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Cytotoxicity of Betaine in 4T1 and L929 Cells: In Silico ADMET Profiling

Elif Ece Akgün[1], Büşra Gülbenli Türkoğlu[2], Yasemin Sunucu Karafakıoğlu[3], Senem Akkoç[4]

ABSTRACT

Betaine, a naturally occurring zwitterion, was evaluated for its anticancer potential against murine 4T1 breast cancer cells and non-cancerous L929 fibroblast cells. Cells were treated with various concentrations for 48 hours, and viability was assessed using the MTT assay. Betaine showed weak, dose-dependent cytotoxicity with an IC_{50} value of 859.10 µg/mL. *In silico* ADME analysis showed high solubility, low lipophilicity (LogP: –2.18), and poor blood-brain barrier permeability. ProTox-II predicted low hepatotoxicity, nephrotoxicity, and immunotoxicity, but possible neurotoxic and cardiotoxic effects. Betaine was non-carcinogenic and showed no significant interaction with major metabolic enzymes. These findings suggest that while betaine has limited direct cytotoxicity, its favorable safety and pharmacokinetic profile support its potential as an adjuvant in cancer therapy.

Keywords: *Antiproliferative Acitivity, Betaine, Breast Cancer, Toxicity.*

1. INTRODUCTION

Betaine (trimethylglycine) is an endogenous osmoprotectant and methyl donor abundantly found in various dietary sources, including wheat bran, spinach, and whole grains. Functioning as a critical component of one-carbon metabolism, betaine facilitates the remethylation of homocysteine to methionine, thereby modulating DNA methylation patterns and broader epigenetic regulation [1]. Owing to its biochemical role, betaine has attracted increasing attention for its potential implications in cancer biology.

A growing body of epidemiological and experimental evidence supports the inverse association between dietary betaine intake and the incidence of several malignancies, including breast, colorectal, liver, lung, esophageal, ovarian, and prostate cancers [2-5].

[1] Corresponding author: ece.akgun@atauni.edu.tr Assist Prof., Atatürk University, Faculty of Veterinary Medicine, Department of Histology and Embryology, 25240, Erzurum, Türkiye, Orcid: 0000-0002-2982-0307
[2] Research Assist., Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pathology,15030, Burdur, Türkiye Orcid: 0000-0001-6666-8992
[3] Assoc. Prof., Uşak University, Faculty of Education, Department of Mathematics and Science Education, Department of Science Education, Uşak, Türkiye Orcid: 0000-0002-9219-8696
[4] Prof Dr., Suleyman Demirel University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Isparta, 32260, Türkiye & Bahçeşehir University, Faculty of Engineering and Natural Sciences, Istanbul, 34353, Türkiye Orcid:0000-0002-1260-9425

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Mechanistically, betaine exerts its protective effects through multiple pathways, such as attenuation of oxidative stress, suppression of pro-inflammatory responses, and regulation of apoptotic signaling cascades [6]. These pleiotropic properties underscore its potential as a candidate for cancer chemoprevention and adjuvant therapy.

Beyond its oncological relevance, betaine also demonstrates substantial neuroprotective efficacy. Recent *in vivo* and *in silico* studies have shown that betaine ameliorates behavioral deficits and restores redox balance in models of sodium fluoride–induced neurotoxicity, highlighting its antioxidant, anti-inflammatory, and antiapoptotic mechanisms of action [7]. Such finding extends the therapeutic relevance of betaine beyond cancer and into the realm of neurotoxicity and possibly neurodegenerative disorders.

From a drug development perspective, *in silico* ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling has become an essential tool for predicting pharmacokinetic behavior and preclinical safety [8]. While betaine demonstrates high aqueous solubility and low lipophilicity, its gastrointestinal absorption and membrane permeability are limited. Moreover, ProTox-II toxicity analysis indicates minimal hepatotoxicity or nephrotoxicity, yet suggests potential risks for neurotoxicity, cardiotoxicity, and respiratory toxicity [9]. These findings highlight the importance of balancing therapeutic efficacy with safety considerations when evaluating betaine's clinical potential.

