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Investigation of the anticancer effect of Ramelteon on different human cancers: An in vitro study

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MAIN POINTS

- Antiproliferative effect of Ramelteon: It reduced cell viability in tumour cell lines (A2780, MCF-7, Caco-2, LNCaP).
- Ramelteon exhibited anti-genotoxic properties by suppressing DNA damage.
- Clinical potential: The demonstration of anti-cytotoxic and anti-genotoxic effects of Ramelteon across different tumor cell lines suggests that it may be a candidate for cancer therapy; however, advanced in vitro and in vivo studies are required to elucidate the underlying mechanisms.

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ABSTRACT

Aim: To describe the various methods used to treat cancer, one of the diseases with the highest mortality rates worldwide. Among these methods, the use of agents exhibiting antioxidant activity has increased significantly. Ramelteon, a melatonin receptor agonist, stands out for its antioxidant and anti-inflammatory properties. In this study, we investigated the cytotoxic and genotoxic effects of Ramelteon in different human cancer cell lines.

Materials and Methods: In the study, Ramelteon was applied at concentrations of 5, 10, 25, 50, 100, and 500 µM to human ovarian (A2780), breast (MCF-7), colon (Caco-2), and prostate (LNCaP) cancer cell lines, which were exposed and incubated for 24 hours. The MTT assay was used to measure changes in viability. After obtaining data on dose-dependent effects, inhibitory concentration values were determined. Genotoxicity was examined using the Comet assay at the determined effective dose. Intergroup comparisons were performed using the Kruskal-Wallis H test. When statistically significant differences were found between groups, multiple comparisons were performed using the Bonferroni-corrected Mann-Whitney U tests ($p<0.05$).

Results: Ramelteon significantly reduced cell viability ($p<0.05$) and exhibited genotoxic effects in the A2780, MCF-7, Caco-2, and LNCaP cell lines ($p<0.05$).

Conclusion: Research findings indicate that Ramelteon exhibits anticancer potential in A2780, MCF-7, Caco-2, and LNCaP cell lines, suggesting that the drug may be considered a promising candidate for the development of new cancer treatment strategies.

Keywords: Colon cancer, Melatonin, Breast cancer, Prostate cancer, Ramelteon, Ovarian cancer

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INTRODUCTION

When a cell reaches a certain level of maturity, it divides to promote growth and development. When this process proceeds normally, the organism remains healthy; however, if control of cell division is lost for various reasons, cancer can develop [1]. Results indicate that anti-inflammatory and antioxidant compounds used to reduce inflammation closely associated with carcinogenesis promote programmed cell death (apoptosis) [2-4]. Therefore, the search for effective cancer treatments has led to speculation about the therapeutic potential of certain agents with anti-inflammatory properties. Investigating the anticancer potential of pharmacological agents currently in clinical use offers significant strategic and translational advantages, particularly with regard to compounds exhibiting anti-inflammatory and antioxidant properties. The pharmacokinetic and safety profiles of these agents are well understood; thus, safety concerns can be significantly reduced in early-phase clinical trials [5]. Because chronic inflammation and oxidative stress drive tumor growth and spread (formation, progression, and metastasis), these drugs may fundamentally affect the core determinants of anticancer biology [6].

Melatonin, which has demonstrated anti-inflammatory and antioxidant effects and is produced endogenously, has been studied extensively and shown to exert anticancer effects in multiple reports. Other findings indicate that melatonin promotes autophagy and induces apoptosis, in addition to exhibiting anticancer effects [7-9]. Currently, exogenous forms

of melatonin are used as receptor agonists in pharmacological treatments. The most important characteristics of these pharmacological agents that distinguish them from melatonin are their higher receptor affinity, their greater absorption, and their longer half-life [10]. Ramelteon, a tricyclic synthetic analog of melatonin, is used as a hypnotic agent for the treatment of insomnia. Approved for use in 2005, this agent has a higher affinity for melatonin receptor types 1a (MT1) and 1b (MT2) than melatonin [11]. Short- to intermediate-acting Ramelteon binds to MT1 and MT2 receptors in the suprachiasmatic nucleus, the central circadian pacemaker [12]. Other studies indicate that its half-life is approximately 1–2.6 hours, which is longer than melatonin's half-life of 30 minutes [13, 14]. Research findings indicate that Ramelteon also contributes to memory development by increasing neuronal activity during wakefulness [15]. In addition, Ramelteon has been shown to reduce postoperative delirium, which is commonly encountered in elderly patients with esophageal cancer [16]. Published studies have shown that Ramelteon reduces the viability of neuroblastoma and prostate cancer cell lines by 50% [17]. Another study also suggests that Ramelteon, with its melatonin-like activity, has the potential to be a candidate for the treatment of recurrent endometrial cancer [18].

Although Ramelteon has been reported to be clinically reliable and to provide indirect benefits in oncology practice, and the literature indicates its efficacy in certain cancer types, a detailed review of these studies reveals that its effects on other cancer types have not yet been investigated. This study investigates the effects of Ramelteon on several cancer types, including ovarian, breast, colon, and prostate cancer.

In this context, we aimed to investigate the effect of Ramelteon on ovarian, breast, colon, and prostate cancer cell lines.

