CHRONIC ARTIFICIAL LIGHT AT NIGHT EXPOSURE DISRUPTS CIRCADIAN RHYTHMS AND MODULATES *P*53 GENE EXPRESSION IN A RAT MODEL OF COLORECTAL CANCER

ABSTRACT

Background: Artificial light at night (ALAN) is an increasingly prevalent environmental exposure that disrupts circadian organization and is classified as a probable human carcinogen. Circadian disruption alters physiological rhythms and molecular pathways involved in tumor suppression, oxidative balance, and inflammation, which is associated with various health risks, including cancer. Colorectal cancer (CRC) remains a leading cause of cancer-related mortality worldwide, yet the role of ALAN in modulating CRC progression is not fully understood.
Methods: Male Wistar rats were assigned to control (12 hrs light/12 hrs dark), carcinogen-exposed (1,2-dimethylhydrazine + dextran sulfate sodium; DMH/DSS), or carcinogen plus ALAN exposure (18 hrs light/6 hrs dark; 430 lux) groups for 12 weeks, six rats per group. Circadian rhythmicity was assessed using core body temperature and Cosinor analysis. Oxidative stress markers (malondialdehyde [MDA], total antioxidant capacity [TAC]), plasma carcinoembryonic antigen (CEA), colonic *P*53 gene expression, and histopathological changes were evaluated.
Results: ALAN exposure significantly attenuated nocturnal core body temperature, delayed the acrophase and reduced the amplitude of the temperature rhythm. Carcinogen exposure increased MDA, reduced TAC, elevated CEA, downregulated *P*53 expression, and induced colitis-like histological alterations. Combined exposure to carcinogens and ALAN resulted in a further increase in CEA, altered oxidative stress dynamics, significant upregulation of *P*53 gene expression relative to carcinogen-only animals, and progression from inflammatory pathology to dysplastic glandular changes in the colon.
Conclusion: Chronic ALAN exposure disrupts circadian organization and potentiates colorectal cancer progression in the presence of carcinogenic insult. These findings provide experimental evidence that environmental light exposure acts as a disease modifier in colorectal carcinogenesis, highlighting circadian health as a potential target for cancer prevention in modern light-polluted societies.

Keywords: artificial light at night, circadian disruption, colorectal cancer, *P*53, oxidative stress, carcinoembryonic antigen, Wistar rats

INTRODUCTION

The modern lifestyle, characterized by increased exposure to artificial light at night (ALAN), has significantly disrupted natural circadian rhythms (Rabiu *et al*, 2020; Barber *et al*, 2024). This disruption, referred to as circadian rhythm disruption (CRD), has been associated with various adverse health outcomes, including metabolic disorders, sleep disturbances, and cancer (Shafi and Knudsen, 2019; Yan *et al,* 2023). Circadian rhythms generated by the internal biological clocks that regulate various physiological and behavioral processes, are essential for maintaining homeostasis and health. These rhythms are primarily driven by the internal clock and modulated by light-dark cycle. The circadian rhythms are influenced by environmental cues, particularly light (Lourdes *et al*, 2023). The circadian system is a fundamental biological system present in living organisms that is regulated by the circadian clocks located centrally in the suprachiasmatic nucleus of the hypothalamus and peripherally in all tissues (Saper *et al,* 2005). The circadian clock in mammals is driven by an auto-regulatory feedback loop of transcriptional activators and repressors (Reppert and Weaver, 2002; Mohawk *et al,* 2012). Circadian locomotor output cycles kaput (CLOCK), and brain and muscle arnt-like protein 1 (BMAL1) are transcription factors form heterodimers that induce expression of period protein genes (*Per1*, *Per2*, and *Per3*) and cryptochrome protein genes (*Cry1* and *Cry2*) through E-box enhancers (Jin *et al.,* 1999). Period (PER) and cryptochrome (CRY) proteins accumulate in the cytoplasm throughout the circadian day. Upon reaching critical levels, PER and CRY form a complex that translocates back to the nucleus to associate with CLOCK and BMAL1 and repress their own transcription. The process takes approximately 24 hours to complete a full cycle (Lee *et al*, 2007). A number of other clock control genes (retinoic acid- related orphan nuclear receptors, *Reverb* α and *Rora*,) are necessary for generation of precise circadian rhythms (Zhang and Kay 2010). Ablation of any transcription factors and core clock genes; *Clock*, *Bmal1*, *Per1, Per2*, *Cry1*or *Cry2* disrupts circadian physiology (Ko and Takahashi, 2006). Human and animal

studies showed that exposure to light disrupt circadian rhythm (Rabiu *et al*, 2020; Barber *et al*, 2023). Disruption of the circadian rhythm has been linked to cancer development (Shafi and Knudsen, 2019). The circadian system plays a crucial role in regulating various cellular processes involved in tumor development, such as DNA damage response and cell cycle (Shafi and Knudsen, 2019). Circadian disruption is classified as a probable human carcinogen (IARC 2020). However, conflicting viewpoints regarding the strength and validity of this association have been presented in systematic reviews and meta-analyses (Zhang and Papantoniou 2019, Dun *et al*, 2020; Rivera *et al*, 2020).

Colorectal cancer (CRC) is the third most common diagnosed cancer worldwide and rank second globally in terms of cancer-related mortality (WHO, 2024). The International Agency for Research on Cancer (IARC) has been estimated that between 2020 and 2040 the global liability of colorectal cancer will grow by 56%, meaning more than 3 million cases per year. The estimated number of death cases from this cancer will rise to 69% and about 1.6 million death cases in 2040 (IARC, 2020). Several risk factors for CRC, including bacterial infections, metabolic diseases, genetic predisposition, lifestyle behaviors and environmental exposures, have been well-documented (Obidike *et al,* 2019; Ebrahimi *et al,* 2020; Eyvazi *et al,* 2020; Saiedeh *et al,* 2021). However, emerging evidence suggests that circadian rhythm disruption due to ALAN may also contribute to the development and progression of CRC (Ariadna *et al,* 2020). Molecular and genetic findings have revealed a connection between clock genes, tumor supressor genes, and colorectal tumors, as well as associations between the expression of clock genes and the pathogenesis, progression, aggressiveness, and prognosis of colorectal cancer in human and animals (Momma *et al,* 2017; Jung *et al*, 2020; Razi *et al,* 2021).

