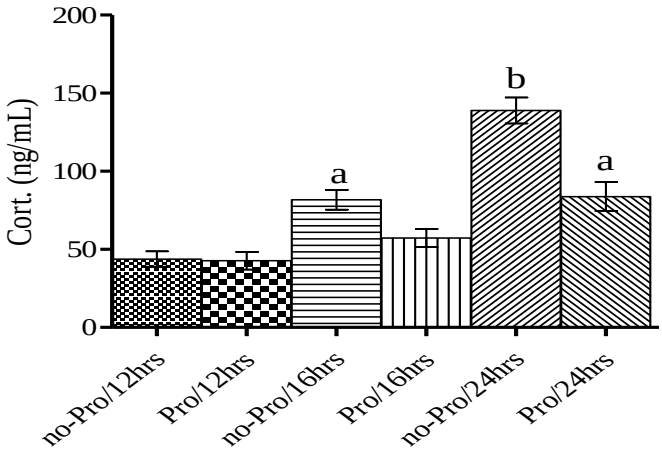

Figure 4:
Corticosterone level following probiotic administration and exposure to light. [a] indicates statistically significant difference from bars without asterisk while [b] is significantly different from [a] at P<0.05.

Light pollution induces Stress in male rats: Figure 4: shows that the Corticosterone level in no-Pro/16hrs, no-Pro/24hrs, and Pro/24hrs groups significantly rose compared with other groups. However, the no-Pro/16hrs and Pro/24hrs groups are significantly lower than the no-Pro/24hrs group.

Gut Microbiome supplementation mitigates Redox imbalance following exposure to light pollution: Figure 5A shows no significant difference between the Malondialdehyde concentration in no-Pro/12hrs and Pro/12hrs groups. While there is a significant increase in no-Pro/16hrs, no-Pro/24hrs, and Pro/24hrs groups compared with any other group, the no-Pro/24hrs group is significantly higher than no-Pro/16hrs and Pro/24hrs groups. Figure 5B shows a significant decrease in no-Pro/16hrs and 24 hours groups compared with other groups. There is no significant difference between no-Pro/12hrs and Pro/12hrs groups and also, no significant difference between no-Pro/24hrs and Pro/24hrs groups. However, the no-Pro/16hrs is significantly lower than the Pro/16hrs groups. Figure 5C shows a significant decrease in the catalase level of no-Pro/16hrs, no-Pro/24hrs, and Pro/24hrs groups compared with other groups. There is no significant difference between no-Pro/12hrs and Pro/12hrs groups and also, no significant difference between no-Pro/24hrs and Pro/24hrs groups. Also, the no-Pro/16hrs is significantly lower than the Pro/16hrs groups. Figure 5D shows that there is no significant difference between the glutathione concentration of no-Pro/12hrs and Pro/12hrs group. The no-Pro/12hrs, Pro/12hrs, and Pro/16hrs rose significantly compared with other groups. Pro/24hrs group is significantly higher than no-Pro/16hrs and no-Pro/24hrs groups, but significantly lower than other groups.

DISCUSSION

The most important finding from this study is that light pollution has a detrimental effect on male reproductive function on the one hand, but supplementation with probiotics during exposure to light pollution, on the other hand; can mitigate this effect. Light pollution is one of the fastest-growing environmental pollutions and it is increasing between 2.2- 6% per year globally (Holker *et al*., 2010; Kyba *et al*., 2017), and consequently potent a significant threat to animal reproductive behavior, reproduction, and biodiversity because the fine regulation of spermatogenesis yields to the influence of the circadian rhythm (Holker *et al*., 2010).

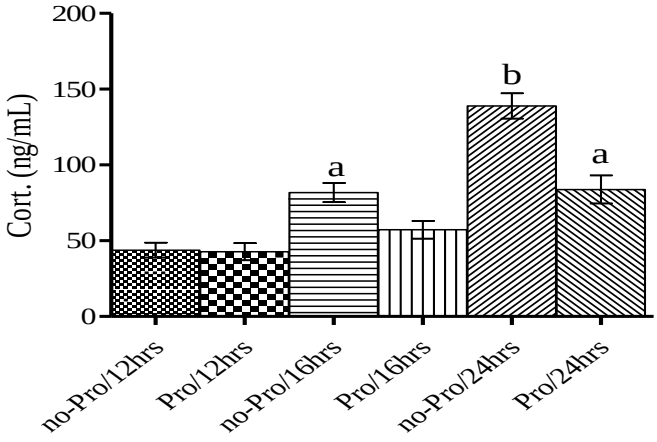

Figure 4:
Corticosterone level following probiotic administration and exposure to light. [a] indicates statistically significant difference from bars without asterisk while [b] is significantly different from [a] at P<0.05.

