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Dual Action of Resveratrol: Inhibition of Human Glutathione Transferase and its Antagonism with Tamoxifen on Breast Cancer Cell Viability

Abeer Shokeer,[a*] Eman R. Zaki,[a] Nevein Salah El-Din Saleh,[a] Rasha A. Guneidy[a]

[a] *Molecular Biology Department, Biotechnology Research Institute, National Research Centre, Cairo12622, Egypt*

Abstract

Breast cancer is considered as the second leading cause of death among women. Resveratrol, known as phytoestrogen, exhibits anti-cancer, anti-oxidant, and anti-inflammatory properties. It is a trans-stilbene and a non-flavonoid polyphenol. This study examined the impact of resveratrol on human glutathione transferase P1-1 (hGST P1-1) activity and its efficacy when combined with tamoxifen using MCF-7 breast cancer cells. Steady-state kinetics and molecular docking were demonstrated to show the effect of resveratrol inhibition on hGST P1-1. Resveratrol acts as a mixed noncompetitive inhibitor for hGST P1-1 by using glutathione (GSH) as a substrate, altering both Vmax and KM. The Ki values were investigated of 71±12.6 µM for GSH, suggesting that resveratrol can interact with the free enzyme and the enzyme-substrate complex. Molecular docking studies revealed interactions between both resveratrol and tamoxifen with the dimer interface of hGST P1-1. The combination of resveratrol and tamoxifen showed an antagonist effect on MCF-7 cell viability, suggesting potential complexities and challenges in their combined use for therapeutic purposes. The antagonistic effect suggests that resveratrol could interfere with the therapeutic efficacy of tamoxifen, potentially through molecular mechanisms. This study investigates the importance of understanding drug interactions at the molecular level and highlights the necessity for careful consideration when combining therapeutic agents.

Keywords: Glutathione transferase P1 inhibition; trans-Resveratrol; Structure-function relationship; Tamoxifen

1. Introduction

Breast cancer is the second leading cause of death among women. It is a hormone-dependent disease, and estrogen is secreted from mammary tumor cells [1]. Numerous types of cancer, including breast cancer, have a significant correlation with dietary intake and exercise [2, 3]. The stilbene backbone class of polyphenols exhibits various biological activities and beneficial effects, including anti-oxidant and anti-inflammatory properties, mainly due to its ability to scavenge free radicals. Consequently, it can positively influence pathologies associated with oxidative stress, including cancer [4-6]. Resveratrol is classified as a phytoestrogen because of its similarity in structure to estrogen diethylstilbestrol which allows interaction with the human estrogen (α and ß) receptors and activates estrogen receptor-dependent transcription in human breast cancer cells [7,8]. It acts as an anti-tumor agent in breast cancer [9-11] by affecting several intracellular mediators involved in cancer initiation, promotion, and progression, as well as apoptosis and cell-cycle arrest [12-16]. Additionally, resveratrol acts as a potential multidrug-resistant inhibitor in different drug-resistant cancer cells [17].

Due to its important properties, the stilbene scaffold has been introduced to many synthetic anti-cancer drugs such as toremifene, raloxifene, and tamoxifen. The tamoxifen [(z) -1- [4- [2-(dimethylamino)ethoxy] phenyl] -1,2-diphenyl-1-butene] is a trans-stilbene synthetic compound [18]. It is defined as a non-steroidal selective estrogen receptor modulator that can help in treating early and advanced human breast cancer. Additionally, it can potentially prevent breast cancer in humans in specific cases [19]. It works by blocking the effects

*Corresponding author e-mail: abeergs70@hotmail.com.; (Abeer Shokeer).
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of estrogen, thereby reducing estrogen production in breast cancer cells [20].

The relationship between chemical structure and biological activity has been extensively studied and is still being debated due to its significance. The stilbene backbone class includes trans-resveratrol (3,4′,5-trihydroxystilbene, monomeric stilbene, Fig.1). Although resveratrol and tamoxifen share a stilbene backbone, they have different mechanisms of action as anti-cancer drugs as shown above.