MATERIALS AND METHODS

Cell culture

The present study was conducted in the Department of Physiology, Faculty of Medicine, Inonu University. Ovarian (A2780), breast (MCF-7), colorectal (Caco-2), and prostate (LNCaP) cancer cell lines were utilized in this study. The cells were multiplied under suitable conditions [19]. Cultures were maintained at 37°C with 5% CO_2 in an incubator (Esco, Singapore) and were provided with fresh media every three days. When the cells became confluent, they were harvested from the flasks using trypsin-EDTA and stained with 0.4% trypan blue. The number of viable cells was determined by counting using an inverted microscope, and the experiment was initiated only when cell viability was 90% or greater [20, 21]. For the cell viability experiments, 1.5×10^5 cells were plated into a 12 × 8–well plate. Following a 24-hour incubation period, the cells within the plate were exposed to culture medium, dimethyl sulfoxide (DMSO), and Ramelteon. Post hoc power analysis indicated a calculated power [1-beta] of approximately 1, with a type I error (alpha) of 0.05, a sample size of 10, and an effect size of 7.7 for cell viability.

MTT assay method

Following cell seeding, Ramelteon was prepared in 1% DMSO and added to the wells at final concentrations of 5 µM, 10 µM, 25 µM, 50 µM, 100 µM, and 500 µM [17, 18]. The control group received only the cells' own medium, while the solvent group received the same medium containing 1% DMSO. The cells were then incubated for an additional 24 hours, and cell proliferation was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). MTT penetrates the cell membrane of viable cells and, after various enzymatic reactions, is reduced to form a blue-violet product [22, 23]. The MTT assay is employed for cell viability testing precisely because this reaction is restricted to metabolically active cells. Spectrophotometric measurement of the resultant color allows correlation of absorbance with the number of living cells. For analysis, MTT was dissolved in phosphate buffer of 0.5 mg/mL and sterilized via filter. Cell culture media and Ramelteon were removed from the plates, and 50 µL of MTT solution was added to the wells, which were then incubated for 3 hours. After incubation, the MTT solution was removed from the wells, and 100 µL of DMSO was added [24, 25]. The light intensity from the cells in the wells was measured at a wavelength of 550 nm using a BioTek Synergy HTX plate reader (Netherlands) [26]. The average absorbance values obtained from the control wells were accepted as 100% viability, and the results were obtained by comparing the absorbance value obtained from the control group with the average [27]. The absorbance values obtained from the ramelteon and solvent groups were also compared with the control group to calculate the % cell viability [25]. The experimental design of the study is shown in Figure 1. These study steps were repeated at least 10 times on different occasions. After the effects of Ramelteon on cell viability were determined by the MTT assay, the inhibitory concentration 50 (IC_{50}) values of these compounds were calculated using GraphPad Prism 8 software and subsequently used for the Comet assay.

Single-cell gel electrophoresis comet assay methods

Comet analysis, also known as single-cell gel electrophoresis, is widely used to assess DNA damage (genotoxicity) [26]. Comet analysis was performed using the method developed by Devlin et al. [27]. In the first step, microscope slides were coated with 0.65% high-melting-point agarose prepared in phosphate buffer and then incubated in the dark for one day. Ramelteon at the determined IC_{50} concentrations was prepared and used to incubate A2780, MCF-7, Caco-2, and LNCaP cells. The cells were then spread onto slides prepared with low-melting-point agarose, and the slides were covered with coverslips. The protocol began by storing the prepared slides in the dark at +4°C for 20–25 minutes. After removing the coverslips, the slides were lysed for 1 hour at +4 °C

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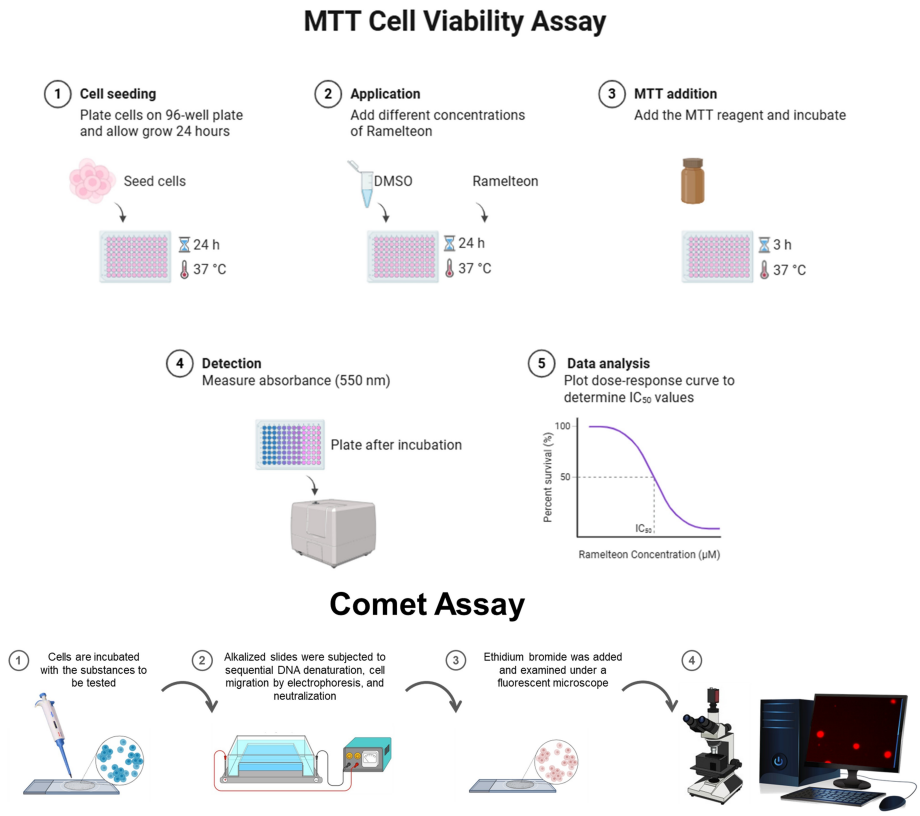