The present study elucidated the mechanism in which ALAN, potentiates the development of cancer, especially colorectal cancer. Therefore, the study investigated the effect of circadian disruption due to prolonged exposure to ALAN on *P*53 tumor suppressor gene expression,

Carcinoembryonic antigen (CEA), oxidative stress (malondialdehyde and total antioxidant capacity) and colorectal tissue cytoarchetecture in colorectal cancer modeled male Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Thirty male Wistar rats (3-4 weeks old) were sourced from the Animal House of the Department of Human Physiology, Bayero University Kano, Nigeria. The rats were housed in designed light experiment box, at room temperature and well ventilated environment. Rodent chow and water were available only at night throughout the study period (acclimation and intervention periods) (Dissi *et al,* 2020). Prior to commencing the experiment, all animals were maintained in a natural light/dark cycle for 3 weeks to ensure that the animals entrained to the light/dark cycle (Arble *et al*, 2009). The weights of the rats were monitored throughout the study period. The research was conducted at the Environmental and Chronophysiology Unit, Department of Human Physiology, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria. Ethical approval was obtained from animal care and use research ethics committee, Bayero University Kano, Nigeria (Approval No. BUK/ACUREC/CAP/PG95). Animal care, handling and use were carried out in accordance with National Institute of Health Guide for the care and use of laboratory animals (National Research Council, 2011).

Experimental Design

The rats were randomly assigned into three different groups, six rats each (n=6). Group A (control group) were maintained under natural 12hrs:12hrs light / dark cycle. Group B were also maintained under natural 12hrs:12hrs light / dark cycle, but with carcinogen (1,2-Dimethylhydrazine and dextran sulfate sodium). Group C were exposed to 6hrs ALAN (18hrs:6hrs light / dark cycle) and were also exposed to carcinogens. The light intensity used

was 430 lux which is within the range of 200 to 500 lux (Moore-Ede *et al,* 2017). The study last for 12 weeks after acclimatization (Dzhalilova *et al*, 2023).

Induction of Colorectal Cancer (CRC): CRC was induced by administering 30 mg/kg of 1,2-dimethylhydrazine (DMH) dissolved in 0.9% saline via a single intraperitoneal injection, followed by the administration of 2% (w/v) Dextran Sodium Sulfate (DSS) in drinking water for 7 consecutive days, beginning one week after DMH injection, as previously described (Tanaka et al., 2003; Congcong et al., 2022). The first carcinogen exposure was initiated after three weeks of acclimatization, which coincided with the first week of exposure to ALAN. Exposure to ALAN began simultaneously with the initiation of the carcinogen treatment and continued throughout the 12-week experimental period, in accordance with the model described by Dzhalilova *et al,* (2023). The mortality was recorded during acclimation period (5 rats) but no mortality was recorded during the course of induction and intervention period. One rat presented with the right sided hemiparesis after carcinogen induction and survived to the end of 12-week experimental period.

Exposure to ALAN: The rats in groups C were exposed to 6 hours ALAN for 12 weeks. The light was on at 18:00 hrs and off at 24:00 hrs (Dissi *et al,* 2020).

Body Weight Changes: The initial weight for each rat was taking before acclimatization at 18: 00 hrs to avoid fluctuations due to food intake. The weights were also be measured after the 3 weeks acclimatization period. The weights were continuously been measured at 3 weeks, 6 weeks, 8 weeks, 10 weeks, 11 weeks and 12 weeks post-acclimation, using same electronic balance (CAMRY, made in China), time of day and handling method to minimize variations (Potrebić *et al*, 2022)

Assessment of Circadian Rhythmicity: Two days prior to termination of the study, the core body temperature of the animals were measured at three hours interval (10hrs, 13hrs, 16hrs, 19hrs,

22hrs, 1hr, 4hrs and 7hrs) for 24 hours using Digital Infra-red Thermometer (LZ600, made in China). The adopted distance used was about 5-10 cm away from the direction of the ear lobe. The values of core body temperature were used in the determination of circadian rhythmicity in the control and interventions groups (Rabiu *et al,* 2024).

Samples Collection

At the end of the twelve weeks, the rats were anesthetized using Ketamine/Xylazine (65+7.5mg/kg) in sterile saline (1:10 v/v) by intraperitoneal injection (Oh and Narver, 2024). Blood samples were collected by cardiac puncture; about 4ml of blood samples were collected from rats into an EDTA container and centrifuge at 3000 rpm for 30 minutes (relative centrifugal force=504G). The plasma was collected in plain containers and store at -20^{o}C (Ma *et al,* 2018). The middle and distal colon were harvested and the colon sample from each rat was divided into three portions: The first potion was immediately stored in RNA Stabilization Solution (GDSBio, made in China) for *P*53 gene expression analysis. The second portion was stored in phosphate buffer saline for MDA and TAC analyses and the third portion was preserved in 10% formalin for histological analysis. The samples in RNA Stabilization Solutions and phosphate buffer saline were stored at -20^{o}C for two weeks before the analyses.

P53 gene expression

The samples in RNA Stabilization Solution (GDSBio, China) were homogenized with tissue homogenizer (LABGIC, China). the homogenates were centrifuged at 10,000g for 15 minutes at 4°C. The supernatant were collected for total RNA extraction (Castagliulo *et al,* 2001). Total RNA was extracted with PrimeWay Total RNA Extraction Kit (1st BASE, Malaysia) according to the manufacturer's instructions. The quality of the extracted RNA was assessed using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). The total RNA was reverse transcribed using a cDNA Synthesis SuperMix kit (Guangzhou Saturn Health

Technology Co. Ltd, China). Reaction mixtures were incubated at 42°C for 2 min for genomic DNA removal. The reaction mixtures were then incubated (for reverse transcription reaction) in thermal cycler (Sure Cycler, Agilent Technologies. USA) for 15 min at 55°C, followed by 5 min at 85°C. The real-time reverse transcription polymerase chain reaction was conducted in an Mx3005P Real-Time PCR System (Agilent Stratagene, USA) using the SYBR Green PCR Master Mix (Guangzhou Saturn Health Technology Co. Ltd, China) according to the manufacturer's instructions. The PCR conditions were as follows: pre-denaturation at 95°C for 2 minutes, denaturation at 95°C for 10 seconds, annealing at 60°C for 30 seconds and 40 cycles of extension at 72 °C for 30 seconds. Quantitative PCR data were normalized using GAPDH mRNA levels as the internal standard. The relative changes in gene expression were calculated using the $2^{-}(\Delta\Delta CT)$ method. The primers [*P*53 target gene-specific primers (Forward primer: 5′-TCTGTGGCATCTTGGGTTCC-3′; Reverse primer: 5′-AAGAAAGCTGGGCTCCATCC-3′) and GAPDH primers (Forward primer: 5′-ACCACAGTCCATGCCATCAC-3′; Reverse primer: 5′-TCCACCAGCCTGTTGCTGTA -3′)] were synthesized by Inqaba Biotec West Africa Ltd.