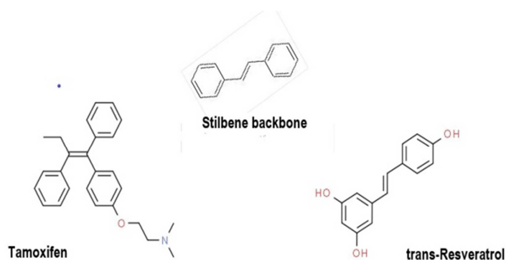

Figure 1: Chemical structures of stilbene backbone, tamoxifen, and trans-resveratrol. The chemical structures were drawn by the ACD/ChemSketch 2018.2.1 program (ACD/Labs, Toronto, ON, Canada).

Combination treatment with resveratrol has been a promising strategy to overcome tamoxifen resistance [21]. Resveratrol is a proven antioxidant that can protect normal cells from damage that is produced by anticancer medicines during chemotherapy [22]. Human glutathione transferases (EC 2.5.1.18; GSTs) belong to the enzymes involved in detoxication enzymes (phase II). They are divided into three families according to their sub-cellular distributions including cytosolic, mitochondrial, and microsomal [Membrane-Associated Proteins in Eicosanoid and Glutathione (MAPEG)] [23]. Cytosolic GSTs represent the large family. They have been divided into seven classes based on their amino acid sequences and structural similarities into, Alpha (A). Mu (M), Pi (P), Theta (T), Sigma (S), Omega (O), and Zeta (Z) [24-26]. Cytosolic GSTs are made up of two subunits, each of which is about 25 kDa. Each subunit of dimeric form constitutes two domains, N-terminal and C-terminal domains [25-27]. The N-terminal domain includes a hydrophilic binding site (G-site) which is specific for GSH [28]. The C-terminal domain constitutes a hydrophobic substrate binding site (H-site), allowing the enzyme to interact with a wide range of electrophilic substances. The enzyme�even's active site is formed by the G and H sites [29-31]. The dimer interface that is located between the two dimers, is supposed to facilitate the release of toxic substances from the cell [32].

GSTs facilitate the GSH thioether conjugate with potentially harmful electrophilic intermediates. Additionally, GSTs play an important role in post-translational modifications, c-Jun N-terminal kinase (JNK) signaling pathways, and Cyclin-dependent kinase 5 (Cdk5) kinase activity, as well as mechanisms related to cell death and survival [27]. Elevation of GSTP production has been found in tumors and is believed to be related to tumor cell resistance to therapy. For instance, research has shown that GSTP expression is linked to drug resistance and poor diagnosis in breast cancer patients [33]. Numerous studies also indicate that the presence of GSTP predicts poor pathological response in estrogen receptor α-negative breast cancer during neoadjuvant therapy [34, 35]. It was also suggested that GSTP expression, along with loss of estrogenic receptor α at the mRNA or protein level has been linked to poor prognosis in breast cancer as well as the mechanism of drug resistance [35-37].

This study aims to investigate the effects of resveratrol on hGST P1-1 catalytic activity as well as its efficacy in MCF-7 breast cancer cells, both separately and in combination with tamoxifen. Furthermore, the study will use molecular docking techniques to elucidate the molecular interactions between resveratrol, tamoxifen, and hGST P1-1. The results of this study may reveal the complexity of drug interactions at the molecular level and highlight the necessity for careful consideration when combining therapeutic agents. Understanding these interactions is crucial for optimizing breast cancer treatment strategies and improving patient outcomes.

2. Experimental

Chemicals

In this study, resveratrol, Tamoxifen, reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), LB broth media, lysozymes, isopropyl-β-D-thiogalactoside (IPTG), Sodium dodecyl sulphate (SDS), dimethyl sulfoxide (DMSO), and Ni-IMAC affinity gel were obtained from Sigma-Aldrich, USA. (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) (MTT) and human breast cancer cell lines (human Caucasian breast adenocarcinoma (MCF-7) were obtained from the Bioassay-Cell Culture Laboratory, National Research Centre, Cairo, Egypt.