Among triple-negative breast cancer (TNBC) models, the 4T1 murine mammary carcinoma cell line is widely recognized for its high metastatic potential and its close resemblance to stage IV human breast cancer, particularly in terms of immune evasion and tumor aggressiveness [10]. Owing to its resistance to conventional hormonal or HER2-targeted therapies, 4T1 serves as a robust experimental model for evaluating novel therapeutic agents with cytotoxic or immunomodulatory potential. Despite betaine's well-documented biochemical properties, its direct cytotoxic activity on 4T1 cells—representing an aggressive breast cancer phenotype—remains underexplored.

Therefore, the present study aims to assess the *in vitro* antiproliferative effects of betaine on 4T1 cells, characterize its physicochemical and pharmacokinetic attributes through *in silico* tools. This integrative approach seeks to advance understanding of betaine's therapeutic applicability in triple-negative breast cancer models and inform future strategies for its clinical development.

2. MATERIALS AND METHODS

2.1. Betaine structure

Betaine was purchased commercially from Sigma, USA (Cat no. 61962). The open structure of betaine is given in Figure 1.

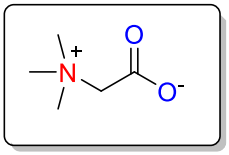


Figure 1. Open structure of betaine

2.2. Preparation of solution

0.038 g of betaine was weighed and dissolved in 1 mL of phosphate buffer saline (PBS). The solutions were freshly prepared and used in cell culture studies as stock solutions.

2.3. Cell propagation and maintenance

The murine breast cancer cell line (4T1) and the murine fibroblast non-cancerous cell line (L929) were cultured in RPMI 1640 and EMEM, respectively. Both media were supplemented with 10% fetal bovine serum (FBS) and 1% GlutaMAX, and the cells were maintained in a humidified incubator with 5% CO2 at 37 °C. When the cells reached approximately 90% confluency, the culture medium was aspirated from the flask. Subsequently, the cells were rinsed twice with PBS to remove any residual medium. Trypsin-EDTA (Thermo Fisher Scientific) was then applied to detach the adherent cells, facilitating their passage. The removed cells were transferred to a 15 mL centrifuge tube containing 3 mL of medium. Centrifuged at 1450 rpm for 5 min. The supernatant was discarded carefully, and 5 mL of fresh medium was added to the bottom pellet. After thorough mixing, cell counting was performed using a Thoma slide.

2.4. *In vitro* antiproliferative activity studies

Cytotoxicity evaluation was carried out based on MTT protocols reported in the literature [11-13]. Firstly, cells were seeded in sterile 96-well culture plates with 5×10^3 cells per well and kept in the incubator for 24 hours to allow the cells to adhere. The old medium was then aspirated, and 100 µL of fresh medium was added to each well. At this stage, cells were incubated with 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 µL volumes of solution (equivalent to final concentrations of 1900, 950, 475, 237.5, 118.75 and 59.375 µg/mL, respectively) for 48 hours. At the end of the incubation, the medium was carefully removed, 5 mg/mL MTT stock solution was added to the wells, and the plates were kept in the incubator for another 2 hours. At the end of the incubation period, the medium was gently

aspirated under light-tight conditions, and the formazan crystals formed were dissolved with 200 µL DMSO. The mixture was shaken at room temperature in the dark for 30 minutes. Absorbance measurements were performed at 630 nm with an Agilent BioTek ELISA plate reader; IC_{50} values were calculated using GraphPad Prism 5 software.

2.5. ADME and toxicity (ADMET) properties

The SMILES representation of betaine C[N+](C)(C)CC([O-])=O was generated using ChemBioDraw Ultra 15.0 software. This structure-based code served as the input for *in silico* evaluation of pharmacokinetic and toxicity profiles. ADME-related parameters were predicted via the SwissADME online tool [14], while toxicity assessments were carried out using the ProTox-II platform [9].