Measurement of CEA

Plasma concentration of CEA was measured by Enzyme-Linked Immunosorbent Assay (ELISA) using commercially available CEA ELISA kit (Biovantion Inc, Beijing China), according to manufacturer's protocol. The CEA ELISA kit uses sandwich principle. The absorbance was read using Ensight Multimode Microplate Reader (PerkinElmer, USA).

Measurement of Oxidative Stress

Colorectal tissue homogenates were used to evaluate oxidative stress biomarkers, lipid peroxidation by measuring MDA using the method of Albro *et al,* (1986). TAC was measured

using the Ferric Reducing Antioxidant Power (FRAP) method (Iris and Strain 1996). MDA and TAC were measured using colorimetric method based on Beer-Lambert law.

Histological Analysis of Colorectal Tissues

Colonic tissues were immediately fixed in 10% neutral buffered formalin, processed through graded alcohol dehydration, cleared in xylene, and embedded in paraffin wax. Serial sections (5 µm) were cut using a microtome, mounted on albumin-coated slides, and stained with hematoxylin and eosin (H&E) following standard protocols. Histological sections were examined by a pathologist, after being blinded to grouping, using a Leica DM750 light microscope (Leica Microsystem, USA), and photomicrographs were captured with a Leica DM750 digital camera (Leica Microsystem, USA).

Statistical Analyses

Data were analyzed using IBM SPSS Statistics version 23. Normality was assessed using the Shapiro-Wilk test and Z values of kurtosis and skewness. Data were expressed as mean ± standard deviation. Circadian rhythmicity was analyzed using Cosinor.Online (Molcan, 2019). Paired sample t- test was computed to determine the different between diurnal and nocturnal temperature within each group. Student's t-test for independent groups was utilized to estimate the effect of carcinogen only and that of ALAN on core body temperature. One way ANOVA with Bonferroni correction for multiple comparisons was computed to determine the potentiation effect of ALAN on colorectal cancer outcomes. Statistical significance was set at $P \leq 0.05$.

RESULTS

Diurnal and Nocturnal Core Body Temperature Variations

Core body temperature (CBT) rhythms were analyzed by comparing diurnal (DCBT) and nocturnal (NCBT) averages within each experimental group (Figure 1). Across all groups, nocturnal CBT was significantly higher than diurnal CBT ($P \leq 0.05$), indicating preservation of a diurnal–nocturnal thermoregulatory pattern. However, the magnitude of nocturnal CBT elevation differed among groups. The control (Group A) exhibited a robust nocturnal increase in CBT, whereas animals exposed to carcinogens (DMH+DSS) plus 6hrs ALAN (Group C) showed a comparatively attenuated nocturnal rise. These results demonstrate that although nocturnal elevation of CBT was maintained in all groups, experimental interventions modified the amplitude of the circadian thermoregulatory response.

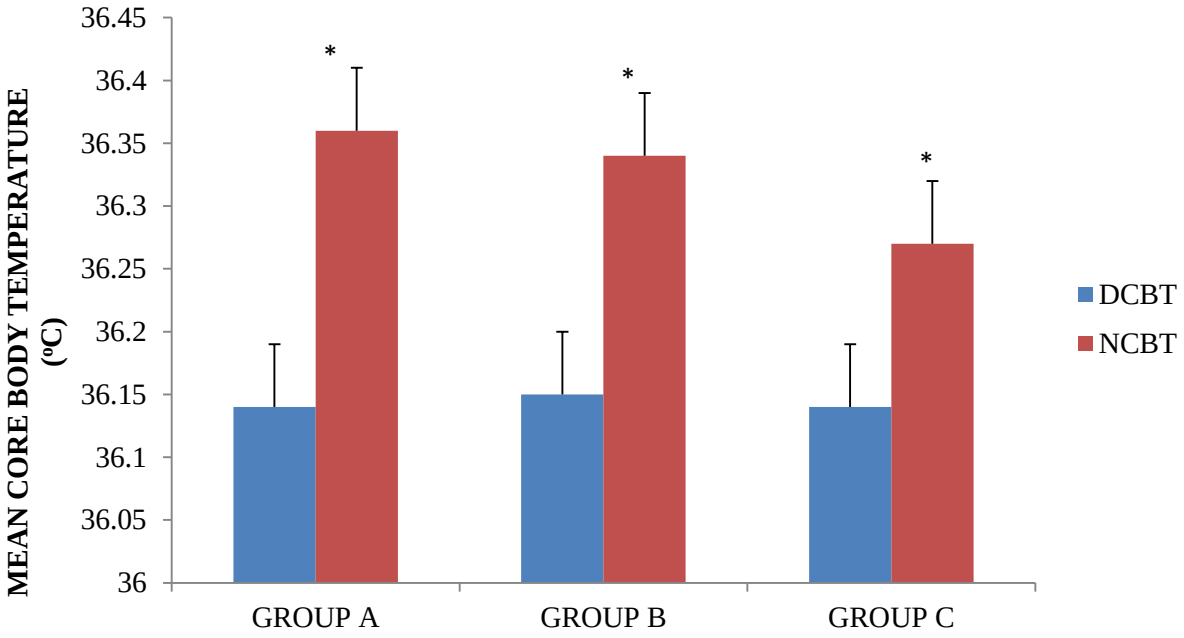

Figure 1: Diurnal vs. Nocturnal Core Body Temperature (CBT) Within Groups. Values are presented as mean ± SD (n= 6 rats). CBT was measured at 3-hour intervals over 24 hours and averaged for diurnal (DCBT, daytime) and nocturnal (NCBT, nighttime) periods. Group A (control), Group B (exposed to carcinogens only), Group C (exposed to carcinogens plus 6hrs ALAN) * Significant differences between DCBT and NCBT within each group were assessed using paired sample t-test ($P \leq 0.05$).