2.1. Expression and purification of recombinant human GST P1-1

The wild-type recombinant hGST P1-1 was kindly provided by Prof. Bengt Mannervik (Stockholm University, Department of Biochemistry and Biophysics). The hGST P1-1 was transformed in XL1- Blue strain of E. coli with the plasmid pKK-D. The cells had been cultured to reach the absorbance of 0.2 at OD600 before induction with 0.2 mM IPTG.

After 18 hours of incubation at 37°C, the cells were collected by centrifugation at 3000 xg for 10 minutes. The bacterial pellets were suspended in 20 mM phosphate buffer, pH 7.4, 1.0 mM EDTA, 0.2 mM DTT, 0.2 mg of lysozyme\ mL, and kept on ice for 30 minutes. Sonication was performed in three 60-second treatments, with 60-second intervals. The resulting suspension was centrifuged at 30,000 xg for 30 minutes at 4°C, and the supernatant containing hGST P1-1 was saved for further purification.

N-terminal 6XHis-tag hGST P1-1 was purified in a single step using Ni-IMAC affinity chromatography. The matrix was equilibrated with 20 mM sodium phosphate buffer, pH 7.4, 0.2 mM DTT, 500 mM NaCl, and 100 mM imidazole (buffer A). The bound GST had been eluted with buffer A which included 500mM imidazole, and dialyzed against 10 mM Tris-HCl, pH 7.8, containing 0.2 mM DTT, and 1mM EDTA. All of the procedures had been carried out at 4 °C. The purity of hGST P1-1 protein was checked by SDS-PAGE [38]. Protein concentration was determined by Bradford method using Coomassie brilliant blue (G-250) dye and bovine serum albumin serving as the standard [39].

2.2. GST P1-1 activity assay

The specific activity of recombinant hGST P1-1 was determined as described by [40]. The assay mixture, with a total volume of 1.0 mL, included 0.1 M potassium phosphate buffer, pH 6.5, 1.0 mM CDNB in ethanol, 1.0 mM GSH in buffer, and 150 µM enzyme. The assay was measured at an absorbance of 340 nm, using an extinction coefficient of 9.6 mM-1cm-1 [40].

2.3. Resveratrol inhibition effect on hGST P1-1

The potency (Ki), and the type of inhibition of resveratrol were followed by using a steady-state kinetics inhibition study. The apparent KM and Vmax values for GSH were determined at pH 6.5, with GSH concentrations ranging from 0.1 to 2.0 mM with a constant CDNB concentration of 2.0 mM. The selection of pH 6.5 for the assay was chosen to optimize hGST P1-1 activity and stability. The chosen concentration range of GSH was relevant for assay optimization, as it allows for accurate measurment of hGST P1-1 specific activity as well as avoiding substrate inhibition effect that can occur at high level of concentrations. The experiment was carried out with three different concentrations of resveratrol (30, 50, 70µM). The apparent KM, Vmax, and Ki values were calculated by using Michaelis-Menten and Lineweaver–Burk plots. Another method (Dixon plot) for determining the Ki value was suggested by [41]. The reaction's initial velocity was recorded as resveratrol concentrations at three fixed GSH concentrations.

2.4. Molecular docking of resveratrol and tamoxifen with GST P1-1.

The crystal structure of recombinant hGST P1-1 enzyme (PDB: 6GSS) in complex with a substrate (GSH) was obtained from the Protein Data Bank (www.rcsb.org) in PDB format [42]. The information on the enzyme structure is described in text files containing crucial information such as the number of atoms, their names, angles, dihedral angles, bond distances, and numbers of residue. The three-dimensional structures of the ligands of interest (resveratrol and tamoxifen) were obtained from PubChem(https://pubchem.ncbi.nlm.nih.gov\compounds). These ligand structures were optimized through geometry adjustments and energy minimization, and then saved as mol2 format. Graphical representations were created using the UCSF-Chimera program, which is a visualization system for analysis and exploratory research [43]. Molecular docking was employed to predict the optimal orientation of a protein when attached to another compound in a stable complex. Most docking programs assume the target is an inflexible while allowing for ligand flexibility [44]. The molecular docking was performed using SwissDock server, an online tool based on the EADock DSS engine that employs a multi-objective scoring functions designed around the CHARMM22 force field and FACTS solvation [45].