Figure 1. MTT and Comet Assay experimental design (DMSO: dimethyl sulfoxide, IC_{50}: Inhibitory concentration 50, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

in the dark using a cold lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10) containing 1% Triton X-100 and 10% DMSO. Post-lysis, the slides were placed in a horizontal electrophoresis tank (Bio-Rad, USA) filled with cold electrophoresis buffer, and electrophoresis was performed in the dark. Neutralization was then carried out three times, for 5 minutes each, at +4 °C using neutralization buffer (0.4 M Tris, pH 7.5). The slides were then stained with 50 μL of ethidium bromide and incubated for 20–30 minutes. Imaging was performed using a Leica fluorescence microscope, and scoring was performed with Comet IV software. At least 25 cell images per slide were randomly selected for analysis, allowing determination of head intensity and length, tail intensity and length, and olive tail moment for each experimental group. Analyzing the changes in these five parameters was key to assessing the presence and degree of DNA damage [25]. Figure 1 shows the experimental design of the study.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 24.0 (Armonk, NY: IBM Corp.). Normality was confirmed by the Kolmogorov–Smirnov test. Based on the study results, comparisons between groups were conducted using a one-way ANOVA. When significant differences were identified among the groups, post hoc pairwise comparisons were performed using the Bonferroni test. All p-values < 0.05 were considered statistically significant. Based on the MTT assay results, the IC_{50} values of Ramelteon for all cell lines were determined using GraphPad Prism 8. Figures for the study were generated using the BioRender platform.

RESULTS

Investigation of the effects of Ramelteon on cell viability

Cell viability (%) of cancer cell lines treated with Ramelteon at different concentrations after 24 hours of incubation is shown in Figure 2 and Table 1. Exposure of A2780 cells to Ramelteon reduced cell viability compared with the control, and this effect was most pronounced at 50, 100, and 500 μM (p = 0.001) (Figure 2A). Similarly, 24-hour Ramelteon exposure significantly decreased cell viability in the MCF-7 cell line at all tested doses compared with the control (p = 0.001) (Figure 2B). As shown in Figure 2C, Ramelteon also reduced cell viability in the Caco-2 cell line at all doses except the 5 μM dose (p = 0.02). Furthermore, in LNCaP cells, a statistically significant reduction in viability was observed at the higher doses (100 and 500 μM) (p = 0.02) (Figure 2D). Additionally, numerical data related to cell viability are presented in Table 1. Table 2 summarizes the IC_{50} values determined by the MTT assay for A2780, MCF-7, Caco-2, and LNCaP cells after 24 hours of Ramelteon treatment. These IC_{50} findings collectively demonstrate that Ramelteon reduces cell viability by 50% in various cancer cell lines at the lowest tested concentration (Table 2).

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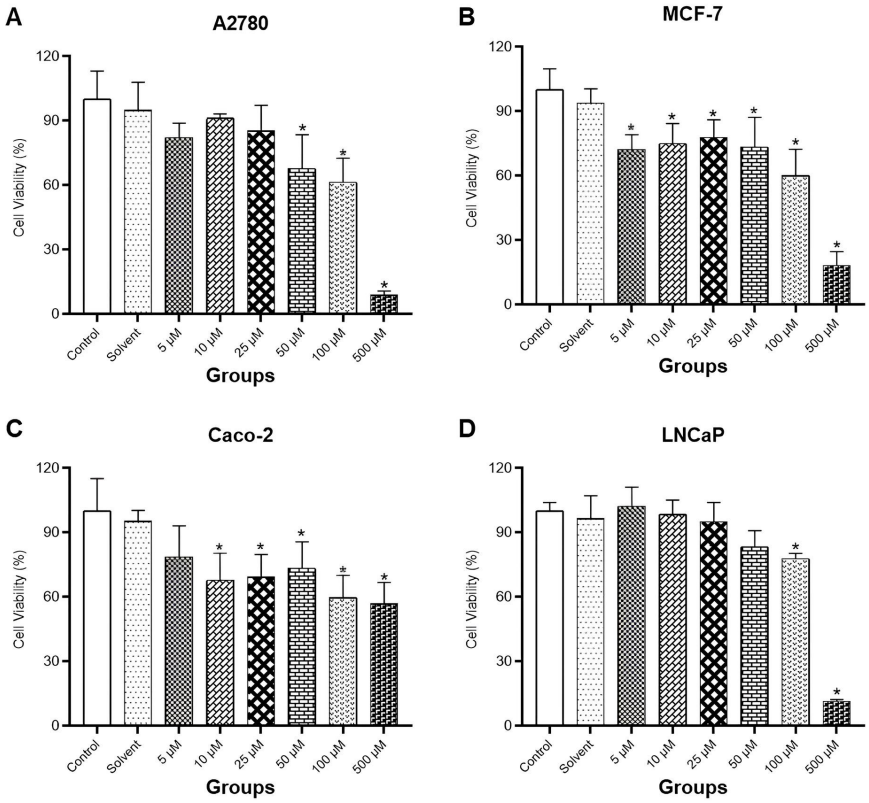

Figure 2. Dose-dependent cell viability results of A2780, MCF-7, Caco-2 and LNCaP cells after 24 h incubation of Ramelteon agents. Each data point is the average of 10 viability measurements. (*) shows p<0.05. (A, A2780 Cell Lines; B, MCF-7 Cell Lines; C, Caco-2 Cell Lines; D, LNCaP Cell Lines).

