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Investigating the cytotoxicity and genotoxicity of Vortioxetine with in vivo and in silico methods

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Vortioxetine is a multimodal antidepressant with reported benefits on cognitive functioning, social functioning, and fatigue, however its potential genotoxic risks have not been adequately studied. Therefore, this study aims to investigate the cytotoxicity and genotoxicity of vortioxetine in vivo and in silico. Genotoxic effects were assessed using Chromosome aberration (CA) and micronucleus (MN) assays in cultured human peripheral blood lymphocyte. Cytotoxicity was evaluated through Mitotic index (MI), and DNA interaction was analysed by UV titration and agarose gel electrophoresis. In silico analyses were performed with Attraction Cavities and AutoDock Vina methods. Experimental results demonstrate that vortioxetine binds to Calf Thymus DNA (CT-DNA) through intercalative interactions and cleaves pBR322 DNA in the presence of hydrogen peroxide. DNA binding studies indicated that groove binding is the effective interaction between vortioxetine and CT-DNA (Kb: 6.25×10^5 M^{-1}). It was also supported by molecular docking results, where binding affinities of vortioxetine and escitalopram were − 7.29 and − 7.69 kcal/mol for Attracting Cavities and − 6.01 and − 6.57 kcal/mol for AutoDock Vina. When comparing vortioxetine to escitalopram, both drugs were found to be potentially genotoxic. These findings suggest a potential genotoxic risk with prolonged use and provide valuable insight for clinicians in evaluating long-term safety.

Keywords Vortioxetine, Cytotoxicity, Genotoxicity, Depression, Molecular docking

Major depressive disorder (MDD) is a condition that results in an impairment of the psychosocial function of the central nervous system[1]; and affects people's emotions and behavior, causing persistent sadness, loss of enjoyment, insomnia, appetite problems, and inability to concentrate[2,3]. MDD is characterized by high rates of recurrence and chronicity, resulting in both physical and psychosocial disability, and it is often accompanied by cognitive, motor, and somatic symptoms. The pharmacotherapy used to treat depression alters the synthesis, release, conversion, and reuptake of monoamines, including norepinephrine, serotonin, and dopamine[4]. This treatment encompasses tricyclic antidepressants and monoamine oxidase inhibitors, known as first-generation antidepressants, as well as selective serotonergic reuptake inhibitors (SSRIs) and serotonergic and norepinephrine reuptake inhibitors (SNRIs), referred to as second-generation antidepressants.

Vortioxetine is a multimodal antidepressant that acts as a serotonin modulator and stimulant. It functions by directly modulating the serotonin receptors and easily crosses the blood-brain barrier. This molecule is designed to alleviate depressive symptoms and maintain treatment response by functioning as an antagonist, agonist, and partial agonist at multiple serotonin receptors[5,6]. In addition to its antidepressant and anxiolytic effects, vortioxetine also provides improvements in cognitive function[7]. Vortioxetine has approval from both the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). It is complemented with a starting dose of 10 mg daily, increasing up to 20 mg daily.

Due to the lack of sufficient studies on pregnant women and the risks to the fetus shown in animal studies, we have classified vortioxetine as pregnancy category C. Preliminary information on vortioxetine toxicity has been gathered to address specific scenarios, such as embryonic brain and central nervous system development during pregnancy and lactation. Motivated by these considerations, this study aims to evaluate the genotoxic effect of vortioxetine doses, commonly used in psychiatric clinical practice, on lymphocyte cells isolated from human

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blood, by in vitro tests such as micronucleus, mitotic index, and abnormality. Additionally, the interactions of vortioxetine with DNA were determined by in vitro and in silico methods and it was evaluated whether it has a potential genotoxic effect. Based on these considerations, it was hypothesized that vortioxetine might exhibit dose-dependent cytotoxic and genotoxic effects on human peripheral lymphocytes, mediated primarily by its interaction with DNA, as evaluated through in vitro and in silico approaches. The findings from these studies could offer helpful details about the safety profile of vortioxetine, particularly concerning its long-term use in clinical settings. Further research is necessary to elucidate the precise mechanisms underlying its cytotoxic and genotoxic effects, as well as to explore potential protective strategies against such outcomes.

Vortioxetine is a third-generation multimodal antidepressant approved by both FDA and EMA. However, its long-term safety, especially regarding DNA interaction, has not been fully studied. Unlike other antidepressants such as escitalopram, there is little data on vortioxetine's genotoxicity. Because antidepressants are often utilized for long periods, understanding their potential DNA-related side effects is important. This study is the first to investigate vortioxetine's genotoxic effects using in vitro assays and in silico molecular docking, providing a broader view of its safety.

Results

In vitro genotoxic and cytotoxic effects of Vortioxetine

To determine the genotoxic and cytotoxic potential of vortioxetine in vitro on human lymphocytes, six different indicators (MN, BN, TN, CA, MI) were evaluated at three concentrations (5, 10, 20 µg/mL) for 48 h exposure. The effects of vortioxetine on Micronucleus (MN), Binucleus (BN), MN frequency in BN cells (MNBN) and Tetra nucleus (TN) frequency are shown in (Table 1).

The formation of MN, BN and TN was induced by vortioxetine in a dose-dependent manner, whereas only BN was triggered significantly depending on dose (Fig. 1). A dose of 20 mg/kg vortioxetine and escitalopram significantly induced micronucleus formation when compared with the negative control ($p<0.005$), although this potential was not as significant as the positive control.

In our study, vortioxetine and escitalopram exhibited similar effects on the formation of MN, indicating that both compounds are safe in terms of MN formation. In terms of BN formation, vortioxetine at 20 mg/kg is more effective than escitalopram in inducing BN formation (p:0.008). Concerning TN formation, while there is a dose-dependent increase in TN, all doses of vortioxetine and escitalopram have a similar potential to induce TN formation and are safe when assessed in terms of this parameter (Table 2).

The highest dose of vortioxetine (20 mg/kg) and escitalopram (20 mg/kg) significantly increased the frequency of chromosomal aberrations compared to the negative control ($p<0.005$). A marked decrease in the mitotic index was observed at all doses of vortioxetine and escitalopram (Table II). The results demonstrated that the occurrence of chromosomal anomalies increased in a concentration-dependent manner. However, the frequency or index of chromosomal anomalies in cells treated with vortioxetine or escitalopram did not differ significantly from the positive control (Fig. 2).

DNA binding

The UV-Vis absorption titration method is widely employed to investigate the interaction of small molecules with DNA[8,9]. Therefore, we used the UV-Vis absorption titration method in our study to investigate the interaction of vortioxetine with DNA. Figure 3 illustrates the changes in the UV-Vis absorption spectrum of vortioxetine upon the incremental addition of CT-DNA. The results demonstrate a 27.43% hypochromism (i.e., a reduction in absorption intensity) and a 7 nm bathochromic shift (redshift) at 232 nm, indicating significant interactions between vortioxetine and CT-DNA.