2.5. Cell culture for breast cancer cells

MCF-7 breast cancer cells were utilized in this study. A laminar flow chamber biosafety class II level (Baker, SG403INT, Sanford, ME, USA) was used to perform all procedures in an atmosphere of sterility. The cells were cultured in DMEM medium HCT116 containing 1% antibiotic-antimycotic solution (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine at 37ºC with 5% CO2. Cells were cultured in batches to grow for 10 days after then transfered at a concentration of 10x103 cells/well to new complete growth media in 96-well microtiter plastic plates at 37ºC for 24 hours under 5% CO_2 through a carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). After aspirating the cultural media, a fresh medium (without serum) was then added and the cells were cultured with several concentrations of the sample

(resveratrol, tamoxifen, and their combination) or alone (negative control) to achieve the final concentration of 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, and µM/ml.

2.6. Cell viability measurement

To measure the viability of cells, the mitochondria-dependent reduction in yellow color of MTT into purple formazan was assessed [45]. The cultural medium was aspirated after 48 hours of incubation process, and 40ul of MTT salt with a concentration of 2.5µg/ml was added to every well. The mixture was then incubated further for 4.0 hours at 37ºC with 5% CO_2. To terminate the reaction and dissolve the crystals that had formed, 200µL of 10% SDS solution was applied to each well and maintained at 37ºC overnight [47]. The absorbance was then determined at a wavelength of 595nm using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA). A statistical significance was tested between samples and the negative control (cells containing vehicle) using a t-test (SPSS 11 program). The concentration of DMSO reagent used for dissolving the tested compounds was less than 0.2%. A probit analysis was performed to determine IC50 and IC90 using the SPSS 11 program. The percent change in viability was estimated using the following formula:

[(Reading of compound/Reading of negative control) – 1] x 100

2.7. Statistical analysis

All tests were done in triplicate, and their results are displayed as mean ± standard deviation (SD). GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA) had been used for statistical analysis. The One-way ANOVA test followed by the Kruskal-Wallis test was used to assess the significance of differences among samples. A p-value of 0.05 or less was regarded as a statistical significance.

3. Results and discussion

3.1. Inhibitory effect of resveratrol on hGST P1-1

Elevated expression of GST P1-1 is linked to drug resistance in cancer cells, including breast cancer [35, 48, 49]. By inhibiting hGST P1-1 activity, selected inhibitors, including resveratrol, could potentially counteract this resistance mechanism, causing the cancer cells more sensitive to chemotherapy treatments. Furthermore, resveratrol has anti-oxidant and anti-inflammatory properties that could provide additional benefits in cancer treatment [50-51]. Resveratrol's anti-oxidant mechanisms can play a crucial role in mitigating oxidative stress, which is linked to many human diseases [52], by reducing oxidative stress and inflammation, resveratrol could protect healthy cells from chemotherapy-induced damage [53]. Steady-state kinetics inhibition analysis was used to determine resveratrol's inhibition potency (K_i) and identify the inhibition type in the enzymatic reaction.

The data were represented by the Michaelis-Menten, Lineweaver–Burk plot, and Dixon plot of hGST P1-1 kinetics (Figure 2A, B, and C, respectively). The results indicated that resveratrol acts as a mixed inhibitor toward GSH. This suggests that resveratrol can bind to both free enzyme (E) and enzyme-substrate (ES) complex, leading to the formation of enzyme-inhibitor (EI) or enzyme-substrate-inhibitor (ESI) complexes. The mixed inhibition of resveratrol was confirmed by altering both K_M and V_{max} values among different concentrations of resveratrol (Table 1). Specifically, the V_{max} and K_M values, which indicate the maximum reaction velocity and the Michaelis constant, respectively, were affected. The K_i value was found to be 71 ± 12.6 µM using GSH as a substrate as shown in Table 1.