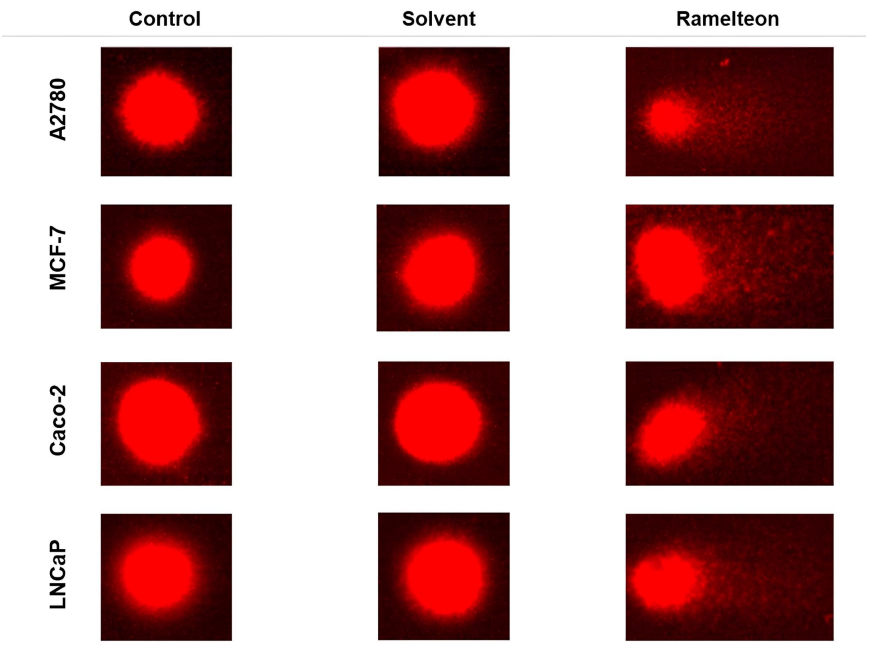

Figure 3. Comet assay images obtained from various cancer cells treated with Ramelteon.

Investigation of the effects of Ramelteon on DNA damage

The extent of DNA damage in cancer cell lines after Ramelteon treatment was quantified using the Comet assay, and the results are presented in Figures 3, 4 and Table 3. The microscopic data (Figure 3) provide qualitative evidence that Ramelteon induced comet formation in the cell lines. Comet assay results showed that Ramelteon significantly increased both head intensity (Figure 4A) and head length (Figure 4B) in A2780, MCF-7, Caco-2, and LNCaP cells ($p = 0.004$). Interestingly, the critical DNA damage parameters tail intensity

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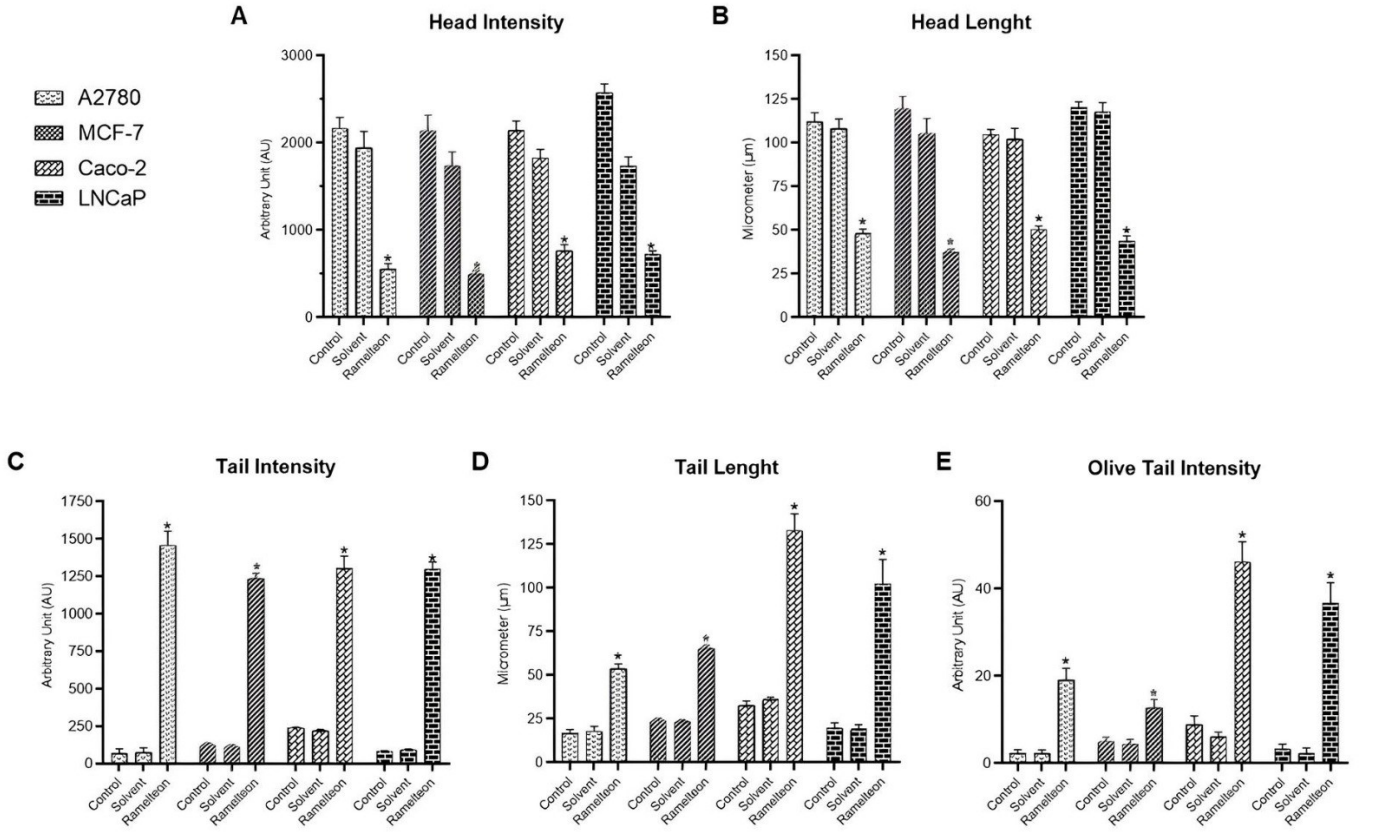