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Effect of Carcinogen Exposure on Core Body Temperature

The impact of carcinogen administration (DMH + DSS) on thermoregulation was evaluated by comparing diurnal (DCBT), nocturnal (NCBT), and 24-hour average CBT in carcinogen treated group (Group B) (Figure 2). No significant differences were observed between DCBT, NCBT, and 24 HRS CBT in this group compared to control ($p > 0.05$), indicating that carcinogen exposure alone did not alter the overall pattern or magnitude of circadian temperature variation. This suggests that DMH + DSS exposure does not intrinsically disrupt core thermoregulatory rhythms.

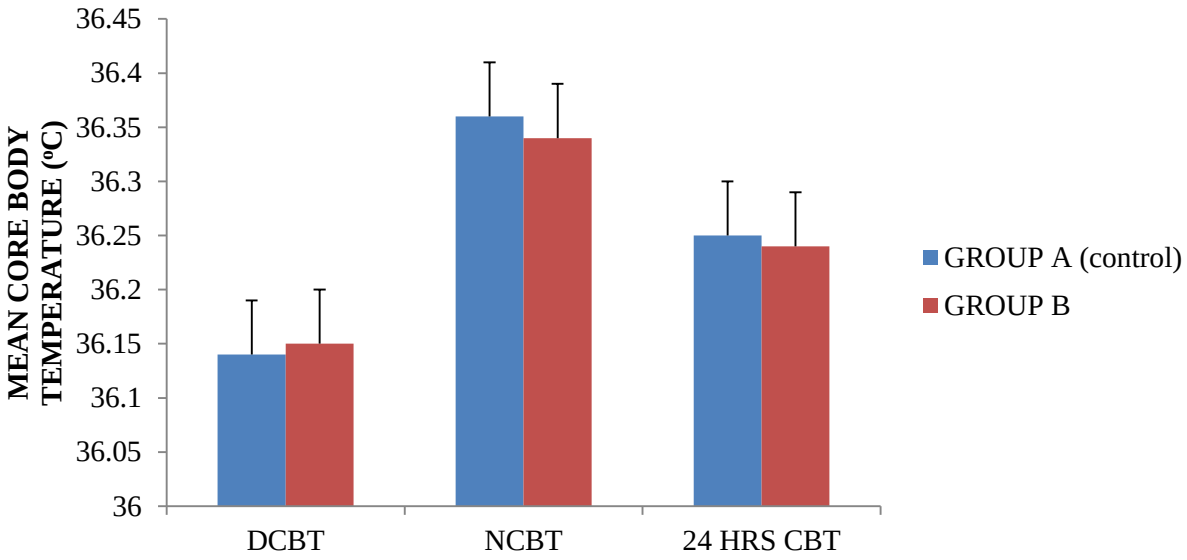

Figure 2: Effect of carcinogen exposure on diurnal, nocturnal and 24-hour core body temperature (CBT). Bars represent mean ± SD (n= 6 rats) for Group A (control) and Group B (exposed to carcinogens only). DCBT = Diurnal Core Body Temperature, NCBT = Nocturnal Core Body Temperature, 24 HRS CBT = 24-hour average core body temperature. Significance between groups was assessed using an independent t-test ($P \leq 0.05$).

Effect of Artificial Light at Night on Core Body Temperature in Carcinogen Exposed Rats

To isolate the effect of ALAN on circadian thermoregulation in a carcinogenic context, we compared Group C (carcinogens+6hrs ALAN) with Group B (carcinogen only) across DCBT, NCBT, and 24 hour CBT (Figure 3). ALAN exposure resulted in a significant reduction in NCBT and 24 HRS CBT compared to the carcinogen-only group ($P < 0.05$), while DCBT remained unaffected.

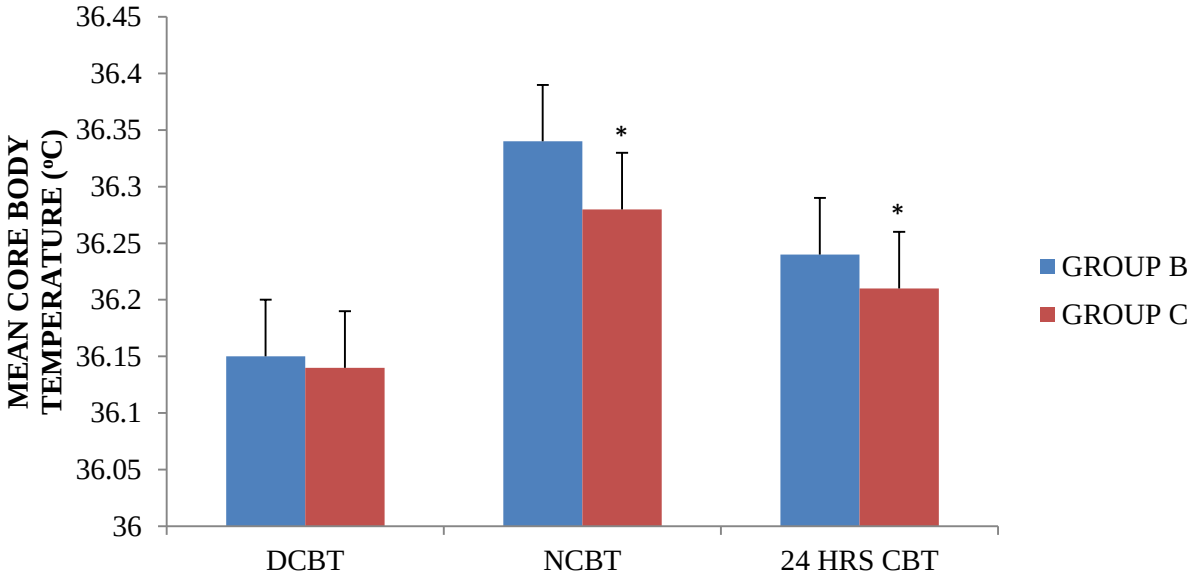

Figure 3: Effect of 6-hour ALAN exposure on diurnal, nocturnal, and 24-hour core body temperature (CBT) compared to carcinogen-only group. Values are presented as mean ± SD (n= 6 rats) for Group B (exposed to carcinogens only) and Group C (exposed to carcinogens+6hrs ALAN). DCBT = Diurnal CBT, NCBT = Nocturnal CBT, 24 HRS CBT = 24-hour average CBT, * Significant difference between groups was assessed using an independent t-test ($P \leq 0.05$).