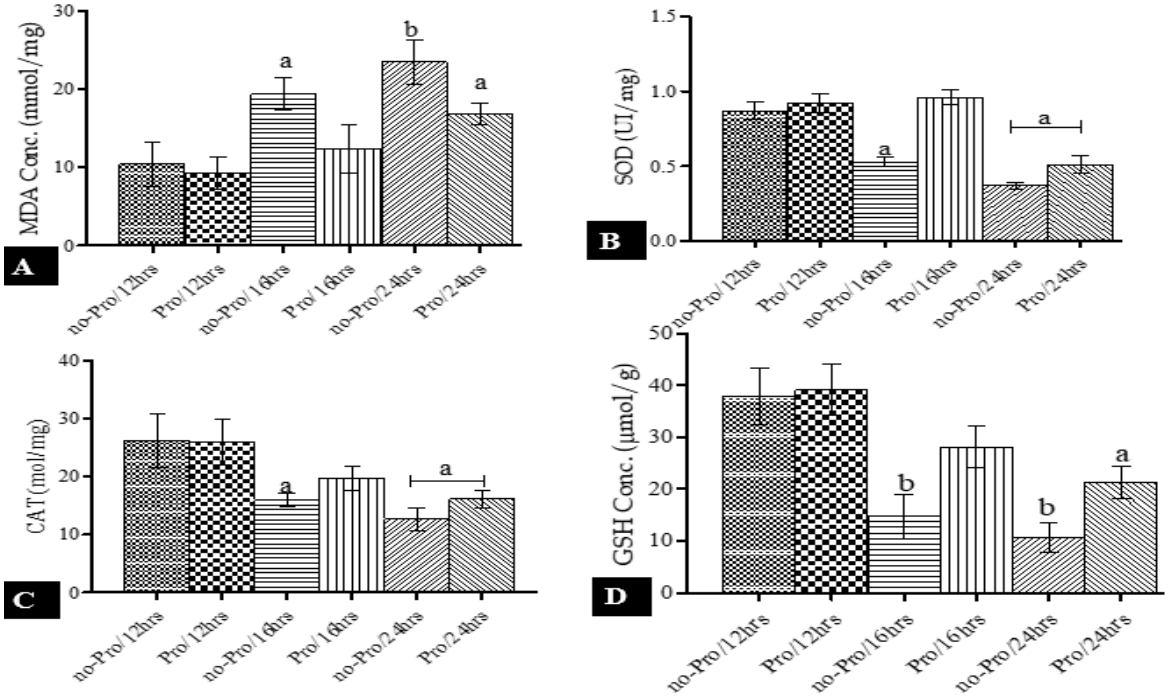

Figure 5:
Pro-and anti-oxidants parameters following exposure to light pollution and probiotic administration. In 5A, [a] indicates a statistically significant difference from bars without asterisk while [b] is significantly different from [a] In 5B, [a] indicates a statistically significant difference from bars without asterisk while [b] is significantly different from [a]. In 5C, [a] indicates a statistically significant difference from bars without asterisk while [b] is significantly different from [a]. In 5D, [a] indicates statistically significant difference from bars without asterisk while [b] is significantly different from [a]

Alteration to this biological rhythm as demonstrated in this investigation depresses sperm concentration significantly. Increasing the duration of daily exposure to light by just a third, for a period of consecutive 14 days produced about a 50% decrease in sperm concentration while continuous exposure to light, for the same number of days, reduced sperm production by about 60%. This suggests that significant disruption in rat spermatogenesis is possible even in a situation of additional 4 hours of light per day. Stated differently, our data suggest that the daily duration of exposure to light pollution does not produce a corresponding linear reduction in sperm concentration as it took only 4 hours of light exposure at night to observe a 50% reduction in sperm output while 12 hours of light exposure at night resulted in about 60% reduction in sperm concentration. In addition, this also suggests that even continuous exposure to light round the clock might not obliterate sperm production. This is valid for the length of our experiment.