The intrinsic binding constant Kb (binding constant) was calculated using Eq. 1 below.

$$[\mathrm{DNA}]/(\varepsilon\mathrm{A}-\varepsilon\mathrm{f}) = [\mathrm{DNA}]/(\varepsilon\mathrm{B}-\varepsilon\mathrm{f}) + 1/\mathrm{Kb}(\varepsilon\mathrm{B}-\varepsilon\mathrm{f}) \quad (1)$$

In the formula, [DNA] represent the concentration of DNA base pairs, while the apparent molar absorption coefficients for the compound in the presence of DNA, in its free form, and when fully bound to DNA are denoted as εa, εf, and eB, respectively. The value of Kb is ascertained from the ratio of the slope to the intercept. The calculated Kb value for vortioxetine, indicating hypochromism, was $6.25\times10^5\ \mathrm{M}^{-1}$.

Treatments	Concentration	MN	BN	MNBN	TN	Abnormal Cells*	%Abnomality Frequency
dH_2O	-	4.5 ± 1.17	8.8 ± 1.23	2.00 ± 0.47	2.3 ± 1.49	17.6 ± 2.76	1.61 ± 0.20
MMC	0.3 µg/mL	20.1 ± 4.20	34.6 ± 2.76	8.10 ± 1.60	8.70 ± 2.5	71.5 ± 5.13	6.44 ± 0.69
Vortioxetine	5 µg/mL	4.90 ± 0.88	10.3 ± 0.83	2.00 ± 0.47	2.50 ± 1.43	19.7 ± 2.06	1.81 ± 0.22
	10 µg/mL	6.30 ± 1.06*	12.1 ± 1.52	2.30 ± 0.83	2.60 ± 1.35	23.3 ± 2.45	2.15 ± 0.26
	20 µg/mL	7.40 ± 1.07*	16.0 ± 1.63	2.30 ± 0.68	3.10 ± 1.29	28.8 ± 2.30	2.73 ± 0.25
Escitalopram	20 µg/mL	7.50 ± 0.85*	12.5 ± 1.51	2.00 ± 0.82	2.40 ± 0.83	24.4 ± 1.65	2.2 ± 0.83

Table 1. The comparison of micronucleus (MN), binucleate (BN), BN + MN, tetranucleate (TN), total abnormal cells and abnormality frequency values between the groups for 48-h treatment periods. Data are expressed as mean ± SD ($n=10$) and representing values per 1000 cells. MMC are represented as Mitomycin C.

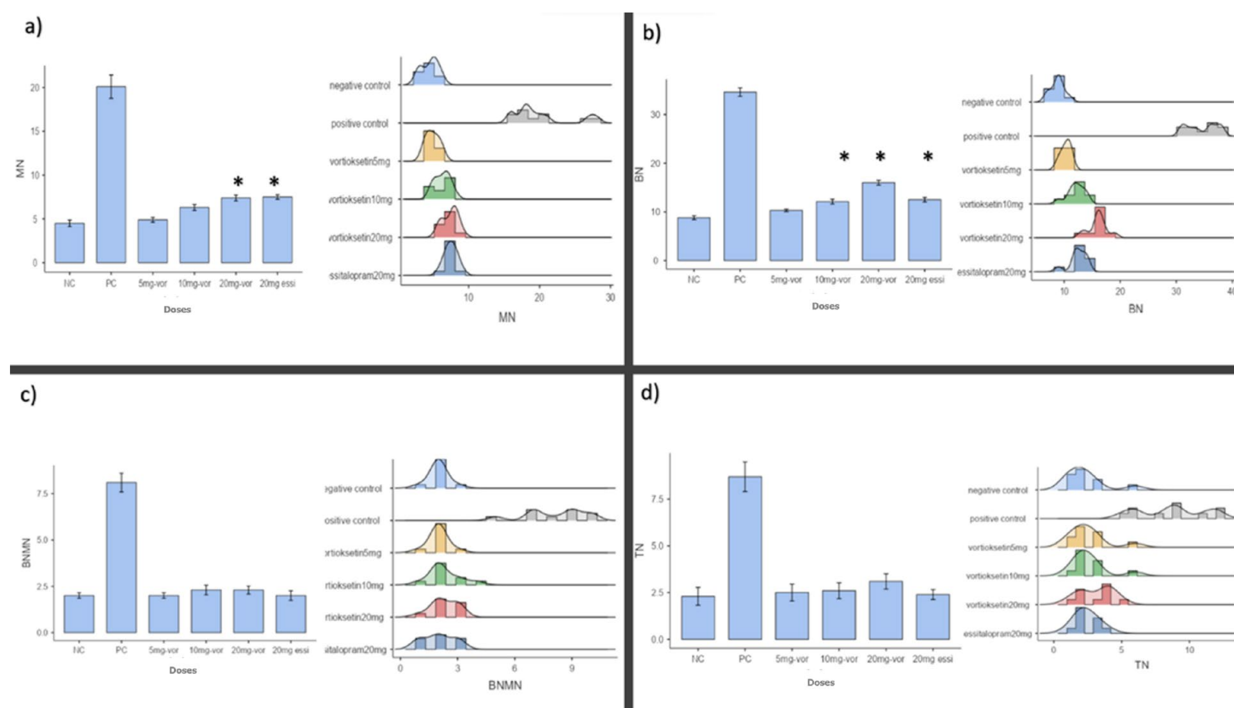

Fig. 1. Distribution of (**a**) micronuclei, (**b**) binuclei, (**c**) Micronuclei in binucleated cells, (**d**) tetra nuclei between groups (right) and individuals (left) Data are expressed as mean ± SD in ten healthy individuals, analyzed per treatment; Significance level tested by Kruskal Wallis followed by Dunn's test, Significant from negative control (dH_2O). *$p < 0.05$.

Treatments	Concentration	Chromosomal Anomaly Frequency	Chromosomal Anomaly Index	Chromosomal Anomaly Mitotic Index
dH_2O	-	5.48 ± 0.66	2.80 ± 0.38	51.00 ± 1.04
MMC	0.3 µg/mL	48.60 ± 2.23	13.96 ± 0.72	28.76 ± 1.65
Vortioxetine	5 µg/mL	5.35 ± 0.87	2.52 ± 0.38	47.24 ± 1.47
	10 µg/mL	6.95 ± 1.28**	3.24 ± 0.61	46.64 ± 1.61
	20 µg/mL	9.84 ± 1.61**	4.28 ± 0.68*	43.56 ± 1.89
Escitalopram	20 µg/mL	9.08 ± 1.53**	4.32 ± 0.67*	47.72 ± 2.47
	ε^2	0.832	0.804	0.785
	p	< 0.001	< 0.001	< 0.001

Table 2. Consequences of chromosomal aberrations of Vortioxetine and escitalopram. Data are expressed as mean ± SD ($n = 10$), MMC are represented as Mitomycin C, Kruskal-Wallis, Significant from negative control (dH_2O). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

In silico DNA binding study

The binding affinities of two drugs, vortioxetine and escitalopram, with DNA (PDB ID: 195D) using two computational methods: the AutoDock Vina method and the Attracting Cavities method. The interactions between molecules and DNA can be broadly categorized into two main types: covalent and/or non-covalent interactions (intercalation, electrostatic or groove binding). Docking studies revealed that both Vortioxetine and Escitalopram bind the minor grooves of DNA with binding in both methods.