3. RESULTS AND DISCUSSION

3.1. Antiproliferative activity studies

The cytotoxic effect of betaine on 4T1 murine breast cancer cells was evaluated using the MTT assay after 48 hours of treatment at varying concentrations (1900–59.375 µg/mL) (Figure 2). Cell viability decreased in a dose-dependent manner with increasing concentrations of betaine. However, compared to standard chemotherapeutic agents, betaine exhibited relatively low cytotoxic potency under the tested conditions. The half-maximal inhibitory concentration (IC_{50}) was calculated as 859.10 µg/mL, indicating that a high concentration of betaine is required to inhibit 50% of cell proliferation in the 4T1 cell line (Table 1).

To further evaluate whether this effect is cancer-specific, the cytotoxic activity of betaine was also assessed on a non-cancerous cell line (L929 fibroblasts). The IC_{50} value obtained for L929 cells (970.40 µg/mL) was comparable to that observed in 4T1 cells (859.10 µg/mL), indicating that betaine does not exhibit strong selectivity toward cancer cells under the tested conditions (Table 1). This finding suggests that the observed antiproliferative effect may primarily result from general cytotoxicity at high concentrations rather than a cancer-specific mechanism.

Nevertheless, the relatively low level of cytotoxicity observed in both cancerous and non-cancerous cells may support the notion that betaine possesses a favorable safety profile. This characteristic could be advantageous in the context of long-term use or combinational therapeutic strategies, where minimizing toxicity to healthy cells is critical.

Overall, these findings indicate that betaine is unlikely to be effective as a standalone cytotoxic agent against aggressive breast cancer models such as 4T1. Instead, its potential utility may lie in supportive or adjuvant roles rather than direct anticancer activity.

Table 1. The half-maximal inhibitory concentration (IC_{50}) values of betaine in 4T1 and L929 cell lines.

Compound	IC_{50} (µg/mL)	
	4T1	L929
Betaine	859.10	970.40

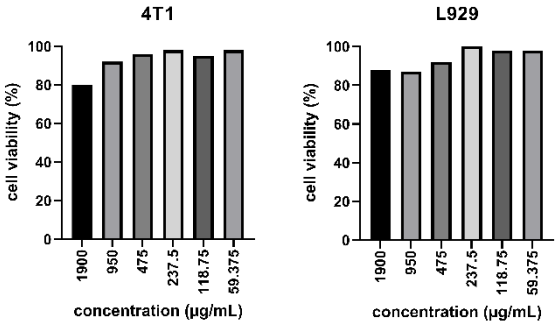


Figure 2. Cytotoxicity analysis of betaine. Cell viability ratio of 4T1 breast cancer cells and L929 fibroblast cells after 24h treatment with various concentrations of betaine (59.375–1900 µg/mL).

3.2. ADME and toxicity (ADMET) properties

Betaine's molecular formula is $C_5H_{11}NO_2$, and its molecular weight is calculated as 117.15 g/mol. The molecule has a total number of heavy atoms of 8 and contains no aromatic heavy atoms. The Csp^3 fraction of the molecule is 0.80, indicating a highly saturated carbon character. Furthermore, two rotatable bonds, two hydrogen bond acceptors, and the absence of a hydrogen bond donor increase the molecule's flexibility while limiting its capacity to form hydrogen bonds. The total polar surface area (TPSA) was determined to be 40.13 $Å^2$, which is generally considered within the appropriate range for small molecules with good bioavailability. Physicochemical, lipophilicity, pharmacokinetics water solubility properties of betaine are given in Figure 3.