Figure 4. Comet assay obtained from various cancer cells treated with Ramelteon. (*) shows p<0.05.

Table 1. Cell viability results calculated for A2780, MCF-7, Caco-2 and LNCaP cells after a 24-hour incubation with ramelteon compounds.

	Cell Viability (%)			
Groups	A2780	MCF-7	Caco-2	LNCaP
Control	100.00±13.11	100.00±9.85	100.00±15.16	100.00±4.01
Solvent	94.94±13.05	93.94±6.62	95.23±5.12	96.51±10.59
5 µM	82.23±6.71	72.38±6.80*	78.80±14.38	102.30±8.84
10 µM	91.25±2.01	75.04±9.29*	67.96±12.46*	98.51±6.66
25 µM	85.36±11.89	77.83±8.30*	69.31±10.52*	95.13±8.87
50 µM	67.92±15.61*	73.59±13.63*	73.54±12.24*	83.40±7.57
100 µM	61.44±11.24*	60.06±12.28*	59.63±10.55*	77.86±2.51*
500 µM	8.83±1.93*	18.11±6.56*	56.86±10.01*	11.47±0.80*

Each data point is the average of 10 viability measurements. (*) shows p<0.05.

Table 2. IC_{50} (µM) concentrations calculated for A2780, MCF-7, Caco-2 and LNCaP cells in the GraphPad Prism 8 program of 24 h incubation of compounds Ramelteon.

	IC_{50} Concentrations			
Ramelteon (µM)	A2780	MCF-7	Caco-2	LNCaP
	114.8	111.3	186.6	205.2

(Figure 4C), tail length (Figure 4D), and olive tail moment (Figure 4E) were assessed.

The tail moment (Figure 4E) was observed to decrease significantly in all four cell lines following Ramelteon exposure compared with the control (p=0.004). Additionally, numerical data related to DNA damage are presented in Table 3.

DISCUSSION

Studies concerning the antineoplastic effects of melatonin and its analogues have proliferated recently [28]. The anticancer potential of melatonin has been documented; this stems from its ability to inhibit cancer cell proliferation, induce apoptosis, and increase DNA damage [28]. Numerous experimental studies have demonstrated that melatonin has oncostatic effects in several cancers, including breast, ovarian, prostate, oral, stomach, and colorectal cancer [29]. The mechanisms involved include numerous molecular pathways, such as antioxidant activity, modulation of MT1 and MT2, and control of crucial tumor behaviors. These behaviors include apoptosis, metabolism, angiogenesis, invasion, inhibition of metastasis, and induction of epigenetic alterations [30-32]. Melatonin receptor agonists (such as Agomelatin, Tasimelteon, and Ramelteon) are prominent not only are significant therapeutic options for sleep disorders but also for their antioxidant, anti-inflammatory, and antiproliferative effects [33]. In this context, Ramelteon, a selective agonist of the MT1 and MT2 melatonin receptors, was recently approved for the treatment of insomnia characterized by difficulty falling asleep [34]. A review of the literature revealed that Ramelteon reduced cell viability in neuroblastoma, prostate cancer [17], and endometrial cancer cells, and that it exerted its effects via MT1/MT2 receptors

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Table 3. The extent of DNA damage in A2780, MCF-7, Caco-2 and LNCaP cancer cell lines head density, head length, tail density, tail length and olive tail moment were calculated for each experimental group after Ramelteon treatment.

Head Intensity				
Ramelteon (μM)	A2780	MCF-7	Caco-2	LNCaP
Control	2166.84±126.35	2136.180±181.74	2144.05±106.06	2577.52±98.03
Solvent	1941.62±190.47	1731.120±165.610	1827.41±98.22	1737.21±100.78
Effective Dose (IC_{50})	553.62±66.37*	494.240±44.110*	763.98±72.05*	727.12±36.45*
Head Length				
Ramelteon (μM)	A2780	MCF-7	Caco-2	LNCaP
Control	107.75±1.7	120.880±7.37	104.96±2.88	120.24±3.29
Solvent	107.38±3.04	112.300±8.7	102.04±6.3	117.96±5.24
Effective Dose (IC_{50})	47.23±2.14*	36.45±1.75*	50.32±2.05*	43.84±2.92*
Tail Intensity				
Ramelteon (μM)	A2780	MCF-7	Caco-2	LNCaP
Control	67.19 ±31.12	122.120±15.79	239.71±4.89	80.76±5.36
Solvent	73.04±32.17	112.200±14.65	219.78±8.27	92.38±6.28
Effective Dose (IC_{50})	1455.57±96.61*	1233.570±36.870*	1301.15±85.37*	1297.98±48.64*
Tail Length				
Ramelteon (μM)	A2780	MCF-7	Caco-2	LNCaP
Control	19.12±4.52	21.16±4.49	31.34±7.3	24.75±3.31
Solvent	21.91±3.36	20.8±5.74	29.11±6.01	25.08±5.20
Effective Dose (IC_{50})	57.85±5.15*	64.61±4.14*	137.52±10.64*	108.50±5.74*
Olive Tail Moment				
Ramelteon (μM)	A2780	MCF-7	Caco-2	LNCaP
Control	2.23±0.9	4.91±1.13	8.88±1.99	3.36±1
Solvent	2.29±0.8	4.39±1.12	6.07±1.05	2.25±1.3
Effective Dose (IC_{50})	19.01±2.83*	12.62±2*	46.16±4.64*	36.65±4.84*