The Attracting Cavities Method reveals that vortioxetine's binding affinity increases to -7.29 kcal/mol, while escitalopram's affinity strengthens further to -7.69 kcal/mol (Table 3). Considering that the AC score of vortioxetine (53.7650) significantly exceeds that of escitalopram (6.18), it can be deduced that the binding surface of the vortioxetine molecule on DNA is more extensive. RMSD value reported by the Attracting cavities method (e.g. 32.4385 Å for vortioxetine and 153.6072 Å for escitalopram) are not comparable to classical docking metrics such as those from AutoDock Vina. This value is likely calculated as spatial deviations from seed conformation in a large simulation box and therefore does not indicate binding stability. For this reason, these RMSD values are not used as indicators of docking quality. Instead, we compared binding affinities and interaction types across docking methods (Fig. 4).

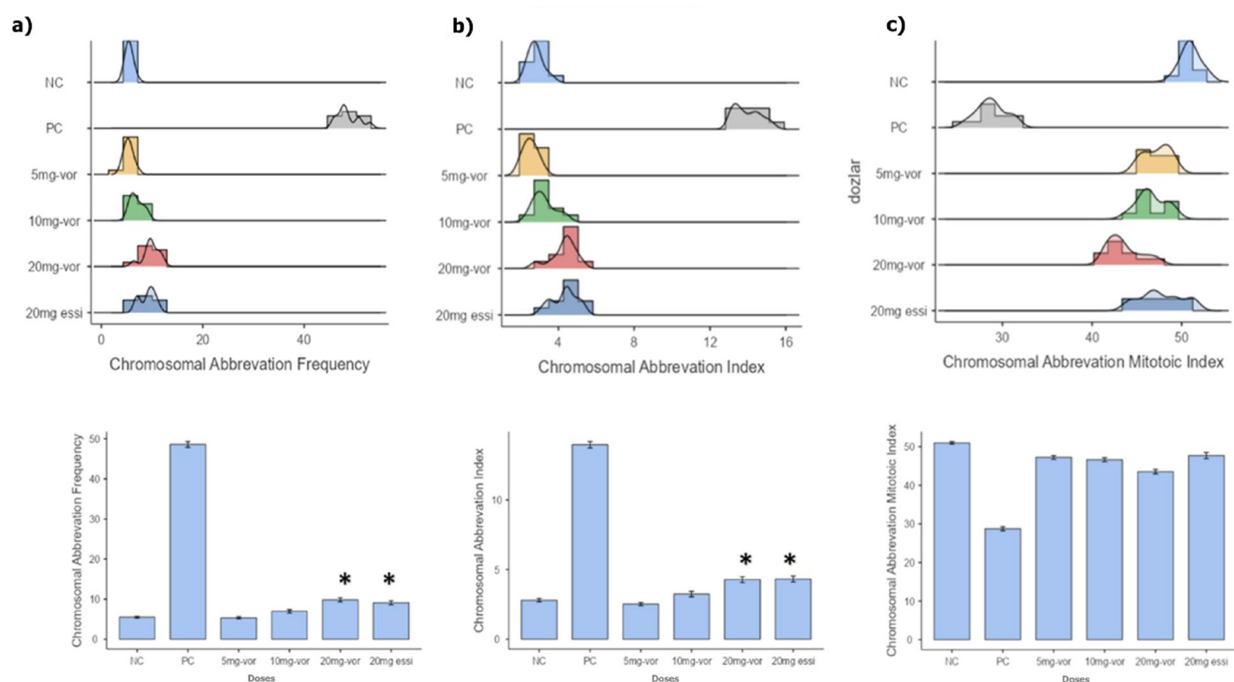

Fig. 2. Distribution of (**a**) chromosomal abbreviation frrequency (**b**) chromosomal abbreviation index (**c**) chromosomal abbreviation / Mitotic Index (**d**) mitotic İndex between groups (right) and individuals (left) Data are expressed as mean ± SD in ten healthy individuals, analyzed per treatment; Significance level tested by Kruskal Wallis followed by Dunn's test, Significant from negative control (dH_2O). *$p < 0.05$.

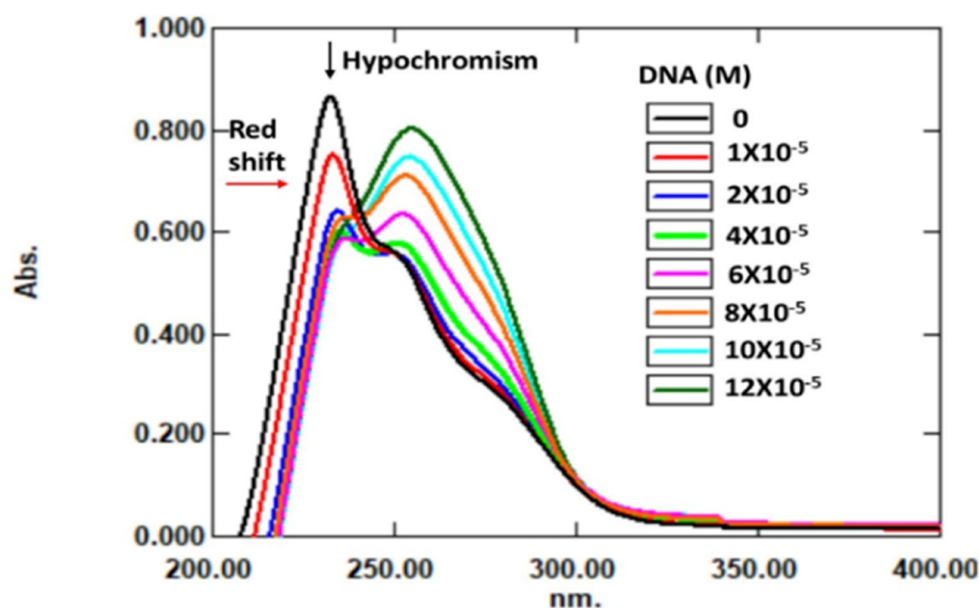

Fig. 3. Interaction of vorteoksetine with CT-DNA, UV–Visible absorption spectra of vortioxetine in the presence of increasing concentrations of CT-DNA ($1–12 \times 10^{-5}$ M) in Tris-HCl buffer (pH 7.2).

Molecular docking results indicate that Vortioxetine primarily forms hydrogen bonds with cytosine (CYT9), adenine (ADE19), and thymine (THY17), while Escitalopram interacts similarly with thymine (THY18) and adenine (ADE19). The distances between these bonds range between 2.5 and 3.0 Å. Additionally, both Vortioxetine and Escitalopram show electrostatic interactions with adenine (ADE19), with an interatomic distance of approximately 5 Å.