Circadian Rhythm of Core Body Temperature

The circadian rhythmicity of core body temperature (CBT) was analyzed using Cosinor analysis across the three experimental groups (Table 1). The control group (Group A) exhibited a significant circadian rhythm, characterized by a MESOR of 36.25 °C, an amplitude of 0.26 °C, and an acrophase at 19.37 hrs ($P < 0.05$). Similarly, rats exposed to carcinogens only (Group B) showed a statistically significant rhythmic pattern, with a comparable MESOR (36.24 °C), amplitude (0.25 °C), and acrophase (19.39 hrs; $P < 0.05$). In contrast, the carcinogens + ALAN group (Group C) demonstrated a reduced rhythm amplitude (0.15 °C), a delayed acrophase (20.25 hrs) and a phase shift of 48 minutes delayed with no statistically significant circadian rhythmicity detected ($P > 0.05$). These findings indicate an attenuation and disruption of the normal CBT circadian rhythm in animals exposed to artificial light at night compared with both control and DMH + DSS exposed groups.

Table 1. Cosinor analysis of 24-hour core body temperature rhythm in control and intervention groups.

GROUPS (n=6)	MESOR (^{O}C)	AMPLITUDE (^{O}C)	ACROPHASE (hour)	PHASE SHIFT (DL or AV)
GROUP A (control)	36.25*	0.26	19.37	control
GROUP B (DMH+DSS)	36.24*	0.25	19.39	2 minutes DL
GROUP C (ALAN+Carcinogens)	36.20	0.15	20.25	48 minutes DL

MESOR= Midline Estimating Statistic of Rhythm, n= number of rats per group, ^{O}C= Degree Celsius, carcinogens (DMH + DSS), DMH= Dimethylhydrazine, DSS= Dextran sulfate sodium, ALAN= Artificial light at night, DL = delay, AV = advanced, * = Significant (P<0.05) values

Effects of the experimental interventions on body weight changes across the study period.

Body weights were monitored weekly and are presented according to experimental groups from baseline through post-induction (PI). At baseline, there were no marked differences in body weight among the groups. Over the course of the intervention, differential patterns of weight change were observed. Control animals showed a progressive increase in body weight across the study period. In contrast, animals subjected to carcinogens only (Group B) exhibited reduced weight gain. Similarly, carcinogens + ALAN group (Group C) demonstrated altered weight trajectory compared with control, characterized by attenuated weight gain during the exposure period.

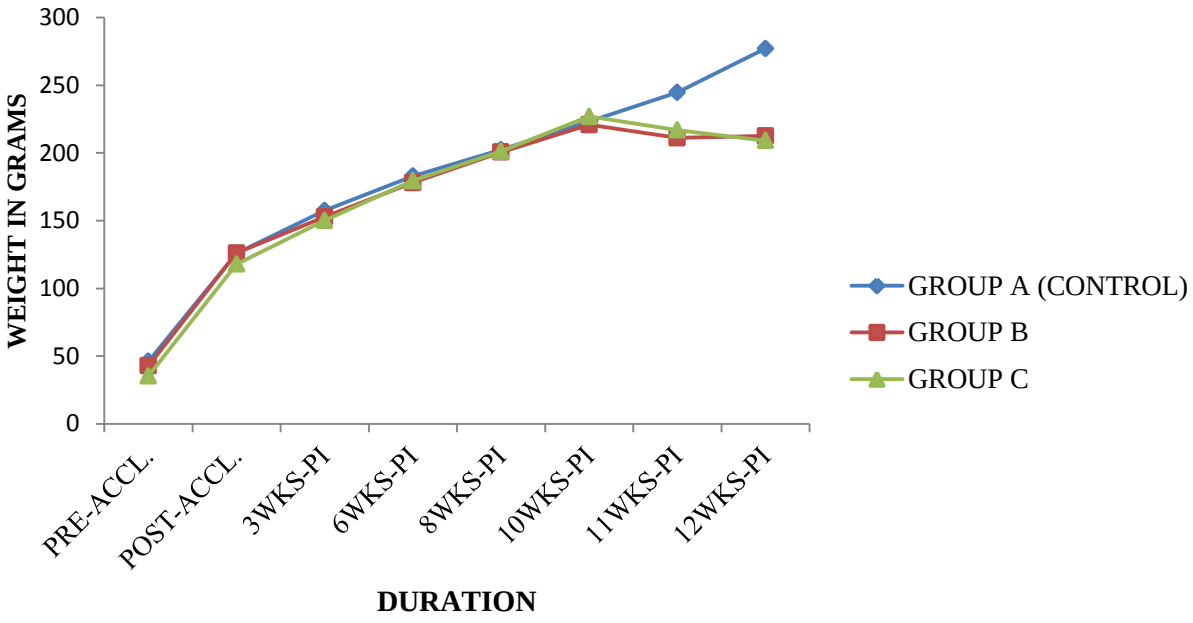

Figure 4: Effect of interventions on weights. GROUP A (control), GROUP B (exposed to carcinogens only), GROUP C (exposed to carcinogens + ALAN), ALAN= Artificial light at night, WKS= weeks, PI= post-induction.

Effect of ALAN on MDA, TAC, CEA and *P*53 Gene Expression in Carcinogens Exposed Rats.

As presented in Figure 5A, serum MDA levels were significantly elevated in the carcinogen-exposed group (Group B) compared with the control (Group A) ($P < 0.05$). However, MDA levels were significantly lower in carcinogens plus ALAN-exposed group (Group C) compared with Group B ($P < 0.05$), indicating a reduction in lipid peroxidation following combined carcinogen and ALAN exposure. Figure 5B demonstrates a significant decrease in TAC in Group B relative to Group A ($P < 0.05$). Although TAC values in Group C were lower than those observed in Group B, this reduction is not statistically significant ($p > 0.05$). Serum CEA levels (Figure 5C) were significantly elevated in the carcinogen-exposed group (Group B) compared with the control (Group A) ($P< 0.05$). A further significant increase in CEA concentration was recorded in combined carcinogen and ALAN exposure (Group C) relative to carcinogen-exposed group (Group B) ($P < 0.05$). Figure 5D shows that *P*53 gene expression was significantly downregulated in carcinogens-exposed group (Group B) compared with control (Group A) ($P < 0.05$). Notably, *P*53 expression was significantly upregulated in combined carcinogen and ALAN exposure (Group C) compared with Group B ($P < 0.05$).