The Dixon plot (Figure 2C) shows the reciprocal of the reaction velocity (1/v) plotted versus the x-axis, confirming mixed noncompetitive inhibition. This intersection point provides the K_i value, which concludes that resveratrol can bind to both the free enzyme (E) and the enzyme-substrate (ES) complex. Additionally, the alpha factor value was calculated to be 2.15 ± 0.8. The alpha factor indicates that resveratrol decreases the enzyme's affinity for GSH. This alteration in affinity is a characteristic of mixed inhibition, in which the inhibitor influences the enzyme's binding to its substrate and the overall catalytic activity.

Table 1: Kinetic parameters of hGST P1-1 with different concentrations of resveratrol

Resveratrol concentration (µM)	[a]V_{max}^{GSH} (µM\min\mg protein)	[b]K_M^{GSH} (mM)	[c]K_i (µM)	[d]Alpha factor
0	202 ± 8.00	0.380± 0.04	71 ± 12.6	2.15 ± 0.8
30	185 ± 10.3	0.459± 0.06		
50	163 ± 6.99	0.469± 0.05		
70	135± 9.66	0.472± 0.09		

[a]Vmax is the maximum enzyme velocity, expressed in the same units as µM\min\mg protein.
[b]**K_M**is the MichaelisMenten constant, expressed in mM
[c]**K_i**is the inhibition constant, expressed in µM**.**
[d]**Alpha factor** value determines the degree to which the binding of resveratrol changes the affinityof the hGST P1-1for substrate. Its value is always greater than zero.

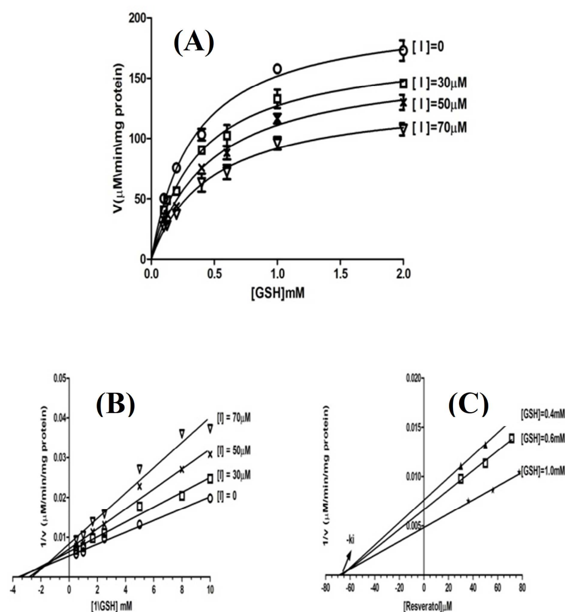

Figure 2: Steady-state kinetics for the inhibition of hGST P1-1 by resveratrol.

(A) Michaelis-Menten plot shows the inhibition of hGSTP1-1 by resveratrol at three concentrations (0, 30, 50, and 70µM). The concentration of GSH was varied from 0.1 to 2.0 mM at constant concentration of CDNB at 2 mM. This plot demonstrates how resveratrol affects the enzyme's activity in the presence of varying GSH concentrations.

(B) Lineweaver-Burk plot (double reciprocal plot) indicates mixed noncompetitive inhibition when $\alpha > 1$. The value of α is crucial as it helps determine the mechanism of inhibition exerted by resveratrol on hGST P1-1.

(C) Dixon plot presents 1\v as a function of inhibitor concentration for mixed noncompetitive inhibition at three different GSH concentrations. The K_i value for resveratrol is determined from negative x-axis value at the intersection point of three lines, providing insight into the potency of resveratrol as an inhibitor of hGST P1-1.