These interactions suggest stable binding of both molecules to DNA. Further, both Vortioxetine (Pi-alkyl: 5.36 Å) and Escitalopram exhibit Pi interactions with DNA. Attracting Cavities analysis indicates that the hydrogen

Molecule	Vortioxetine	Escitalopram
Binding Affinity (kcal/mol)	-7.29	-7.69
RMSD (Å)	32.4385	153.6072
AC Score	53.76	6.18
Binding Type	Groove Binding	Groove Binding
Electrostatic Bond	LIG: N2 - :ADE19:O1P (5.28 Å)	68P1:N03 :ADE19:O1P (4.98 Å)
Conventional Hydrogen Bonds	LIG: H14 -:THY18:O2 (2.76 Å), LIG: H15 - :THY18:O2 (2.29 Å)	-
Carbon Hydrogen Bonds	LIG: H12 -:CYT9:O2 (2.73 Å), LIG: H12 - :THY17:O2 (2.55 Å), LIG: H13 - :CYT9:O2 (2.86 Å), LIG: H16 - :THY18:O3' (2.49 Å), LIG: H16 - :ADE19:O4' (3.09 Å), LIG: H17 - :ADE19:O4' (3.04 Å)	68P1:H5 - :ADE19:O4' (2.71 Å), 68P1:H6 - :THY18:O2 (2.42 Å), 68P1:H13 - :THY17:O3 (2.57 Å), 68P1:H13 - :THY18:O1P (2.84Å), 68P1:H15 - :CYT9:O2 (2.64Å), 68P1:H16 - :CYT9:O2 (2.87 Å), 68P1:H16 - :THY17:O2 (2.54 Å)
Pi-Donor Hydrogen Bond	GUA16:H21 -LIG (3.22 Å)	GUA16:H21–68P1 (3.07 Å)
Hydrophobic (Pi-Alkil)	GUA10 -LIG: C1 (5.37 Å), CYT11 -LIG: C1 (4.92 Å)	-

Table 3. The binding affinities and the molecular interactions between vortioxetine, escitalopram, and target DNA, obtained by molecular Docking simulations by attracting cavities method. The RMSD values obtained from attracting cavities are not conventional RMSD values as calculated in AutoDock Vina-Based docking. These represent spatial deviation from seed conformations and are not used as indicators of docking accuracy.

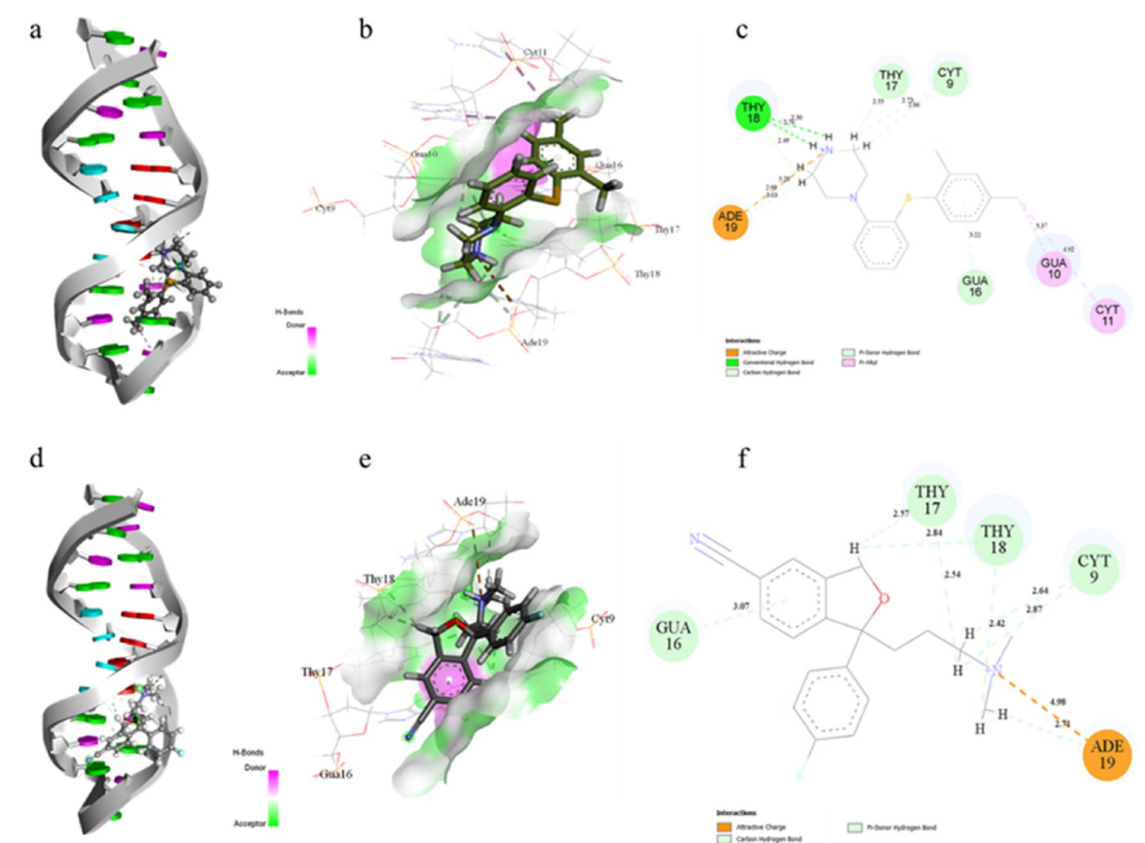

Fig. 4. The crystallographic structure of molecular target under PDB code (195D.pdb) with Vortioxetine (**a**) and Escilatopram (**b**) using the Attracting Cavities method, showing the intermolecular interaction of Vorteoxetine (**b** and **c**) and Escilatopram (**e** and **f**).

bonding and electrostatic interactions are concentrated near the minor groove regions of DNA's smaller base pairs, suggesting that both molecules predominantly bind to the DNA minor groove.

The AutoDock Vina method was used to determine the precise binding energies of vortioxetine and escitalopram to DNA to thoroughly assess their binding energies. According to AutoDock Vina results, vortioxetine showed a stronger predicted interaction with DNA (-6.572 kcal/mol) than escitalopram (-6.01 kcal/mol), as lower (more negative) binding energies reflect higher binding stability. Consequently, Vortioxetine

Molecules	Vortioxetine	Escitalopram
Binding Affinity (kcal/mol)	-6.572	-6.01
RMSD (Å)	0.00	0.00
Binding Type	Groove Binding	Groove Binding
Hydrogen Bond (Conv.)	LIG1:H-: CYT11:O2’ (2.70 Å)	LIG1:H-: THY6:O2’ (2.60 Å)
Hydrogen Bond (C-H)	LIG1:C-: GUA16:N3’ (3.38 Å) LIG1:C-: CYT11:O3’ (3.54 Å) LIG1:C-: GUA16:O4’ (3.44 Å)	LIG1:C-: THY6:O3’ (3.52 Å) LIG1:C-: ADE20:N3’ (3.65 Å)
Pi-Sigma	LIG1:THY17:H4’ (2.63 Å)	-

Table 4. The binding affinities and the molecular interactions between vortioxetine, escitalopram, and target DNA, obtained by molecular Docking simulations by AutoDock Vina method.

