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GENOTOXIC AND CYTOTOXIC EVALUATION OF AGOMELATINE IN HUMAN PERIPHERAL LYMPHOCYTES USING INTEGRATED IN VITRO AND IN SILICO APPROACHES

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BACKGROUND AND AIM: Agomelatine is a widely used antidepressant; however, robust cytogenetic safety data remain limited, particularly regarding potential dose-dependent genotoxicity at therapeutic and supra-therapeutic exposures. To address this gap, we performed a dual in vitro cytogenetic evaluation in human peripheral blood lymphocyte cultures—combining the cytokinesis-block micronucleus (CBMN) assay and the chromosomal aberration (CA) assay—and integrated these findings with in silico docking analyses to explore plausible molecular interactions with DNA and β-tubulin.

The primary aim was to determine whether agomelatine induces (i) chromosomal breakage or missegregation (micronuclei), (ii) structural chromosome damage (chromosomal aberrations), or (iii) cell-cycle suppression/cytotoxicity, across a range of clinically relevant to high concentrations, and to triangulate biological outcomes with mechanistic docking signals.

METHODS (Ethics Committee Approval must be obtained and the number should be specified.): We conducted a controlled experimental study using human lymphocyte cultures with donors treated as biological replicates (n = 10 per group; total N=60), and two technical replicates per donor per condition (averaged before analysis).

Agomelatine was tested at therapeutic doses (25 and 50 mg/L), a lower dose (10 mg/L), and a higher dose (100 mg/L), alongside a negative control and a positive control (mitomycin C), allowing assay validity checks and benchmarking of clastogenic response.

Cytokinesis-block micronucleus assay (CBMN): Micronuclei frequency (MN%) and micronucleated binucleated cell ratio (MNBN%) were quantified as core indicators of chromosomal damage, supported by parallel assessment of proliferation-related indices (e.g., CBPI/cytostasis) and nuclear abnormalities.

Chromosomal aberration assay (CA): Structural aberrations were evaluated via the chromosomal aberration index (CA index) and aberrant cell percentage, with additional reporting of aberration subtypes (e.g., chromatid breaks/fragments), and mitotic activity/dividing cell counts used to contextualize potential cytostatic effects.

In silico analyses: DNA–agomelatine docking and β-tubulin–agomelatine docking were performed to examine whether agomelatine shows energetically plausible binding consistent with intercalation-like genotoxicity or microtubule disruption. Binding affinities and interaction patterns (hydrogen bonds, electrostatic contacts) were compared against canonical reference ligands (ethidium bromide for DNA; colchicine for β-tubulin).

Ethics Committee of Canakkale Onsekiz Mart University, Medical Sciences approved study (10.02.2021, decision No. 2020-02).

RESULTS: Micronucleus-related endpoints (genotoxicity screening): Across all agomelatine concentrations (10–100 mg/L), there was no statistically significant increase in micronuclei frequency (MN%) ($H=9.71$, $p=0.0839$; $\varepsilon^2=0.087$), indicating no detectable elevation in chromosomal breaks or missegregation under the tested conditions. The positive control group showed a significant increase, confirming assay sensitivity and internal validity.

For MNBN%, an overall group difference was detected ($H=22.926$, $p = 0.000349$; $\varepsilon^2 = 0.332$); however, this effect was driven by the positive control: post-hoc testing demonstrated that MNBN% in the 25, 50, and 100 mg/L agomelatine groups was significantly lower than the positive control (adjusted p-values reported), while no significant differences emerged among agomelatine doses. Taken together, the CBMN profile supports a non-genotoxic pattern for agomelatine in this lymphocyte model, especially when benchmarked against the strong clastogenic response of the positive control.

Cell division/cytostasis-related endpoints: Total dividing cells differed between groups ($H=49.997$, $p = 1.39 \times 10^{-9}$; $\varepsilon^2 = 0.833$). As expected, dividing cells were markedly reduced by the positive control, reflecting mitotic suppression from the clastogenic

agent. In agomelatine conditions, values were close to negative control at 10 and 25 mg/L, with only a slight decrease at 50 and 100 mg/L, while remaining significantly higher than positive control at all agomelatine doses—supporting the interpretation that agomelatine does not induce major cytokinesis inhibition, even at the highest concentration.

The tetranucleated cell ratio also differed between groups (H=31.219, $p = 8.48 \times 10^{-6}$; $\varepsilon^2 = 0.486$): it increased significantly in the positive control, whereas all agomelatine groups remained close to negative control and significantly lower than the positive control, supporting preserved cytokinesis integrity under agomelatine exposure.

Chromosomal aberration (CA) outcomes (structural damage): A strong overall group difference was observed for the CA index (H=54.008, $p = 2.09 \times 10^{-10}$; $\varepsilon^2 = 0.908$).