[18]. Studies have reported that Ramelteon exhibits therapeutic effects on inflammation, apoptosis, oxidative stress markers, and DNA damage [35, 36]. Ramelteon's anticancer mechanism primarily revolves around the drug's agonistic effect on melatonin receptors (MT1 and MT2), although receptor-independent pathways have also been reported [37]. When MT1 and MT2 receptors are overexpressed in cancer cells, Ramelteon binding suppresses adenylate cyclase activity, thereby reducing cyclic adenosine monophosphate levels and slowing cell proliferation [18]. Furthermore, it has been suggested that Ramelteon may indirectly affect antioxidant pathways (e.g., the nuclear factor erythroid 2-related factor 2 signaling pathway), thereby reducing oxidative stress and DNA damage by exhibiting melatonin-like effects [38]. It has been shown that at high concentrations, Ramelteon can directly trigger mitochondrial dysfunction independent of the receptor by disrupting the balance of the Bcl-2 family proteins, activating apoptotic pathways, and thereby exerting cytotoxic effects [39]. All of these mechanisms support Ramelteon's potential to regulate metastasis, angiogenesis, and the cell cycle in different cancer cell types. Additionally, among other literature data, it has been reported that Ramelteon reduces cellular cytotoxicity in neuroblastoma cells in which an ischemic model has been established [35] and in which a neurodegenerative disease model associated with mitochondrial dysfunction and oxidative stress has been created [39, 40]. These preclinical findings necessitate a clinical re-evaluation of Ramelteon's effects in different disease models and may expand opportunities to develop new strategies for drug repositioning and cost considerations. Thus, drugs in this class may have synergistic effects with existing treatment regimens, improve side-effect profiles, and increase patient tolerability [5, 6]. In this regard, the existing literature is largely limited to toxicity and protective models and to studies using a single cell line; the direct antitumor activity of Ramelteon across different cancer types, in multiple human cell lines, in dose-response analyses, and especially in vivo tumor models remains insufficiently elucidated. Our study aims to investigate the effects of Ramelteon on cell lines derived from ovarian, breast, colon, and prostate cancers, which are among the most common cancers from an epidemiological perspective. In conclusion, Ramelteon has been shown to reduce cell viability significantly in ovarian, breast, colon, and prostate cancer cell lines, and to exhibit protective effects on DNA integrity, as demonstrated by comet assay analyses. In particular, the increase in head diameter and head density and the decrease in tail length, tail density, and olive tail moment suggest that Ramelteon exhibits both anti-cytotoxic and anti-genotoxic profiles. In this context, considering our findings alongside published literature, we predict that Ramelteon,

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which exhibits antioxidant and anti-inflammatory properties, may also have anticancer properties and be a suitable candidate for cancer treatment. However, a more detailed understanding of the mechanisms of action and their validation in different in vivo models are necessary for the clinical evaluation of Ramelteon's anticancer potential. In this context, Ramelteon's current safety profile and pharmacokinetic properties allow for the evaluation of the drug's repositioning strategy in oncological applications.

CONCLUSION

In our study, the antiproliferative and genotoxic effects of Ramelteon, a melatonergic agonist, on four human cancer cell lines (A2780, MCF-7, Caco-2, LNCaP) were evaluated collectively for the first time. The findings revealed that Ramelteon reduced cell viability in a dose-dependent manner across all cell lines. Ramelteon has shown protective effects against genotoxicity by suppressing DNA damage. In this context, our study demonstrates that Ramelteon exhibits anti-cytotoxic and anti-genotoxic effects in different tumor cell lines, suggesting that Ramelteon may be a potential therapeutic agent for cancer treatment. However, in order to clarify the mechanisms of this effect, comprehensive in vitro and in vivo studies involving advanced molecular-level analyses are required.

Ethics Committee Approval: Ethical approval was not required because it was a cell culture study.

Informed Consent: Informed consent was not obtained due to the cell culture study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare no conflicts of interest.

Author Contributions: Conception: T.K, G.S; Design: T.K, S.I; Supervision: C.T, S.T, Materials: G.S, S.I; Data Collection and/or Processing: T.K, C.T; Analysis and/or Interpretation: T.K, S.I; Literature Review: G.S, S.I; Writing: T.K, G.S, S.T, Critical Review: C.T, S.T.