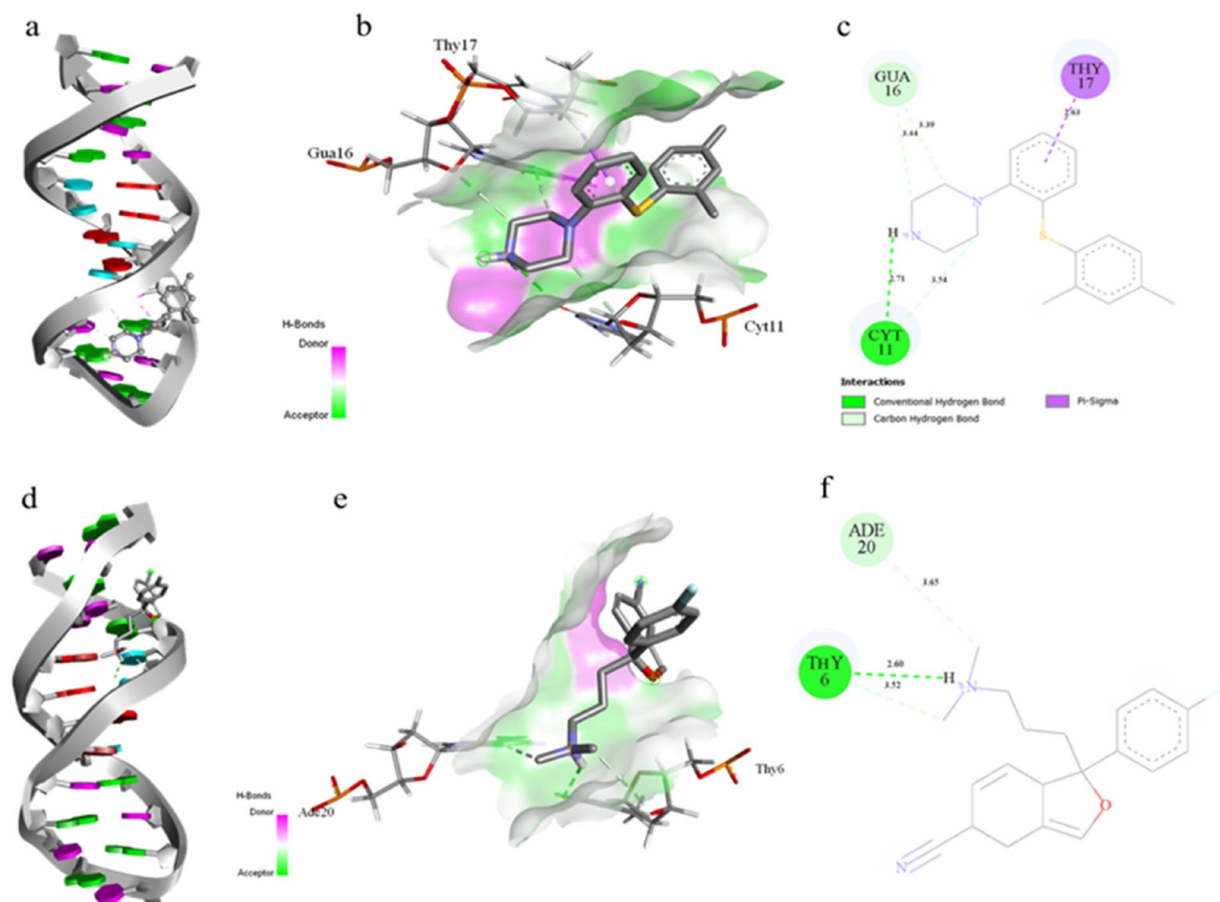

Fig. 5. The crystallographic structure of molecular target under PDB code (195D.pdb) with Vortioxetine (**a**) and Escilatopram (**b**) using the Attracting Cavities method, showing the intermolecular interaction of Vorteoxetine (**b** and **c**) and Escilatopram (**e** and **f**).

demonstrates superior binding affinity to DNA compared to Escitalopram. The outcomes of the analysis conducted using the AutoDock Vina molecular docking technique and the interactions of these two molecules with the DNA molecule are presented in Table 4; Fig. 5.

AutoDock Vina docking results indicate that the molecular interactions of Vortioxetine and Escitalopram with DNA reveal conventional hydrogen bonds, carbon-hydrogen interactions, and pi-sigma interactions. It was established that vortioxetine formed both conventional and carbon-hydrogen bonds with cytosine (CYT11) and guanine (GUA16) bases, as well as Pi-Sigma bonds with thymine (THY17). The distances between the conventional hydrogen bonds and carbon-hydrogen bonds were measured as 2.700 and 3.540 Å for the CYT11 base, and 3.380 and 3.440 Å for the GUA16 base, respectively. The distance of the Pi-Sigma bond formed with the THY17 base was measured at 2.630 Å. The Escilatopram molecule established both conventional and carbon-

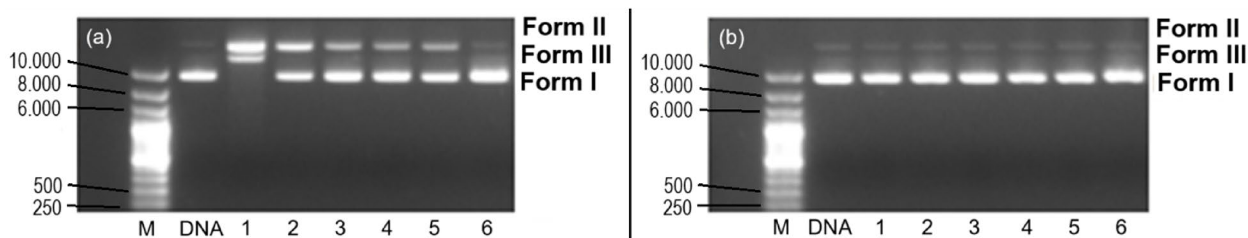

Fig. 6. DNA cleavage activity of vortioxetine. (**a**) Oxidative cleavage in the presence of H_2O_2: M: Marker; Lane 1: DNA + 25 µM compound + H_2O_2, Lane 2: DNA + 50 µM compound + H_2O_2, Lane 3: DNA + 100 µM compound + H_2O_2, Lane 4: DNA + 200 µM compound + H_2O_2, Lane 5: DNA + 400 µM compound + H_2O_2, Lane 6: DNA + H_2O_2. **(b)** Hydrolytic cleavage without H_2O_2: M: Marker; Lane 1: DNA + 25 µM compound, Lane 2: DNA + 50 µM compound, Lane 3: DNA + 100 µM compound, Lane 4: DNA + 200 µM compound, Lane 5: DNA + 400 µM compound, Lane 6: DNA + H_2O. Vertical line separates distinct gels. Equal loading was confirmed via DNA quantification. The gels displayed were cropped to exclude unused lanes and non-informative regions; no image splicing or digital modification has been applied.

hydrogen bonds with thymine (THY6) at distances of 2,600 Å and 3,520 Å, respectively, while it formed solely a carbon-hydrogen bond with adenine at 3,650 Å (Fig. 5).