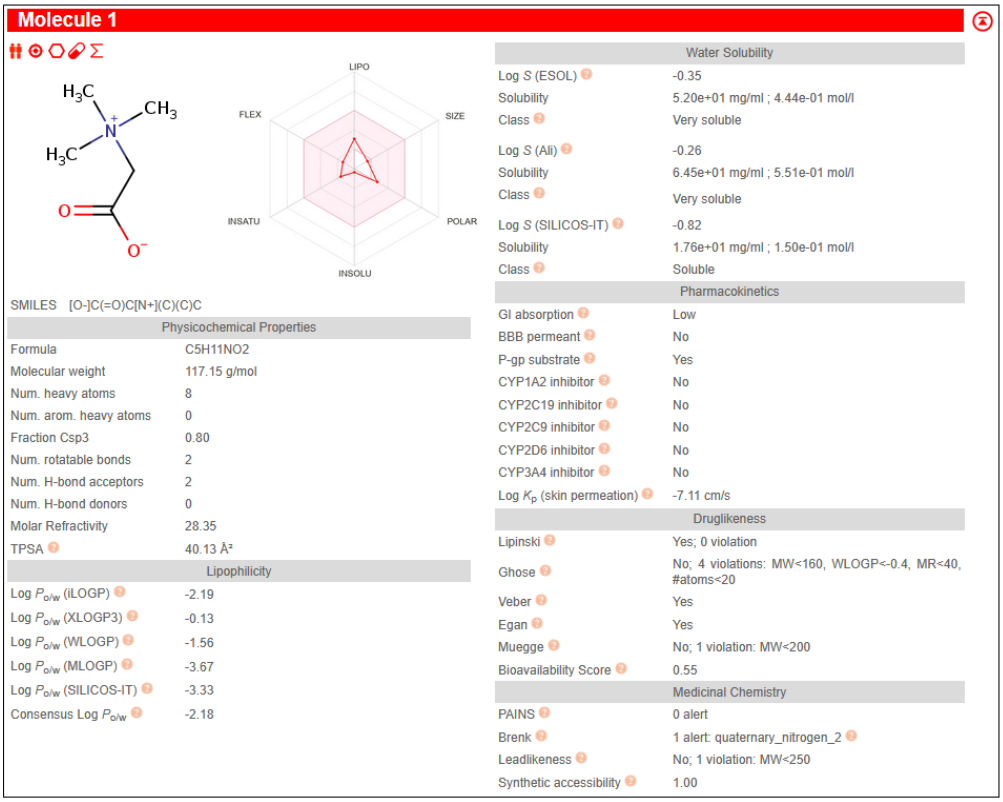


Figure 3. Physicochemical, lipophilicity, pharmacokinetics water solubility properties of betaine

Betaine's lipophilicity values have been calculated using various methods. The consensus LogP value is -2.18, indicating that the molecule exhibits highly hydrophilic properties. This result suggests that betaine has a low permeability to lipophilic membranes and is more soluble in aqueous environments.

Solubility estimations revealed that betaine has high solubility. The ESOL model calculated solubility as 5.20×10^1 mg/mL and the Ali model as 6.45×10^1 mg/mL, both placing the molecule in the "very soluble" category. The SILICOS-IT model predicted a relatively lower solubility (1.76×10^1 mg/mL) and placed it in the "soluble" category. In general, betaine exhibits high solubility in aquatic environments.

Betaine's gastrointestinal absorption was found to be low, and it was also predicted to be unable to cross the blood-brain barrier. The molecule's status as a P-glycoprotein substrate suggests that active transporters play a role in its transport across the cell membrane. Its interaction with CYP enzymes revealed that it is not an inhibitor of the major metabolizing

isoenzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4). Furthermore, the log Kp value, which represents skin permeability, was -7.11 cm/s, indicating that skin absorption would be quite low.

Betaine met the Lipinski, Veber, and Egan criteria for drug similarity, but violated the Ghose and Muegge rules due to its low molecular weight. Its bioavailability score was 0.55, indicating moderate oral bioavailability. While betaine did not have a PAINS warning, the Brenk filter issued a warning due to its quaternary nitrogen structure. The molecule was deemed unsuitable for lead-likeness due to its low molecular weight. However, its synthetic accessibility score was 1.00, demonstrating that it is a highly accessible compound for synthesis.

The boiled-egg model is a visual pharmacokinetic approach used to estimate the oral absorption and blood-brain barrier (BBB) crossing potential of small molecules. In the model, the egg white represents compounds with good gastrointestinal absorption potential, while the yolk represents compounds with high BBB permeability. Boiled-egg model of betaine is given in Figure 4.