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REFERENCES

1. Harris F, Dennison SR, Singh J, Phoenix DA. On the selectivity and efficacy of defense peptides with respect to cancer cells. *Med Res Rev.* 2013;33(1):190-234. doi: 10.1002/med.20252.
2. Zappavigna S, Cossu AM, Grimaldi A, et al. Anti-inflammatory drugs as anticancer agents. *Int J Mol Sci.* 2020;21(7):2605. doi: 10.3390/ijms21072605.
3. Jemel-Oualha I, Elloumi-Mseddi J, Beji A, Hakim B, Aifa S. Controversial effect on Erk activation of some cytotoxic drugs in human LOVO colon cancer cells. *J Recept Signal Transduct Res.* 2016;36(1):21-25. doi: 10.3109/10799893.2014.975246.
4. Chen M, Xu S, Zhou P, He G, Jie Q, Wu Y. Desloratadine citrate disodium injection, a potent histamine H1 receptor antagonist, inhibits chemokine production in ovalbumin-induced allergic rhinitis guinea pig model and histamine-induced human nasal epithelial cells via inhibiting the ERK1/2 and NF-kappa B signal cascades. *Eur J Pharmacol.* 2015;767:98-107. doi: 10.1016/j.ejphar.2015.10.014.
5. Sousa SM, Xavier CPR, Vasconcelos MH, Palmeira A. Repurposing some of the well-known non-steroid anti-inflammatory drugs (NSAIDs) for cancer treatment. *Curr Top Med Chem.* 2023;23(13):1171-1195. doi: 10.2174/1568026623666230130150029.
6. Ioakeim-Skoufa I, Tobajas-Ramos N, Menditto E, et al. Drug Repurposing in Oncology: A Systematic Review of Randomized Controlled Clinical Trials. *Cancers.* 2023;15(11):2972. doi: 10.3390/cancers15112972.
7. Zhao Y, Wang C, Goel A. A combined treatment with melatonin and andrographis promotes autophagy and anticancer activity in colorectal cancer. *Carcinogenesis.* 2022;43(3):217-30. doi: 10.1093/carcin/bgac008.
8. Florido J, Martinez-Ruiz L, Rodriguez-Santana C, et al. Melatonin drives apoptosis in head and neck cancer by increasing mitochondrial ROS generated via reverse electron transport. *J Pineal Res.* 2022;73(3):e12824. doi: 10.1111/jpi.12824.
9. Minocha T, Das M, Rai V, et al. Melatonin induces apoptosis and cell cycle arrest in cervical cancer cells via inhibition of NF-κB pathway. *Inflammopharmacology.* 2022;30(4):1411-29. doi: 10.1007/s10787-022-00964-6.
10. Kast RE. Agomelatine or ramelteon as treatment adjuncts in glioblastoma and other M1-or M2-expressing cancers. *Contemp Oncol (Pozn).* 2015;19(2):157-62. doi: 10.5114/wo.2015.51421.
11. Cajochen C. TAK-375 takeda. *Curr Opin Investig Drugs.* 2005;6(1):114-21. PMID: 15675611.
12. Rawashdeh O, Hudson RL, Stepien I, Dubocovich ML. Circadian periods of sensitivity for Ramelteon on the onset of running-wheel activity and the peak of suprachiasmatic nucleus neuronal firing rhythms in C3H/HeN mice. *Chronobiol Int.* 2011;28(1):31-38. doi: 10.3109/07420528.2010.532894.
13. Stevenson S, Cornelissen K, Clarke E, Hibberd M. Study of the absorption, metabolism, and excretion of (14C)-ramelteon (TAK-375). *Clin Pharmacol Ther.* 2004;75(2):P22-P22. doi: 10.1016/j.clpt.2003.11.084.
14. Kato K, Hirai K, Nishiyama K, et al. Neurochemical properties of ramelteon (TAK-375), a selective MT1/MT2 receptor agonist. *Neuropharmacology.* 2005;48(2):301-10. doi: 10.1016/j.neuropharm.2004.09.007.
15. Kudara M, Kato-Ishikura E, Ikegaya Y, Matsumoto N. Ramelteon administration enhances novel object recognition and spatial working memory in mice. *J Pharmacol Sci.* 2023;152(2):128-35. doi: 10.1016/j.jphs.2023.04.002.
16. Mayanagi S, Haneda R, Inoue M, Ishii K, Tsubosa Y. Ramelteon and suvorexant for postoperative delirium in elderly patients with esophageal cancer. *Esophagus.* 2023;20(4):635-42. doi: 10.1007/s10388-023-01019-7.
17. Balpınar Ö, Nadaroğlu H, Hacımüftüoğlu A. Green synthesis, characterization of melatonin-like drug bioconjugated CoS quantum dots and its antiproliferative effect on different cancer cells. *Mol Biol Rep.* 2023;50(11):9143-51. doi: 10.1007/s11033-023-08817-3.
18. Osanai K, Kobayashi Y, Otsu M, Izawa T, Sakai K, Iwashita M. Ramelteon, a selective MT1/MT2 receptor agonist, suppresses the proliferation and invasiveness of endometrial cancer cells. *Hum Cell.* 2017;30(3):209-15. doi: 10.1007/s13577-017-0169-7.
19. Keser S, Çiftçi EB, Öz S, Tekin S, Kaygılı O. Investigation of in vitro genotoxic, cytotoxic, antiradical and phytochemical properties of endemic Verbascum diversifolium Hochst. *J Phys Chem Funct Mater.* 2025;8(1):15-22. doi: 10.54565/jphcfum.1612825.