The molecular docking analysis results from AutoDock Vina indicate that both Vortioxetine and Escitalopram bind to the minor groove of the DNA molecule. The presence of Pi-Sigma (Vortioxetine) interactions with hydrogen bonds near the DNA base pairs suggests that the molecules are likely to bind to the minor groove. Although UV-Vis absorption measurements indicated intercalative behaviour, molecular docking analysis showed minor groove binding as the most stable pose. This observation reflects methodological differences between solution-phase spectroscopic analysis and static in silico modelling.

DNA cleavage

pBR322 plasmid DNA can be seen in three different forms in gel electrophoresis. These are the supercoiled form of DNA (Form I), the opened circular form (Form II) and the linear form (Form III). Figure 6 displays the gel electrophoresis images of the vortioxetine-pBR322 interaction. In our experiment, vortioxetine showed cleavage activity in the presence of H_2O_2 (oxidative shearing) compared to the DNA control (Fig. 6a). In contrast, vortioxetine had no activity in the absence of H_2O_2 (hydrolytic cutting) (Fig. 6b). As a result, it can be concluded that vortioxetine facilitates oxidative cleavage of DNA in the presence of H_2O_2.

Discussion

To the best of the authors' knowledge, there are no publications on the in vitro genotoxic effects of vortioxetine on human peripheral lymphocytes. Escitalopram was used as a comparative control due to its widespread use as an antidepressant, and the results were compared with previous studies on escitalopram's genotoxicity, demonstrating escitalopram at concentrations of 5 and 10 µg/mL was not cytotoxic to human cells, though it showed genotoxic potential[10]. Other antidepressants, such as venlafaxine and trazodone, have been found to have clastogenic and mutagenic effects[11,12]. Our findings indicate that both vortioxetine and escitalopram, at a concentration of 20 µg/mL, exhibit genotoxic effects in human peripheral blood lymphocytes, as evidenced by statistically significant increases in micronucleus (MN), binucleus (BN), and chromosomal aberration (CA) frequencies at higher doses. However, these effects were not as severe as those observed with the positive control, suggesting that vortioxetine and escitalopram may possess genotoxic and cytotoxic potential at high doses, though they do not reach the level of carcinogenic substances.

In terms of DNA cleavage activity, vortioxetine demonstrated no hydrolytic cleavage ability in the absence of H_2O_2. However, in the presence of H_2O_2 vortioxetine facilitated oxidative cleavage, effectively converting both Form II and Form III of supercoiled DNA. Hydrolytic cleavage results from the hydrolysis of phosphodiester bonds, while oxidative cleavage occurs through the oxidation of deoxyribose sugar or nucleobases[13]. These findings suggest that vortioxetine promotes oxidative DNA cleavage under oxidative conditions, specifically in the presence of H_2O_2.

Computational methods are frequently used in hit identification and lead optimization. These docking methods "dock" small compounds into the structures of macromolecular targets and "score" their potential in a binding site complementary manner. In silico studies hypothetically demonstrate DNA-drug interactions. UV-Vis spectroscopy is essential for determining the binding modes of drugs to DNA[14]. These, we evaluated DNA-drug interaction both in silico and in vitro. Vortioxetine and Escitalopram possess the capability to bind to DNA through both in silico and analytical methods.

We performed absorption measurements in the absence and presence of increasing concentrations of CT-DNA. The drug molecule's binding to DNA can be detected through UV-Vis spectroscopy, which shows a hyperchromic or hypochromic effect with a red or blue shift. Hypochromism and red shift indicate intercalation, while hyperchromism and blue shift indicate electrostatic interaction[15,16].

Our UV-Vis absorption results (Fig. 3) demonstrated a 27.43% reduction in absorbance (hypochromism) and a 7 nm redshift (bathochromic shift) at 232 nm upon the addition of vortioxetine to CT-DNA. These spectral changes are indicative of intercalative binding, where the vortioxetine molecule inserts between the DNA base

pairs. This intercalation disrupts π-π* stacking interactions between the base pairs, causing hypochromism, while the redshift suggests a change in electron density due to ligand binding. The consistency between the docking results, which show vortioxetine intercalating between DNA base pairs, and the UV-Vis results further supports this binding mode.

Upon analysis of the crystal structure of vortioxetine, it is evident that the molecule contains a benzene ring and aromatic features[17]which likely contributes to its ability to intercalate into DNA. The docking images show the ligand positioned between DNA base pairs, consistent with the observed hypochromism and redshift, further indicating intercalative binding. Additionally, the calculated binding constant (K_b=6.25×10^5 M^{-1}) suggests a moderate binding affinity, which is lower than that of common intercalators like ethidium bromide (Kb = 7×10^7 M^{-1})[18]. The result suggests that vortioxetine has a reasonable binding capacity but does not bind as strongly as potent intercalating agents, highlighting its relative safety. This distinction is important as it implies that vortioxetine may have a lower risk of potential side effects associated with stronger intercalators.

In silico findings indicated that Vortioxetine exhibits stronger and more stable binding. The predominant intermolecular interactions were identified as hydrogen bonds and Pi bonds, occurring within the DNA minor groove. Our study concluded that both molecules exhibited stable binding to DNA; however, their binding affinities are insufficient to cause DNA damage. The interactions occur in the minor groove, facilitated by electrostatic and hydrogen bonds, thereby posing no risk of structural disruption to DNA.