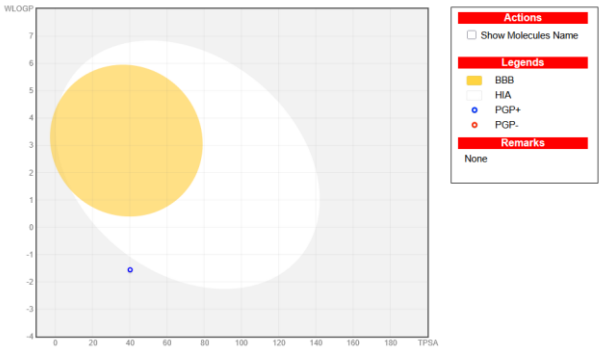
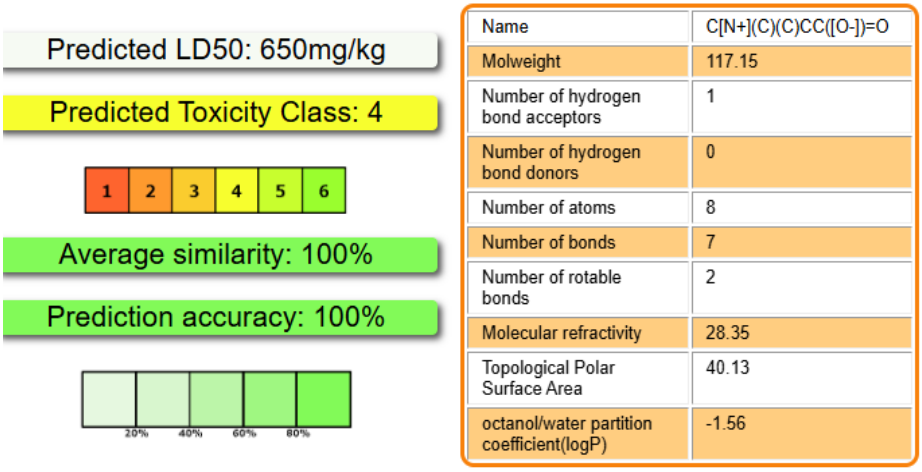


Figure 4. Boiled-egg model of betaine

According to the results obtained for betaine, the molecule is located outside the model or within the white region.

The molecule's hydrophilic properties (negative LogP value, high polar surface area) are consistent with these findings. Therefore, betaine's penetration into the systemic circulation is limited, and its efficacy in central nervous system-targeted applications is not expected. However, due to its high solubility, it can be considered a suitable candidate for local or peripheral pharmacological effects. Oral toxicity prediction results and toxicity model report of betaine are given in Figure 5 and Table 2, respectively.



Name	C[N+](C)(C)CC([O-])=O
Molweight	117.15
Number of hydrogen bond acceptors	1
Number of hydrogen bond donors	0
Number of atoms	8
Number of bonds	7
Number of rotable bonds	2
Molecular refractivity	28.35
Topological Polar Surface Area	40.13
octanol/water partition coefficient(logP)	-1.56

Figure 5. Oral toxicity prediction results for betaine

Table 2. Toxicity model report of betaine

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.99
Organ toxicity	Neurotoxicity	neuro	**Active**	0.76
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.72
Organ toxicity	Respiratory toxicity	respi	**Active**	0.77
Organ toxicity	Cardiotoxicity	cardio	**Active**	0.75
Toxicity end points	Carcinogenicity	carcino	Inactive	0.67
Toxicity end points	Immunotoxicity	immuno	Inactive	0.99
Toxicity end points	Mutagenicity	mutagen	Inactive	0.91
Toxicity end points	Cytotoxicity	cyto	Inactive	0.79

Classification	Target	Shorthand	Prediction	Probability
Toxicity end points	BBB-barrier	bbb	**Active**	0.85
Toxicity end points	Ecotoxicity	eco	Inactive	0.50
Toxicity end points	Clinical toxicity	clinical	Inactive	0.89
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.65
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.95
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99

Classification	Target	Shorthand	Prediction	Probability
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.99
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.99
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.99
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	1.0
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.99
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.69
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.79
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Inactive	0.73
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.95
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.52
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.86
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	1.0
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	1.0