Both non-competitive and mixed types of inhibition are similar but have distinct roles in enzyme kinetics. The similarity is concerned in that the inhibitor can bind to both the free enzyme to give a complex (EI) with dissociation constant K_{ic} and also to the enzyme-inhibitor-substrate complexes (ESI) with a dissociation constant K_{iu} (Fig. 3). Mixed inhibition may occur as a result of reversible interaction to the enzyme-substrate complex (ES) [54,55]. It shows also increasing or decreasing (K_M) depending on whether the inhibitor has more affinity to the enzyme or the enzyme-substrate complex. In addition to resveratrol, catechins have also been shown to exhibit mixed non-competitive inhibition towards hGST P1-1. Our previous study indicates that catechins, a group of flavonoids, alterd the values of V_{max} and K_M, suggesting a similar inhibition mechanism as observed with resveratrol [56]. This comparative analysis highlights the potential for a broader understanding of how various compounds interact with hGST P1-1, which could inform future therapeutic strategies. This understanding can also elucidate the specific binding sites and interactions at molecular level.

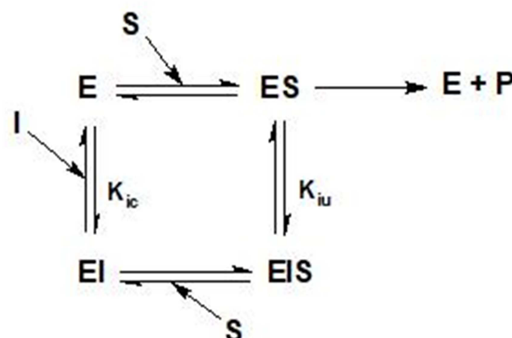

Figure 3: Mechanism that produced mixed inhibition. This figure illustrates the mixed noncompetitive inhibition mechanism of resveratrol (I) on human glutathione transferase P1-1 (E). Resveratrol binds to free enzyme (E) and the enzyme-substrate complex (ES), resulting in enzyme-inhibitor (EI) and enzyme-substrate-inhibitor (EIS) complexes. This dual binding results in alterations to both the maximum reaction velocity (V_{max}) and the Michaelis constant (K_M) using glutathione as substrate (S), indicating mixed inhibition. The inhibition constants (K_{ic}) and (K_{iu}) are depicted in the figure. The (K_{ic}) represents the dissociation constant for the inhibitor binding to the (E) while the (K_{iu}) represents the dissociation constant for the inhibitor binding to the (ESI). These constants highlight the ability of (I) to bind to both forms of the enzyme, thereby interfering with its activity**.**

3.2. Molecular docking of resveratrol and tamoxifen with GST P1-1

The docking results provide detailed structural insights into the binding interactions. Both resveratrol and tamoxifen exhibited affinity binding to hGST P1-1. Tamoxifen showed a slightly higher binding score (delta G= -7.6 Kcal\mol) than resveratrol (delta G= -6.9 Kcal\mol) which indicating a stronger interaction of tamoxifen with hGST P1-1 as shown in figure 4 and 5. This observation is in line with a previous study that has demonstrated the potent anticancer effects of tamoxifen through its ability to modulate estrogen receptor expression and induce apoptosis [57]. On the other hand, both resveratrol and tamoxifen formed hydrogen bonds and hydrophobic interactions with key residues in the dimer interface of hGST P1-1 including Arg13, Glu65, Ser66, Arg70, Glu97, Asp94, Cys101, Lys102, and Trp108 (Fig. 4, 5). Glu65 and Ser66 reidues were highly conserved among GSTs. Arg70 is invloved in key structural region of GST P1-1. The conservations of the residues is thought to play an impotant role in maintaing stability of that regions as well as keep correct folding [58] Trp108 residue that lies within H-site has been identified as crucial for the activity of GST P1-1 [59]. The hydrogen bonds and

hydrophobic interactions observed suggest that both resveratrol and tamoxifen fit well within the GST P1-1 interface, leading to the stabilization of the enzyme-inhibitor complex. These results support the mixed inhibition of resveratrol and un-competitive inhibition of gossypol with hGST P1-1 as shown earlier [56]. The change in inhibition type suggests that while both reveratrol and gossypol may bind similarly [56], they could induce different conformational changes in the enzyme. This could indicates the complexity of enzyme-ligand interactions and emphasize the need to discover how different structural feactures affect the inhibition mechanisms.