It is important to note that AutoDock Vina and Attracting Cavities operate based on different scoring and structural alignment principles. AutoDock Vina calculates RMSD relative to the best-scoring pose or known crystal structure, using a Lamarckian Genetic Algorithm and empirical scoring. On the other hand, Attracting Cavities defines RMSD based on clustering distance from a seed or centroid in an energy grid map, which can yield anomalously high value not representative of ligand-target conformational similarity. Therefore, we rely primarily on binding energies and interaction profiles for assessing docking quality. Using the AutoDock Vina method, vortioxetine displayed a more negative binding affinity (-6.57 kcal/mol), than escitalopram (-6.01 kcal/mol), indicating a stronger predicted interaction with DNA. In contrast, the Attracting Cavities method indicates an improvement in binding affinities for both drugs, with vortioxetine's affinity increasing to -7.29 kcal/mol and escitalopram achieving a slightly stronger binding affinity of -7.69 kcal/mol. This data suggests that attracting cavities method consistently predict escitalopram to have a slightly stronger binding interaction with DNA, as indicated by its more negative binding affinity values. All these values to indicate genotoxic potential, they would need to exceed a certain threshold. Genotoxic agents typically cause permanent damage by inducing significant changes in DNA structure or by targeting specific DNA sequences. With binding affinities around −7 kcal/mol, it's challenging to conclude genotoxicity solely based on these values. However, to better assess potential risk, experimental genotoxicity testing is performed. All these findings suggest that both vortioxetine and escitalopram are capable of binding to DNA; however, the binding strength is not sufficient to cause significant structural damage to DNA. When comparing the two drugs, it can be concluded that while both may induce minor toxicity, this toxicity is not substantial, and both drugs are considered relatively safe in comparison to known carcinogenic compounds used as positive controls. It is important that the binding energy values observed represents moderate interactions and do not necessarily indicate genotoxic harm or use of the term relatively safe is limited to the context of short-term in vitro exposure and DNA binding affinity within the scope of the parameters studied. Long-term in vivo studies and mechanistic validation are needed to establish a more comprehensive safety profile. Notwithstanding the UV-Vis absorption data suggested intercalative interaction due to the observed hypochromism and bathochromic shift, docking studies indicated that vortioxetine binds preferentially to the minor groove of DNA. This divergence is methodologically expected. UV-Vis spectroscopy evaluates global spectral changes in dynamic solution-phase conditions and may capture transient or partial intercalation events. In contrast molecular docking relies on static receptor models and optimized binding energies, often favouring groove binding due to steric and electrostatic compatibility. Together, these findings suggested that vortioxetine may interact with DNA through both intercalative and groove-binding modes depending on the conformational state and local DNA environment.

Given that DNA is a crucial pharmacological target, it is important to consider the potential for marketed drugs to interact with DNA in unintended ways. Our laboratory findings were corroborated by in silico analyses, demonstrating consistent evidence of vortioxetine's capacity to bind to DNA in both experimental and computational contexts. The observed decrease in the mitotic index with increasing doses of vortioxetine may be linked to the inhibition of DNA replication, which could result from its intercalating binding mode. In sum, while vortioxetine and escitalopram bind to DNA and show mild toxicity potential, neither demonstrates significant DNA damage or genotoxic risk at observed levels. Thus, they are considered relatively safe, especially compared to known genotoxic agents.

In conclusion, this study provides a comprehensive analysis of the genotoxicity and cytotoxicity of vortioxetine and its comparator, escitalopram, utilizing both in silico and in vitro methodologies. The DNA binding characteristics of vortioxetine were investigated using spectroscopic techniques, which confirmed its ability to interact with DNA through its aromatic hydrocarbon structure. Additionally, in silico analyses were performed to corroborate these findings. Molecular docking studies further elucidated the nature of drug-DNA interactions, indicating that binding occurs primarily through electrostatic interactions within the minor groove of DNA. While high doses of vortioxetine exhibited cytotoxic and genotoxic effects, these were not as pronounced as those associated with known carcinogenic substances. The observed increase in cell cycle arrest at elevated doses suggests a protective mechanism against genotoxicity. This study primarily focused on assessing the genotoxicity and cytotoxicity of vortioxetine through classical cytogenetic assays (chromosome aberration and micronucleus), DNA interaction analysis and molecular docking simulations. Although these endpoints provide valuable initial insights into potential toxicity mechanisms. Additional mechanistic studies, including apoptosis detection, ROS generation measurements and detailed cell viability analysis, would further elucidate

the underlying biological pathways. Future studies incorporating these mechanistic approaches are warranted to comprehensively characterize the molecular mechanisms behind the cytotoxic and genotoxic responses observed for vortioxetine.

Overall, our study underscores the importance of assessing the genotoxic potential of antidepressants like vortioxetine and emphasizes the need for further investigations to fully understand the implications of drug-DNA interactions in clinical settings. Therefore, clinicians are advised to carefully consider the choice of antidepressants and adjust dosages as necessary to minimize genotoxicity risks while ensuring therapeutic efficacy.

We acknowledge that the sample size used in this study ($n = 10$ lymphocyte donors) is relatively small, which may limit the statistical power and reduce the generalizability of our conclusions. Therefore, the results presented here should be interpreted as preliminary findings. Further confirmatory studies with larger sample sizes and diverse donor populations are needed to more strongly assess the genotoxic and cytotoxic risk profile of Vortioxetine, and to firmly establish its clinical safety. Another limitation of our study is that escitalopram, utilized as a comparative control, was evaluated at only one concentration (20 µg/mL) which is corresponds to a typical therapeutic concentration. Whereas this approach provided clinically relevant comparison data, it did not allow us to understand a complete dose-response relationship. Further experiments involving multiple concentrations of escitalopram would provide more comprehensive insight into its genotoxic profile, dose-dependent cytotoxicity, and comparative safety.

Materials and methods

Materials

Escitalopram was obtained from a 10 mg/mL oral solution of Cipralex® (Lundbeck, Denmark), and vortioxetine was sourced from a 10 mg Brintalix® dissolved in sterile deionized water (Pfizer, New York, NY). In this study, the doses of vortioxetine (5, 10, and 20 mg/kg) were selected as therapeutic equivalents and converted to in vitro concentrations of 5, 10 and 20 µg/mL. The selection of vortioxetine concentrations used in this study was carefully based on clinical pharmacokinetic data to ensure biological relevance in vitro. The in vitro dose of vortioxetine (5, 10 and 20 µg/mL) were chosen with reference to humana therapeutic plasma concentrations reported in European Medicines Agency (EMA) assessment report for Brintellix. Similarly, the escitalopram dose used in this study (20 mg/kg) corresponds 20 µg/mL in vitro.

Blood samples ($n = 10$; 5 men and 5 women, and age between 20 and 30 years) were collected in heparinized tubes from clinically healthy, non-smoking, non-alcoholic individuals with no history of exposure to genotoxic agents. All blood sampling procedures adhered strictly to the Declaration of Helsinki, and ethical approval was obtained from the institutional ethics committee (Ethical Committee Approval Number: 2011-KAEK-27/2021-210014251). Written informed consent was obtained from each donor. Lymphocytes were cultured in chromosome medium B for 72 h, after which they were harvested for the analysis of micronucleus formation and chromosomal aberrations.

Methods

Micronucleus assay

In vitro experiments were performed on peripheral blood lymphocytes obtained from 10 healty volunteers (5 males and 5 females). For each individual and treatment condition, assays were conducted in triplicate. The micronucleus assay was conducted in accordance with the methodology described by Fenech[8]. Under sterile conditions, 0.2 ml of heparinized peripheral blood from the volunteers was mixed with 2.5 ml of Chromosomal medium M and incubated at 37 °C for 72 h. Concurrently, cells without treatment served as the negative control, while cells treated with 0.3 µg/mL mitomycin-C acted as the positive control. To halt cytokinesis, cytochalasin B was added to the medium at a final concentration of 6 µg/mL after the 44-hour incubation period. Following centrifugation, the cells were treated with a hypotonic solution (0.4% KCl) and fixed three times using a methanol-glacial acetic acid solution (3:1, v/v). We assessed the presence of micronuclei (MNs) in 2000 binucleated cells, analysing 1000 cells from replicates.