Relative to negative control, no difference was detected at 10 or 25 mg/L, while significant increases emerged at 50 mg/L (Adj. $p = 0.011$) and 100 mg/L (Adj. $p < 0.001$). Notably, despite this rise, aberration values in agomelatine groups remained well below the positive control. Similarly, aberrant cell percentage differed between groups (H=52.800, $p = 3.70 \times 10^{-10}$; $\varepsilon^2 = 0.885$); differences versus negative control were evident only at 50 and 100 mg/L, while 10 and 25 mg/L were comparable to negative control.

When aberration subtypes were examined, the most frequent abnormalities in agomelatine groups were chromatid breaks and fragments. A dose-related increase was noted at 50–100 mg/L, while chromosomal breaks and dicentrics remained low, indicating that observed damage at higher concentrations was limited in magnitude and pattern compared with classical clastogenic exposure.

In silico docking triangulation (mechanistic plausibility): DNA docking yielded an agomelatine binding affinity of –7.309 kcal/mol, notably weaker than the reference intercalator ethidium bromide (–9.5 kcal/mol). Agomelatine formed multiple hydrogen bonds (ADE17, CYT9, GUA10; 2.32–2.56 Å), consistent with moderate, likely transient DNA binding rather than classic intercalation. Structural inspection supported minor groove residence without base-pair separation, contrasting with ethidium bromide's intercalative distortion. For β-tubulin, agomelatine showed a binding affinity of –7.389 kcal/mol, similar to colchicine (–7.365 kcal/mol), with stabilizing hydrogen-bonding and electrostatic interactions.

CONCLUSIONS: In this integrated in vitro–in silico evaluation, agomelatine demonstrated a reassuring cytogenetic safety profile in human lymphocytes across clinically relevant to high concentrations. The CBMN assay showed no significant increase in micronuclei frequency, while MNBN% findings primarily reflected separation from the positive control rather than dose-dependent damage among agomelatine groups. In contrast, the chromosomal aberration assay detected a dose-linked signal at ≥50 mg/L, with small increased CA index and aberrant cell percentage versus negative control, yet still far below the clastogenic reference condition. Mechanistically, docking results support non-intercalative minor-groove DNA binding with weaker affinity than classical DNA intercalators, aligning with the absence of a robust micronucleus signal.

Overall, the data suggest that therapeutic-range exposure is unlikely to confer meaningful genotoxic risk, whereas supra-therapeutic concentrations may modestly increase structural chromosomal aberrations, warranting attention in contexts such as overdose, impaired clearance, or extreme exposure scenarios. This dual-platform approach provides a strong, award-competitive framework for resolving real-world safety questions by aligning cytogenetic endpoints with mechanistic docking evidence.

Keywords: Micronucleus assay, chromosomal aberration, peripheral blood lymphocytes, OECD TG 487, OECD TG 473, molecular docking

Table 1. Key findings in the Cytokinesis-Block Micronucleus (CBMN) test: No increase in MN in agomelatine groups

Groups (n = 10)	MN [Median (IQR)]	MNBN [Median (IQR)]	MI [Median (IQR)]	Abnormall cell [Median (IQR)]
Negative Control (dH_2O)	6.50 (5.75–8.25)	3.00 (2.00–3.25)	50.80 (50.60–51.50)	7.00 (6.00–7.25)
Positive Control (MMC)	18.50 (17.50–22.50)	8.50 (7.00–9.25)	27.50 (28.60–30.00)	34.50 (33.00–36.25)
Agomelatine 10 mg/L	6.00 (4.75–6.00)	2.00 (2.00–2.00)	48.20 (46.40–49.50)	7.00 (6.00–8.00)
Agomelatine 25 mg/L	7.00 (5.75–8.00)	2.00 (2.00–3.00)	46.00 (45.10–49.20)	10.00 (8.50–11.25
Agomelatine 50 mg/L	8.00 (7.00–8.25)	2.50 (2.00–3.00)	43.60 (42.00–46.90)	13.00 (11.00–16.00)
Agomelatine 100mg/L	9.50 (9.00–11.00)	2.00 (1.75–3.00)	40.60 (39.10–42.80)	17.50 (15.00–19.00)

MN: Micronucleus; MNBN: Micronucleated binucleated cells; MI: Mitotic index; MMC: Mitomycin-C; IQR: Interquartile range.

Table 2. Chromosomal Aberrations: Aberration spectrum and total abnormal cell load

Groups (n=10)	Chr. break	Chr. exchange	Gap	Chroma tid break	Chroma tid exchange	Fragment	Polyploid	Duplication	Abnorm all Cell (total)
Negative Controls	2	6	1	4	2	1	1	2	19
Positive Controls	6	6	3	26	5	8	2	-	56
Agomelati ne 10 mg/L	3	-	-	4	1	1	-	-	9
Agomelati ne 25 mg/L	2	1	1	5	-	3	-	-	12
Agomelati ne 50 mg/L	2	1	-	11	1	4	1	-	20
Agomelati ne 100 mg/L	3	2	2	13	2	5	1	2	30

Kısaltmalar: Chr: Chromosome; MMC: Mitomycin-C.