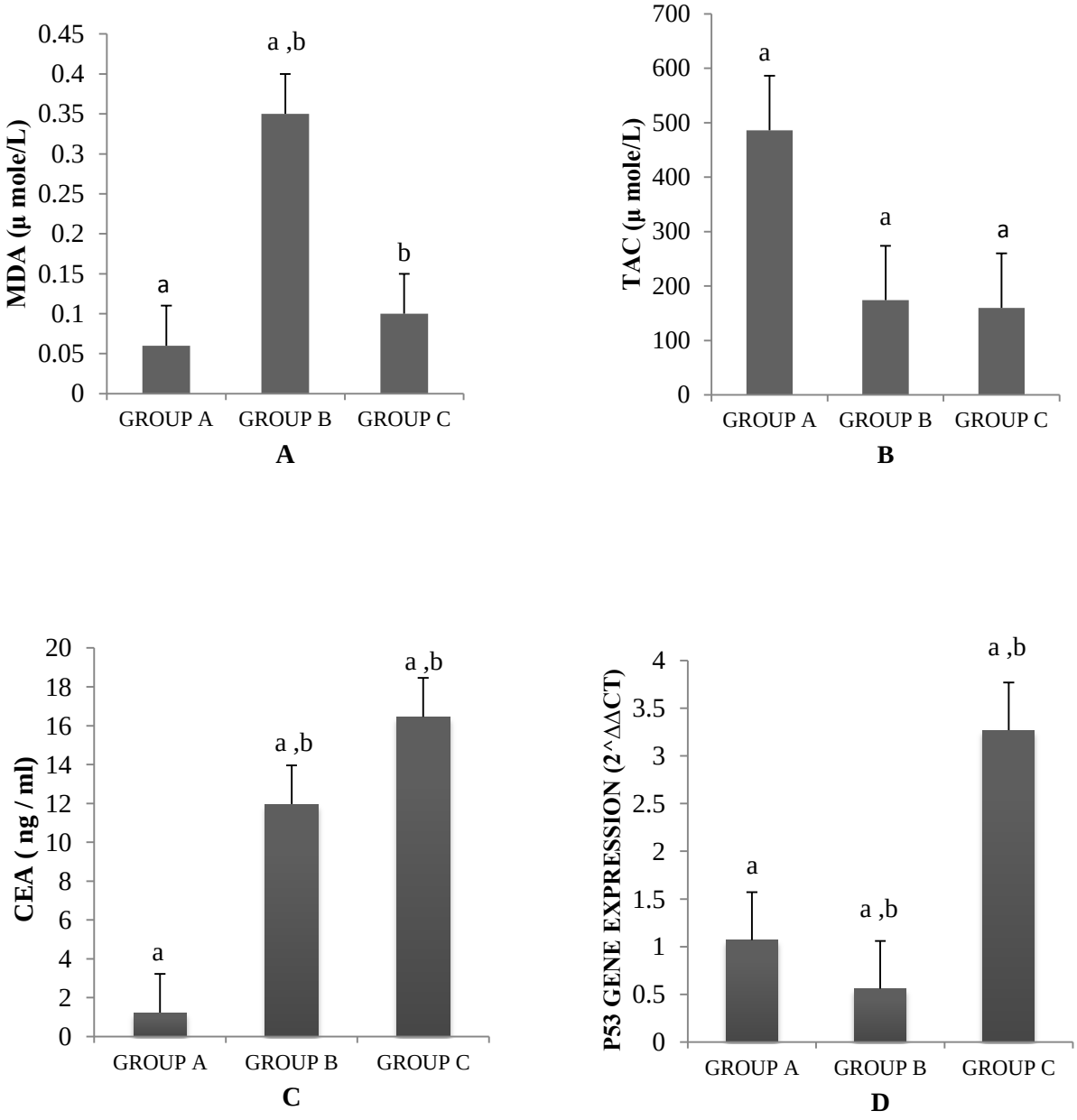

Figure 5: Effect of Artificial Light at Night on MDA, TAC, CEA and *P*53 Gene Expression in Carcinogens Exposed Rats. Malondialdehyde (MDA), total antioxidant capacity (TAC), carcinoembryonic antigen (CEA). Data are expressed as mean ± SD (n = 6 per group). Group A served as the control, Group B received carcinogens alone, and Group C received carcinogens in combination with ALAN exposure. Group with the same lower case letters indicated where there was statistically significant differences ($P<0.05$) by one way ANOVA with Bonferroni's multiple comparisons test.

Effect of ALAN on Cyto-archetecture of Rat's Colon

Histological analysis of H&E-stained colonic sections (×100 magnification) revealed marked differences among the groups (Figure 6). The control group (GR A) showed normal colonic architecture with regularly arranged, straight crypts, intact surface epithelium, preserved goblet cells, and unremarkable muscularis mucosa, submucosa, and muscularis propria, confirming a healthy baseline state (Figure 6a). In contrast, the carcinogen-exposed group (GR B; DMH+DSS) showed marked histopathological alterations. There was pronounced distortion of the mucosal architecture characterized by irregular, shortened, and disorganized colonic crypts with loss of the normal parallel arrangement. Areas of crypt dropout were evident, accompanied by severe inflammatory cell infiltration within the lamina propria (red square). A notable reduction in goblet cell density was observed, suggesting impaired mucosal secretory function. Disruption of the surface epithelium with focal mucosal erosion was also present (blue square). The muscularis propria and serosa remained largely unaffected. Collectively, these changes are consistent with histological features of ulcerative colitis–like pathology.

The combined carcinogen and ALAN exposure group (GR C; DMH + DSS + 6 h LAN) demonstrated more advanced pathological alterations compared with the carcinogen-only group. The colonic mucosa showed crowded, elongated, and tightly packed glands arranged in a back-to-back pattern, with marked loss of normal crypt orientation and reduced intervening lamina propria. These features are indicative of dysplastic glandular changes. Although there was no clear breach of the muscularis mucosae and no evidence of glandular invasion into the submucosa, the presence of submucosal haematoma was noted. Overall, the histological findings in this group are consistent with colitis associated with low-grade dysplasia, representing a clear progression from the colitis state (GR B) toward neoplasia (figure 6b).