Chromosome aberration (CA) and sister chromatid exchange (SCE) assay

The CA and SCE assays were prepared according to Evans and Perry and Thompson[19,20]with minor modifications. Briefly, 0.2 mL of whole blood was combined with 2.5 mL of chromosomal medium B supplemented with 10 µg/mL bromodeoxyuridine.

The cells were incubated in complete darkness for two DNA replication cycles (48 h). Following the first incubation period, cultures were treated with vortioxetine (5, 10 and 20 µg/mL for vortioxetine and 20 µg/mL for escitalopram. The same procedure was used for the negative, DMSO and positive controls. In this study, we utilized DMSO as a solvent at $< 0.1\%$ (v/v) concentration, this level has been known to be non-genotoxic and therefore it was not included as a separate control group.

Cells were treated with colchicine (0.06 µg/mL) for 2 h before harvesting to arrest them in metaphase. Following treatment, the cultures were centrifuged at 1200 rpm for 15 min. The cell pellet was then subjected to hypotonic treatment using 0.075 M KCl for 20 min at 37 °C to induce cell swelling and chromosomal dispersion. The cells were subsequently fixed with a cold methanol: glacial acetic acid solution (3:1) for 20 min at room temperature. The cells were dropped on various sections of glass slides from a height of 40–50 cm and left to air dry for 24 h. The slides were then stained with 5% Giemsa (pH = 6.8) and prepared with Sorensen's buffer (pH:6.8), for 20–25 min to facilitate visualization of the mitotic index and chromosomal abnormalities. Finally, the stained slides were examined under a light microscope to assess chromosomal morphology and any aberrations.

DNA binding

We studied the interactions between vortioxetine and DNA using UV-Vis absorption titration. Calf thymus DNA (CT-DNA) was dissolved in Tris-HCl/NaCl (pH 7.2) buffer to be used in the experiment and the UV-Vis absorbance was expected to be between 1.8 and 1.9 at 260–280 nm, indicating that there is no protein in CT-DNA[21]. Both the DNA and vortioxetine solutions were prepared using double-distilled water. Increasing concentrations of CT-DNA ($1–12 \times 10^{-5}$ M) were added to the solution, while maintaining a constant vortioxetine concentration (3×10^{-5} M). Absorption spectra were recorded by adding equal amounts of CT-DNA to both the vortioxetine solution and the reference solution, ensuring that the absorbance of CT-DNA itself was eliminated from the measurements.

DNA cleavage

Vortioxetine-induced DNA unwinding was assessed by analyzing the electrophoretic mobility of supercoiled pBR322 DNA on agarose gels[22]with the nicked circular form serving as a control. Oxidative cleavage was induced in the presence of H_2O_2, while hydrolytic cleavage was carried out in the absence of H_2O_2. As a positive control, EcoRI restriction digestion of pBR322 DNA was included. We conducted the assay by incubating the reaction mixture in Tris-HCl buffer (pH 7.2) at 37 °C for 3 h. The reaction mixture consisted of 1 µL pBR322 DNA (0.5 µg/mL), vortioxetine or escitalopram, and water for hydrolytic cleavage, or H_2O_2 for oxidative cleavage. Following incubation, the samples were subjected to electrophoresis for 60 min at 60 V on a 1% agarose gel. Ethidium bromide (1 µg/cm³) was incorporated into the gel to visualize DNA bands under UV illumination.

In Silico analyses

Molecular docking analyses of Vortioxetine (PubChem CID: 9966051) and Escitalopram (PubChem CID: 146570), and the receptor molecule (PDB ID: 195D) were carried out using the SwissDock online docking tool. The preparation of receptor DNA (195D.pdb) was performed using the ChimeraX tool. The ligand molecules' 3D structure was downloaded from the PubChem database, optimized using the Avogadro v2.0 tool, and saved in mol2 file format. After that, receptor-ligand interactions were analyzed using the SwissDock platform and both the AutoDock Vina and Attracting Cavity docking methods. Two distinct molecular docking tools, AutoDock Vina and Attracting Cavities, were intentionally employed to investigate ligand-DNA interactions due to their complementary methodological strengths. AutoDock Vina applies empirical scoring functions and a Lamarckian genetic algorithm to efficiently identify the most energetically stable and reliable binding conformations. Conversely, the Attracting Cavities method utilizes a surface-based energy grid approach that emphasizes molecular surface complementarity, thus potentially revealing interaction regions or binding poses that might not be detected using AutoDock Vina alone. Using both docking methods concurrently allows for cross-validation, increases the robustness of docking predictions, and provides a comprehensive and complementary assessment of possible ligand-DNA interaction modes. Docking results were visualized by the viewDock tool on Chimera v1.18, and molecule distances were calculated using BIOVIA Discovery Studio v24.1.0.23298 tool visualizer.

The docking simulations of Vortioxetine /Escitalopram interaction on DNA were performed by B-DNA Dodecamer (PDB ID:195D), which the sequence of DNA (5'-(CpGpCpGpTpTpApApCpGpCpG)-3') in a box with x × y × z directions as 20 20 42 Å centers[23]. The program removed all the water molecules and ions from the DNA (receptor file) and added polar hydrogen atoms to the receptor molecule. The 'grid parameter' files were configured to X = 15, Y = 22, and Z = 9 for vortioxetine, and X = 15, Y = 22, and Z = 10 for escitalopram, while the remaining parameters were assigned the default values specified by the software. The docking simulations of "Vortioxetine" and "Escitalopram" interaction on DNA were done using SwissDock. In addition to the primary docking approach, the attracting cavities method was employed using the same input parameters. The resulting binding affinities and root mean square deviation (RMSD) values were computed to assess docking accuracy. We employed ChimeraX and BIOVIA Discovery Studio to visualize and analyze receptor–ligand interactions, including hydrogen bonding, hydrophobic contacts, and binding poses.

Data analysis

The mean values of triplicate technical replicates per donor were used for statistical analysis. Group comparisons were conducted based on $n = 10$ biological replicates. Statistical analyses were carried out using Jamovi v2.6.26 software. Measurement data were expressed as mean ± standard deviation (SD). The Kruskal-Wallis H test, a nonparametric method, was used to evaluate differences between groups, followed by Dunn's post hoc test for pairwise comparisons. Experimental values were presented as mean ± standard deviation (SD) was used to express the experimental values. A p-value of less than 0.05 was considered statistically significant.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

MMÇ: Conceptualization, Methodology, Investigation Formal analysis, Writing - Original Draft Preparation; Writing - Review & Editing Preparation; Pİ: Methodology, Investigation (In vivo), Writing - Review & Editing Preparation; GA: Performed Experiment, , Investigation (In vivo); MG: Software, Molecular docking, Investigation, Data Curation, Visualization, Writing - Review & Editing Preparation; AÇ: Investigation Formal analysis, Collecting Samples, Review & Editing Preparation. All the authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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