https://doi.org/10.5455/annalsmedres.2025.10.293

20. Karataş MO, Tekin S, Alici B, Sandal S. Cytotoxic effects of coumarin substituted benzimidazolium salts against human prostate and ovarian cancer cells. *J Chem Sci.* 2019;131:1-12. doi: 10.1007/s12039-019-1647-0.
21. Özen F, Tekin S, Koran K, Sandal S, Görgülü AO. Synthesis, structural characterization, and in vitro anti-cancer activities of new phenylacrylonitrile derivatives. *Appl Biol Chem.* 2016;59(2):239-48. doi: 10.1007/s13765-016-0163-x.
22. Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J Immunol Methods.* 1986;89(2):271-77. doi: 10.1016/0022-1759(86)90368-6.
23. Horáková Kn, Šovčíková A, Seemannová Z, et al. Detection of drug-induced, superoxide-mediated cell damage and its prevention by antioxidants. *Free Radic Biol Med.* 2001;30(6):650-64. doi: 10.1016/s0891-5849(00)00508-6.
24. Beytur A, Tekin Ç, Çalışkan E, et al. Hexa-substituted cyclotriphosphazene derivatives containing hetero-ring chalcones: Synthesis, in vitro cytotoxic activity and their DNA damage determination. *Bioorg Chem.* 2022;127:105997. doi: 10.1016/j.bioorg.2022.105997.
25. Ozkaya SN, Keskin T, Tekin C, Tekin S, Beytur A. Investigation of the antitumor effects of anti-inflammatory desloratadine and trimebutine on different types of human cancer cells: An in vitro study. *Ann Med Res*. 2023;30(6):727-32. doi: 10.5455/annalsmedres.2023.05.122.
26. Klaude M, Eriksson S, Nygren J, Ahnström G. The comet assay: mechanisms and technical considerations. *Mutat Res.* 1996;363(2):89-96. doi: 10.1016/0921-8777(95)00063-1.
27. Devlin HL, Mack PC, Burich RA, et al. Impairment of the DNA repair and growth arrest pathways by p53R2 silencing enhances DNA damage–induced apoptosis in a p53-dependent manner in prostate cancer cells. *Mol Cancer Res.* 2008;6(5):808-18. doi: 10.1158/1541-7786.MCR-07-2027.
28. Gatti G, Lucini V, Dugnani S, et al. Antiproliferative and pro-apoptotic activity of melatonin analogues on melanoma and breast cancer cells. *Oncotarget.* 2017;8(40):68338-53. doi: 10.18632/oncotarget.20124.
29. Srinivasan V, Spence DW, Pandi-Perumal SR, Trakht I, Cardinali DP. Therapeutic actions of melatonin in cancer: possible mechanisms. *Integr Cancer Ther.* 2008;7(3):189-203. doi: 10.1177/1534735408322846.
30. Mediavilla MD, Sanchez-Barcelo EJ, Tan DX, Manchester L, Reiter RJ. Basic mechanisms involved in the anti-cancer effects of melatonin. *Curr Med Chem.* 2010;17(36):4462-81. doi: 10.2174/092986710794183015.
31. Sánchez-Hidalgo M, Guerrero JM, Villegas I, Packham G, de la Lastra CA. Melatonin, a natural programmed cell death inducer in cancer. *Curr Med Chem.* 2012;19(22):3805-21. doi: 10.2174/092986712801661013.
32. Li Y, Li S, Zhou Y, et al. Melatonin for the prevention and treatment of cancer. *Oncotarget.* 2017;8(24):39896-39921. doi: 10.18632/oncotarget.16379.
33. Liu J, Clough SJ, Hutchinson AJ, Adamah-Biassi EB, Popovska-Gorevski M, Dubocovich ML. MT1 and MT2 Melatonin Receptors: A Therapeutic Perspective. *Annu Rev Pharmacol Toxicol.* 2016;56:361-83. doi: 10.1146/annurev-pharmtox-010814-124742.
34. Devi V, Shankar PK. Ramelteon: a melatonin receptor agonist for the treatment of insomnia. *J Postgrad Med.* 2008;54(1):45-8. doi: 10.4103/0022-3859.39193.
35. Aslankoc R, Savran M, Doğuç DK, Sevimli M, Tekin H, Kaynak M. Ameliorating effects of ramelteon on oxidative stress, inflammation, apoptosis, and autophagy markers in methotrexate-induced cerebral toxicity. *Iran J Basic Med Sci.* 2022;25(10):1183-89. doi: 10.22038/ijbms.2022.62955.13913.
36. Çatakli D, Sevük MA, Coşan S, et al. Ramelteon protects intestinal tissue against injury caused by methotrexate via showing antiapoptotic, antiinflammatory and antioxidant properties. *Bezmialem Sci.* 2022;10(6):770-76. doi: 10.14235/bas.galenos.2022.70783.
37. Mehrzadi S, Pourhanifeh MH, Mirzaei A, Moradian F, Hosseinzadeh A. An updated review of mechanistic potentials of melatonin against cancer: pivotal roles in angiogenesis, apoptosis, autophagy, endoplasmic reticulum stress and oxidative stress. *Cancer Cell Int.* 2021;21(1):188. doi: 10.1186/s12935-021-01892-1.
38. Yang W, Zhang Y, Lu D, et al. Ramelteon protects against human pulmonary microvascular endothelial cell injury induced by lipopolysaccharide (LPS) via activating nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway. *Bioengineered.* 2022;13(1):1518-29. doi: 10.1080/21655979.2021.2021065.
39. Li C, Zhang Y, Liu R, Mai Y. Ramelteon ameliorated 1-methyl-4-phenylpyridinium (MPP+)-induced neurotoxicity in neuronal cells in a mitochondrial-dependent pathway. *Bioengineered.* 2021;12(1):4868-77. doi: 10.1080/21655979.2021.1960767.
40. Qi X, Tang Z, Shao X, et al. Ramelteon improves blood-brain barrier of focal cerebral ischemia rats to prevent post-stroke depression via upregulating occludin. *Behav Brain Res.* 2023;449:114472. doi: 10.1016/j.bbr.2023.114472.