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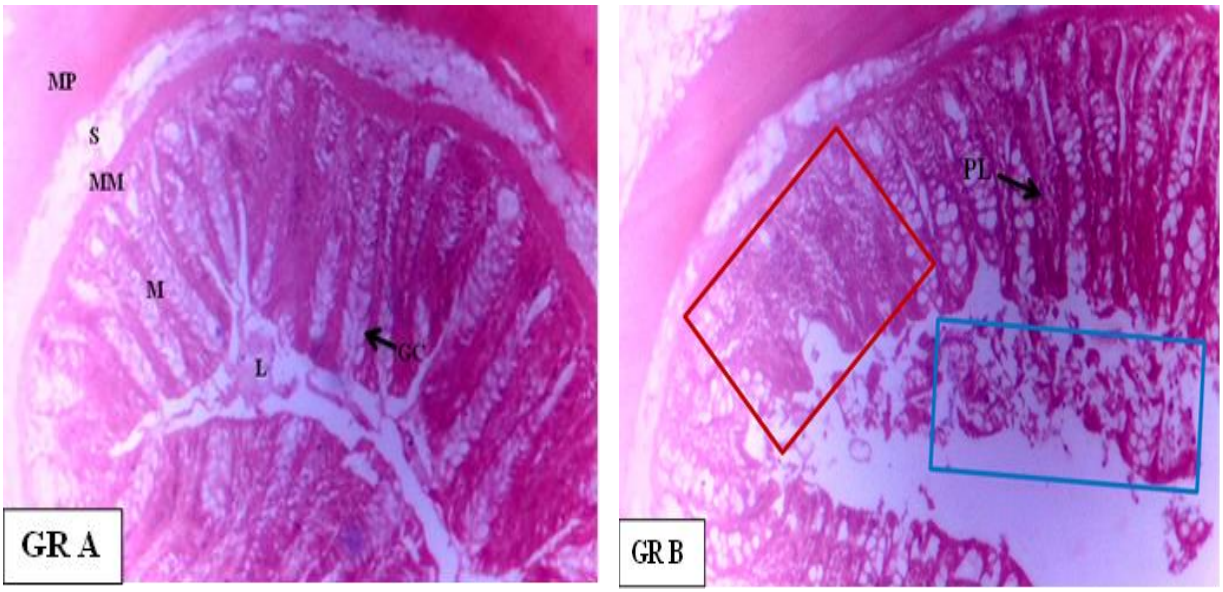
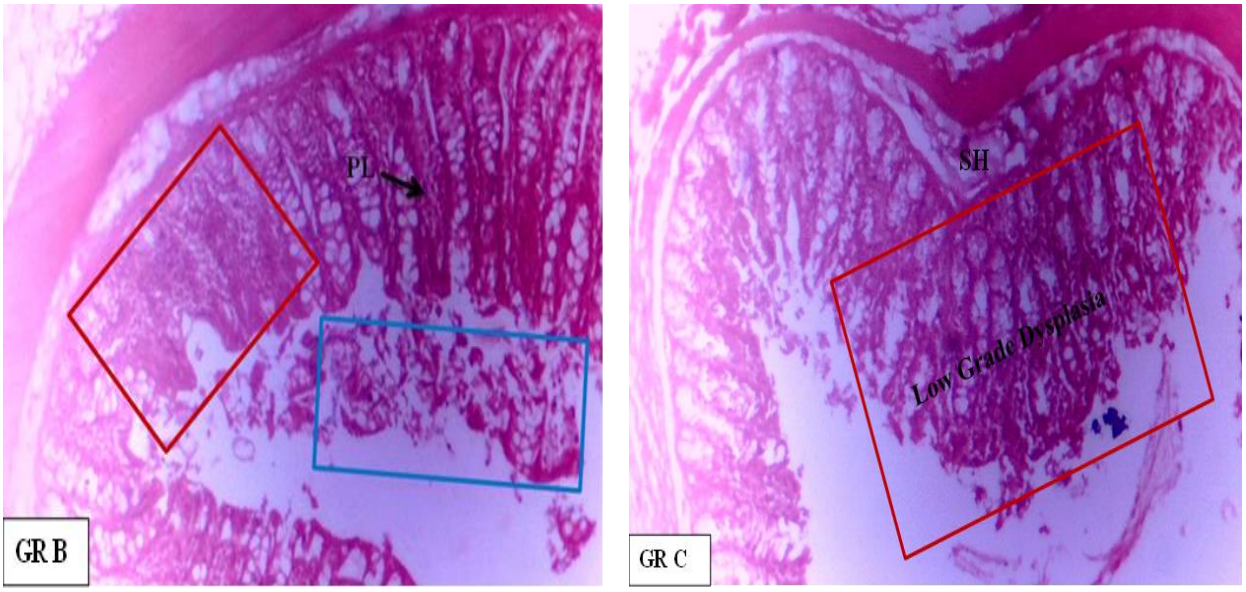


(a)



(b)

Figure 6: Photomicrograph of Rat's Colon of Control Group (GR A), carcinogen- exposed Group (GR B) and combined carcinogens with ALAN exposure group (GR C), M = Mucosa, MM = Muscularis Mucosa, S=Submucosa, MP = Muscularis propria, L= Lumen, GC= Goblet cells, PL = plasma cells, SH= submucosal haematoma, (H&E 100 x).

DISCUSSION

The present study examined the effects of chronic exposure to artificial light at night (ALAN) on circadian rhythmicity and colorectal carcinogenesis in a DMH/DSS-induced colorectal cancer model in male Wistar rats. By integrating physiological, biochemical, molecular, and histopathological outcomes, our findings provide evidence that ALAN-induced circadian disruption modulates tumor-related processes and potentiate colorectal cancer progression.