Previous study has shown that resveratrol interacts with different proteins and enzymes, demonstrating its multifunctional therapeutic potential. For instance, resveratrol's interaction with insulin has been studied using molecular docking, revealing significant binding interactions that contribute to its anti-diabetic properties [60]. They had shown the versatility of resveratrol in modulating different molecular targets, which is consistent with its observed inhibitory effects on hGST P1-1 in the current study.

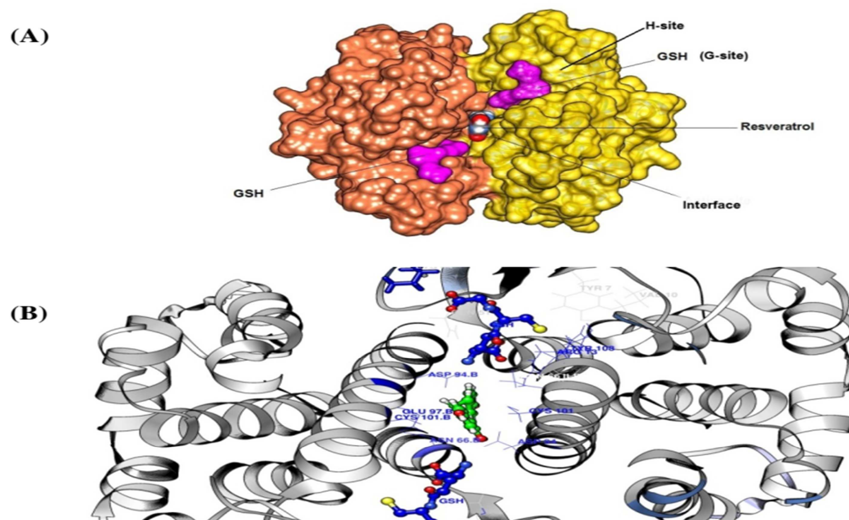

Figure 4: Interaction of trans- resveratrol with hGST P1-1 enzyme. (A) Binding site of the hGST P1-1 (PDB ID: 6GSS) and position of resveratrol at the enzyme interface. (B) Docking pose of resveratrol in hGST P1-1 showing the different amino acid residues of hGST P1-1 interacting with resveratrol, depicted in green, glutathione is shown in blue.

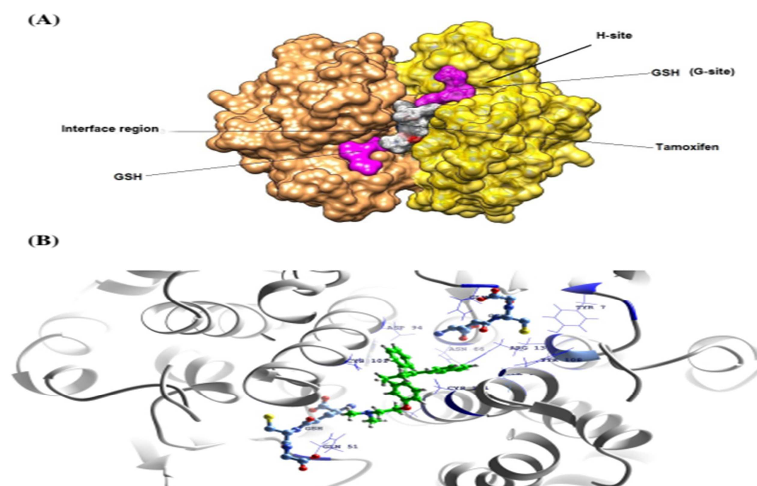

Figure 5: Interaction of tamoxifen with hGST P1-1 enzyme. (A) Binding site of the hGST P1-1 (PDB ID: 6GSS) and position of tamoxifen at the enzyme interface. (B) Docking pose of tamoxifen in hGST P1-1 showing the different amino acid residues of hGST P1-1 interacting with tamoxifen, depicted in green, glutathione is shown in blue.