Supplementation with probiotics appears to offer almost total protection against 4 hours of exposure to light pollution. However, when exposure to light pollution covered the whole night, probiotics supplementation offered only partial protection against light pollution-associated decreased sperm concentration. Nonetheless, it is apparent from the analysis of our data that if animals were treated with probiotics during exposure to 12 hours of light pollution, spermatogenesis will fare better compared with no probiotic supplementation. The role of the gut microbiome in spermatogenesis has been reported in an investigation testing if a high-fat diet causes gut microbiome dysbiosis and it was demonstrated that disruption of the gut microbiome can indeed affect spermatogenesis and sperm motility (Ding *et al.*, 2019)

Constant light exposure has been shown to negatively affect the gut microbiome and its metabolites (Wei *et al.*, 2020). And it has been empirically shown that changes in the gut microbiome directly impair spermatogenesis (Poutahidos *et al.*, 2014). This might explain why animals that were exposed to light pollution in this study exhibited poor sperm parameters. This suggests that one of the pathways that light pollution could impair spermatogenesis is by altering the ecology of the gut microbiome. As shown in the present study, supplementation with probiotics attenuate the poor sperm parameters seen in groups of rats that were exposed to light pollution, clearly suggesting that the gut microbiome was indeed impaired by light pollution. This observation is supported by the fact that supplementation with probiotics without exposure to light pollution made no significant difference to sperm parameters. Stated differently, supplementation of normal rats under normal conditions with probiotics has no appreciable benefits on sperm parameters.

A significant decline in sperm progressive motility was seen in no-Pro/24hrs and Pro/24hrs groups compared with the other groups with no significant reduction in the percentage of sperms with progressive motility following continuous exposure to light for 16 hours. Since sperm motility is linked to a delicate balance between pro-and anti-oxidant systems (Agarwal and Bui, 2017), the present study suggests an imbalance in the redox status as a result of continuous exposure to light for 16 hours did not translate to a significant reduction in sperm motility. To be specific, there is a significant increase in motility in the pro/24hrs group compared with the no-Pro/24hrs, there were no observable significant differences between the 12hrs and 16hrs groups. There is a significant decrease in the percentage of spermatozoa with non-progressive motility in the no-Pro/16hrs group compared with any other group. It is clear that there is no significant difference between the immotile sperms in no-Pro/12hrs and Pro/12hrs groups but on the other hand, the immotile sperms in the Pro/16hrs group are significantly lower than the no-Pro/16hrs, no-Pro/24hrs and Pro/24hrs groups. Non-progressively motile and immotile sperms are of little or no reproductive benefits in nature as they are highly unlikely to progress from site of deposition to site of fertilization.

Although evaluation of sperm motility often makes the determination regarding the proportion of spermatozoa that are immotile and/or motile but exhibit only non-progressive motility; the most important component of sperm paraments is the total number of sperm with progressive motility. This can be calculated by multiplying sperm concentration with the percentage of progressively motile sperm. This means that in the current study, about 32 million spermatozoa exhibited the capacity for the potential to reach the site of fertilization in the group of animals subjected to 16 hours of light daily while only about 19 million spermatozoa in the group exposed to 24hrs of light exhibited the same potential. In the 24-hours-light-exposed group without supplementation, this represents over 40% reduction in the number of sperms that are progressively motile compared with the 16-hour- light-exposed animals and a 70% reduction in the proportion of progressively motile sperm compared with the control.

Exposure to light pollution-induced stress in the animals as elevated corticosterone levels were observed following exposure to light pollution where the corticosterone level in no-Pro/16hrs, no- Pro/24hrs, and Pro/24hrs groups significantly rose compared with other groups. However, the no-Pro/16hrs and Pro/24hrs groups are significantly lower than the no-Pro/24hrs group. Indeed, the increase in corticosterone levels following continuous exposure to light for 24 hours is almost double the levels observed in the animals that had 16 hours of exposure to light. Elevated corticosterone, a glucocorticoid, is an indication of activation of the HPA axis in response to stress (Nargund, 2015). Increased corticosterone, in this study, suggests that the animals perceived light pollution as a threat with energetic demands. This observation aligns with previous studies (Nargund, 2015; Li et al., 2020) that have shown that stress of any type is disruptive to spermatogenesis and male reproductive function.

Spermatogenesis cannot progress normally in the absence of TT or when TT is too low (Ramaswamy and Weinbauer, 2015; Walker, 2021) and as such the current study evaluated both the circulating and testicular levels of TT because the testis has been shown to maintain a local concentration of TT different from that in the circulation (Castro et al., 2002) serum TT concentration in no- Pro/16hrs and no-Pro/24hrs groups are significantly lower compared with any other group.