Classification	Target	Shorthand	Prediction	Probability
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.69
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	0.99
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.65
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.76
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.73
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.94
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.95
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.97
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.88
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.89
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.99
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.9

Betaine was found to be inactive for hepatotoxicity (0.99), nephrotoxicity (0.72), and immunotoxicity (0.99), meaning it did not exhibit significant toxic effects in these organs. However, it was found to be active for neurotoxicity (0.76), respiratory toxicity (0.77), and cardiotoxicity (0.75). This suggests that betaine may pose a potential risk, particularly to the nervous system, respiratory function, and cardiovascular system. Inactive results were obtained for carcinogenicity (0.67), mutagenicity (0.91), cytotoxicity (0.79), and nutritional toxicity (0.65). Furthermore, the risks of immunotoxicity and clinical toxicity were found to be low.

An ecotoxicity estimate of 0.50 categorized betaine as inactive. This suggests that betaine will not exhibit significant environmental toxicity and may be safer for biological systems. Analysis based on the Tox21 database showed that betaine interacted inactively with the nuclear receptors studied, including the aryl hydrocarbon receptor (AhR), androgen receptor (AR), estrogen receptor (ER), and peroxisome proliferator-activated receptor gamma (PPAR-γ). This result suggests that betaine does not exhibit direct toxic or disruptive effects on hormone receptors.

Stress response mechanisms such as the antioxidant response element (nrf2/ARE), heat shock factor response element (HSE), mitochondrial membrane potential (MMP), and p53 pathways were all inactivated. This finding suggests that betaine does not pose a risk of inducing DNA damage, oxidative stress, or apoptosis at the cellular level.

Betaine was found to be inactive against several molecular targets, including thyroid hormone receptors (THRα, THRβ), GABA receptors, NMDA receptors, AMPA receptors, acetylcholinesterase, and voltage-gated sodium channels. This suggests that betaine does not have a direct toxic effect on key neuroreceptors and ion channels.

Betaine was found to have no inhibitory effect on the major CYP450 enzymes in the liver (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, and CYP2E1). This finding demonstrates that betaine has a low pharmacokinetic risk of drug-drug interactions and a metabolically safe profile.

Although a standard chemotherapeutic agent was not included as a physical positive control within the same experimental plates in the current study, our previous investigations using the identical 4T1 cell line and MTT methodology provide a robust comparative baseline. Specifically, in our recent study evaluating abemaciclib—an FDA-approved targeted therapy for breast cancer—against 4T1 cells, the IC50 value was determined to be 14.84 μM [15]. Given the molecular weight of abemaciclib (506.59 g/mol), this concentration is equivalent to approximately 7.52 μg/mL. In stark contrast, the IC50 of betaine in the present study was 859.10 μg/mL, indicating that significantly higher concentrations are required to achieve an antiproliferative effect. Furthermore, the comparable IC50 value obtained for non-cancerous L929 fibroblasts (970.40 μg/mL) confirms that the observed effect represents general cytotoxicity at high doses rather than targeted anticancer selectivity.

In silico ADME/Toxicity profiling revealed that betaine has high aqueous solubility, low lipophilicity, limited gastrointestinal absorption, and low potential to cross the blood-brain barrier, partly due to its negative LogP and high polarity. Although it may be a P-glycoprotein substrate, these physicochemical traits likely hinder its effective cellular uptake and systemic bioavailability, explaining its modest effects in standard *in vitro* assays.

Despite the limited direct cytotoxicity, there is promising evidence that betaine's bioactivity can be significantly enhanced using drug delivery strategies. Notably, when complexed with *p*-sulfonatocalix[4]arene (p-SC4) via host–guest chemistry, betaine exhibited dramatically improved antiproliferative activity: the IC_{50} dropped to 1.6 ± 1.4 µg/mL in MCF-7 breast cancer cells and 3.3 ± 1.1 µg/mL in HeLa cervical cancer cells, compared to negligible effects when used alone [16]. This approach enhances betaine's cellular concentration and selectively releases it in acidic tumor microenvironments, providing a compelling route for therapeutic improvement.