Our data show that exposure to 6 hours of ALAN (430 lux) significantly attenuated the nocturnal rise in core body temperature (CBT) and disrupted circadian rhythmicity, as evidenced by reduced amplitude and delayed acrophase in the carcinogen + ALAN group (Group C). These findings align with previous reports that ALAN impairs circadian entrainment and dampens circadian outputs (Rabiu *et al,* 2020; Barber *et al,* 2024). The preservation of diurnal-nocturnal CBT differences in all groups indicates that the central circadian pacemaker remains functional, but its output is modulated by environmental light exposure. These findings align with experimental and epidemiological evidence demonstrating that exposure to artificial light at night disrupts the central circadian clock in the suprachiasmatic nucleus and desynchronizes peripheral oscillators in tissues like colon (Mohawk *et al,* 2012; Bedrosian and Nelson, 2017; Barber *et al,* 2024). Circadian disruption has been implicated in impaired regulation of cellular processes such as DNA repair, cell cycle control, and apoptosis, all of which are critical in tumor suppression (Shafi and Knudsen, 2019). The observed attenuation of CBT rhythmicity in ALAN-exposed rats suggests systemic circadian misalignment, which may create a permissive environment for colorectal tumor progression.

Altered body weight trajectories were observed in carcinogen-exposed and LAN-exposed animals compared with controls. Reduced weight gain in carcinogens-exposed rats is consistent with previous reports linking carcinogen-induced intestinal inflammation and metabolic stress to

impaired growth (Tanaka et al., 2003; Potrebić et al., 2022). The additional alteration observed in the ALAN-exposed group may reflect circadian misalignment–induced metabolic dysregulation, as ALAN has been shown to impair energy balance, glucose metabolism, and feeding rhythms (Fonken and Nelson, 2014). The attenuated weight gain observed in ALAN-exposed, carcinogen-treated rats may therefore reflect chronometabolic dysregulation rather than direct effects of light exposure alone. Such metabolic disturbances are increasingly recognized as important modulators of cancer susceptibility and progression, particularly in gastrointestinal tissues with strong circadian regulation.

Oxidative stress plays a central role in colorectal carcinogenesis through the induction of DNA damage, lipid peroxidation, and genomic instability (Boakye *et al,* 2020; Vodicka *et al,* 2020; Lucafo *et al,* 2021). In this study, carcinogen exposure significantly increased malondialdehyde (MDA) levels while reducing total antioxidant capacity (TAC), confirming the induction of oxidative stress by DMH/DSS. These findings are consistent with earlier studies demonstrating enhanced lipid peroxidation and compromised antioxidant defenses in experimental CRC models (Eyvazi *et al,* 2020; Congcong *et al,* 2022). Interestingly, MDA levels were significantly reduced in the carcinogens + ALAN-exposed group compared with carcinogens-only animals, while TAC remained low and unchanged. This paradoxical reduction in lipid peroxidation may reflect ALAN altered timing of sample collection relative to circadian phase of MDA production, ALAN-induced changes in lipid composition reduced peroxidizable substrates or a possibility that DMH/DSS-induced oxidative stress had peaked earlier and the ALAN-exposure had induced adaptive response by the rats. Similar non-linear oxidative responses to circadian misalignment have been reported, where altered timing of antioxidant enzyme expression modifies oxidative damage patterns without restoring redox homeostasis (Pekovic-Vaughan *et al,* 2014). Thus, ALAN may reprogram oxidative stress dynamics rather than ameliorating carcinogen-induced oxidative injury. Previous studies have shown that circadian disruption can

alter redox homeostasis and increase reactive oxygen species (ROS) production, contributing to genomic instability and tumor progression (McClean and Davison, 2022).

CEA is a well-established biomarker for CRC progression and metastasis (Ebrahimi *et al,* 2020). The significant elevation of CEA following carcinogen exposure points to a possible tumor development in this model. The further increase observed in ALAN-exposed animals indicates that circadian disruption may potentiates inflammation-associated expression, increased CEA expression per cell due to increased tumor mass or its altered clearance/metabolism. From a chronobiological perspective, ALAN suppresses nocturnal melatonin secretion, a hormone with well-established circadian, antioxidant, and oncostatic properties (Reiter *et al,* 2024). Although the present study could not measure serum melatonin rhythm, its possible suppression by our ALAN exposure, could remove an important temporal constraint on tumor growth, contributing to increased CEA expression.

One of the most notable findings of this study is the significant upregulation of *P*53 gene expression in the combined exposure to carcinogens and ALAN group compared to the carcinogens-only group. This contrasts with the downregulation observed in the carcinogen-only group relative to controls. Carcinogens exposure significantly downregulated *P*53 expression, consistent with previous reports demonstrating suppression of *P*53 during colorectal tumor initiation (Momma *et al,* 2017; Razi *et al*, 2021). *P*53 is a critical tumor suppressor gene involved in cell cycle arrest, DNA repair and apoptosis. Its upregulation in response to ALAN-associated stress may represent a compensatory cellular defense mechanism against increased genomic instability and dysplasia. However, sustained circadian disruption may ultimately lead to *P*53 dysfunction or inactivation, as seen in many cancers (Momma *et al,* 2017). Importantly, increased *P*53 expression may not necessarily equate to functional tumor suppression, particularly if downstream or apoptotic pathways are impaired. The *P*53 can also be mutated and its expression upregulated as a result of disruption of circadian rhythm by the effect of

exposure to ALAN. Since mutant and wild-type *P*53 segregation was not done in this study, clear understanding of the increased *P*53 expression remains our limitation.

Histological examination corroborated the biochemical and molecular findings. While carcinogen-exposed rats exhibited features consistent with colitis-like pathology, combined carcinogens with ALAN exposure resulted in more advanced dysplastic changes, including glandular crowding and loss of normal crypt architecture. These findings suggest a progression from inflammation to dysplasia, a recognized pathway in colorectal carcinogenesis (Tanaka *et al,* 2003). It is also supported by clinical and epidemiological evidence linking circadian disruption to increased CRC risk (Ariadna *et al,* 2020; IARC, 2020). These findings support the concept that ALAN acts as a disease modifier, accelerating progression along the inflammation–dysplasia continuum.

CONCLUSION

Our study demonstrates that chronic exposure to artificial light at night disrupts circadian thermoregulation, exacerbates oxidative stress, elevates CEA, modulates *P*53 gene expression and alters the rectal histology of the model rat. These findings underscore the potential carcinogenic effect of artificial light induced circadian disruption and highlight the need for public health strategies to reduce artificial light at night exposure. Further research is warranted to elucidate the functional status of *P*53, including mutation analysis and downstream apoptotic signaling, rather than relying on gene expression alone.

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest

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