However, no significant difference was observed among the 12hrs, Pro/16hrs, and Pro/24hrs groups. Similarly, testicular TT concentration in no-Pro/16hrs and no- Pro/24hrs groups is significantly lower compared with any other group. There is no significant difference among the 12hrs groups, Pro/16hrs, and Pro/24hrs groups. This observation supports earlier findings that show that the gut microbiota has been found as a major regulator of androgen production and metabolism and could even trespass the blood testis-barrier (BTB) to regulatespermatogenesis (Li *et al*, 2021).

However, studies that make the connections between the androgen-suppressing effects of stress as a possible consequence of the alterations in gut microbiota secondary to stress are lacking in the literature. The current study may as well represent an initial understanding of this connection. Stated differently, stimuli perceived as stressful may change the composition of gut microbiota which may then impact negatively androgen levels in the male leading to all the potential impairment of spermatogenesis and sperm quality.

Our results also indicate that light pollution significantly decreases circulating melatonin in both animals that have additional 4 hours and 12 hours of light exposure. This finding is inconsonant with earlier reports (Kernbach *et al.,* 2020a; 2020b)**.** This finding is important because the male reproductive function and behavior are directly connected with the daily circadian alternations controlled by the hypothalamic suprachiasmatic nuclei which serves as the internal mammalian timekeeping system via the rhythmical secretion of the pineal hormone, melatonin (Dibner *et al.,* 2010). Circulating melatonin peaks in the night and where the duration of night is shortened through light pollution, melatonin secretion may not attain its maximum levels. Previous reports have shown that light pollution can suppress nocturnal melatonin production across many species, including humans **(**Gooley *et al.,* 2011). Rat, although a nocturnal animal, like humans, is not a seasonal breeder. Nonetheless, it is clear that light pollution's suppression of melatonin in rats translates into reduced sperm concentration via impaired testicular redox status (Galano *et al.,* 2011).

From the current study, light pollution disrupts testicular redox balance by accentuating pro-oxidant levels and attenuating testicular anti-oxidants. There is no significant difference between the MDA concentration in no-Pro/12hrs and Pro/12hrs groups. However, there is a significant increase in the level of MDA in the no-Pro/16hrs, no-Pro/24hrs, and Pro/24hrs groups compared with any other group. This may have resulted from the diminished levels of circulating melatonin and increased circulating glucocorticoids indicating that light pollution was perceived as stressful for the animals (Vaz *et al.,* 2021). The elevated level of MDA in the testis as in any cell will cause the peroxidation of the lipid bilayer of membrane-bound organelles such as the mitochondria and the cell, and in this case, those of spermatozoa (Vaz *et al.,* 2021). This may explain, in part, the reduced number of spermatozoa with progressive motility in the groups of animals exposed to light pollution without probiotic supplementation. There was a significant decrease in sperm motility following exposure to light pollution. Sperm motility has been shown to be impaired under elevated oxidative stress levels (Agarwal and Bui, 2017). Our data which indicate that light pollution is associated with increased oxidative stress might explain the observation of decreased sperm motility in light pollution-exposed rats or light- polluted rats.

The testis antioxidant defense system gives protection from indiscriminate assault by pro-oxidants and in the current study, there is a significant decrease in activity levels of SOD in the no- Pro/16hrs and 24 hours groups compared with other groups. However, the no-Pro/24hrs is significantly lower than the no- Pro/16hrs groups which suggest that the longer the duration of light pollution the compromised the activity level of SOD. In the same vein, there is a significant decrease in the catalase activity level of the no-Pro/16hrs, no-Pro/24hrs, and Pro/24hrs groups compared with other groups. Also, the no-Pro/16hrs is significantly lower than the Pro/16hrs groups. However, there is no significant difference between the glutathione concentration of no-Pro/12hrs and Pro/12hrs group whereas, the no- Pro/12hrs, Pro/12hrs, and Pro/16hrs GSH concentration rose significantly compared with other groups. Pro/24hrs group is significantly higher than no-Pro/16hrs and no-Pro/24hrs groups, but significantly lower than other groups.

In conclusion, since day length is a key driver of daily rhythms of physiological activities such as sleep, body temperature, hormone secretion, and even gene expression, light pollution represents a potent disruptor associated with significant impairment of reproductive indices. Our data suggest that this impairment is mediated through changes in hormonal and redox status. More interestingly, our study shows that augmentation of the gut microbiome with probiotics significantly reduced light pollution- induced reproductive impairments by increasing melatonin secretion, testicular anti-oxidants, and improved testicular steroidogenesis.

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