Beyond cytotoxicity, betaine also demonstrates notable anticancer mechanisms. In human prostate DU-145 cancer cells, betaine induced oxidative stress, inflammation, and apoptosis in a dose-dependent manner, and decreased antioxidant defenses, suggesting that at sufficient concentrations it can shift to pro-oxidant activity and suppress tumor cell survival [17]. Additionally, in oral squamous cell carcinoma (HSC-4 and HSC-7 lines), betaine significantly inhibited proliferation, colony formation, invasion, and migration by downregulating MMPs and epithelial-to-mesenchymal transition (EMT) markers [18].

Epidemiological evidence further supports betaine's role in cancer prevention. Meta-analysis data indicate that higher dietary or circulating betaine levels are associated with a lower overall risk of cancer, with relative risks ranging from 0.87 (dietary) to 0.88 (blood levels) [5]. Another pooled analysis suggests that increasing choline plus betaine intake by 100 mg/day may reduce cancer risk by 11 % [4]. These findings suggest that while betaine may not directly kill cancer cells effectively in vitro at low doses, it could contribute to cancer risk reduction in vivo through mechanisms like methylation support and one-carbon metabolism.

A notable discrepancy exists between the in silico predictions of SwissADME and ProTox-II regarding blood-brain barrier (BBB) permeability. While SwissADME predicts that betaine cannot cross the BBB, ProTox-II reports the molecule as 'active' for BBB permeation (probability: 0.85) and concomitantly suggests a high risk of neurotoxicity (probability: 0.76). However, considering betaine's fundamental physicochemical properties –specifically its high hydrophilicity, polar surface area (TPSA: 40.13 $Å^2$), and negative consensus LogP value (-2.18)– passive diffusion across the lipid-rich BBB is biologically highly unlikely, supporting the SwissADME model. The ProTox-II prediction is strictly structure-based and dose-independent, likely representing an algorithm-driven false positive triggered by specific structural fragments like the quaternary nitrogen.

In reality, while betaine poorly crosses the BBB via simple diffusion, it is taken up by the brain through specific active transport systems (such as the betaine/GABA transporter, BGT-1) in living organisms. Once in the neural environment, multiple experimental studies contradict the ProTox-II neurotoxic prediction, demonstrating neuroprotective effects rather than neurotoxicity. For instance, betaine protected neuronal PC12 cells from

rotenone-induced oxidative stress and apoptosis by restoring mitochondrial membrane potential and reducing caspase activation [19]. Similarly, in a rat model, betaine mitigated CuO nanoparticle-induced neurotoxicity by attenuating oxidative damage and improving behavioral function [20]. These findings support a dose-dependent duality, where betaine may exhibit neurotoxicity only at very high concentrations but is otherwise neuroprotective at physiologically relevant levels.

Taken together, these observations suggest that although betaine on its own requires very high doses to exhibit cytotoxic activity in 4T1 cells, its utility lies elsewhere. Betaine shows potential as a preventive agent in epidemiological settings and, more importantly, as an adjuvant or enhancer of anticancer therapeutics when leveraged in advanced delivery systems like host–guest complexation. Its antioxidant and neuroprotective benefits further bolster its suitability for multi-faceted biomedical applications.

4. CONCLUSION AND RECOMMENDATIONS

Future research should therefore prioritize exploring betaine in optimized formulations that improve tumor targeting and cellular uptake such as nanoscale carriers or calixarene-based systems and investigate its modulatory role in oxidative stress and inflammation in vivo. Investigations into low-dose, long-term preventive effects synergistic combinations in animal models would also be highly valuable in defining betaine's translational relevance.

Research and Publication Ethics Statement

The entire process of the manuscript was carried out in compliance with the research and publication ethics guidelines of the Celal Bayar University Journal of Science.

Author's Contributions

Elif Ece Akgün: Drafted and wrote the manuscript, performed the experiment and result analysis.

Büşra Gülbenli Türkoğlu: contributed to the experiment and participated in manuscript drafting.

Yasemin Sunucu Karafakıoğlu: supervised the experiment's progress, result interpretation.

Senem Akkoç: contributed to the study design, methodology, data collection, and analysis.

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