3.3. Cytotoxicity and cell viability

Resveratrol has been demonstrated to increase the effectiveness of some anticancer drugs such as docetaxel, paclitaxel, cisplatin, and doxorubicin, through synergistic effects. This effect can be explained by different mechanisms such as the induction of apoptotic pathways which make cancer cells more susceptible to drug-induced cell death [61]. Additionally, resveratrol has been found to sensitize cancer cells to chemotherapeutic drugs to overcome drug resistance [62]. Resveratrol can also enhance the efficacy of anticancer drugs by modulating various signaling pathways, making them more effective at lower doses [16, 63].

This study investigated the effects of resveratrol and tamoxifen on the cytotoxicity and viability of MCF7 cells across a range of concentrations (0.78 to 100 µM). Both compounds demonstrated a dose-dependent reduction in cell viability. At higher concentrations (100 and 50 µM), both resveratrol and tamoxifen significantly reduced cell viability. As the concentration decreased, resveratrol maintained a higher efficacy in reducing cell viability compared to tamoxifen. At the lowest concentration tested (0.78 µM), both compounds showed a slight increase in cell viability compared to some higher concentrations, suggesting a dose-response or adaptive effect (Fig. 6).

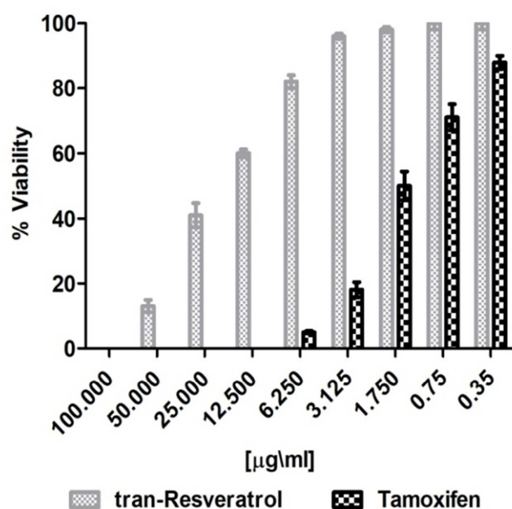

Figure 6: Comparison of cell viability (%) at varying concentrations of resveratrol and tamoxifen. Bars filled with diagonal gray lines represent resveratrol, while bars filled with black dots represent tamoxifen. The x-axis shows concentration values in [µg/mL], and the y-axis indicates the percentage of cell viability. Statistical significance was determined using a one-way ANOVA followed by Kruskal-Wallis test. The value of (p) 0.016, (*$p < 0.05$).

Interestingly, it was found that the combination of resveratrol and tamoxifen showed an antagonistic effect on MCF-7 cell viability (Fig. 7). This antagonistic interaction resulted in higher cell viability in comparison to tamoxifen alone, suggesting that resveratrol reduces the cytotoxic effects of tamoxifen when used in combination. The antagonistic effect observed with the combination of two compounds was calculated using combination index (CI) values, where CI > 1 indicates antagonism.

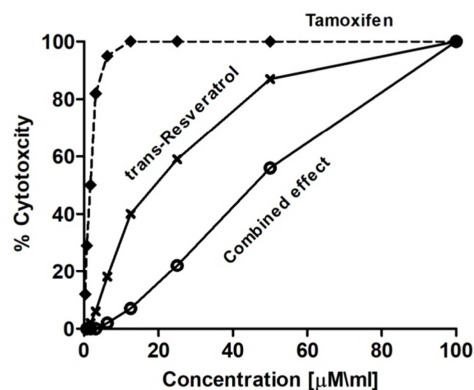

Figure 7: Cytotoxicity percentage as a function of concentration [µM/mL] for resveratrol, tamoxifen, and their combined effect on MCF7 cells. Combined treatment of resveratrol and tamoxifen shows an antagonistic effect compared to the individual treatments. . Statistical significance was determined using a one-way ANOVA with a p-value of 0.016, (*$p < 0.05$).

Previous studies have shown that resveratrol can act as an antagonist for estrogen receptors, which may further complicate its interaction with tamoxifen [7]. Additionally, resveratrol's ability to modulate various molecular targets, including drug- and carcinogen-metabolizing enzymes, supports its multifunctional therapeutic potential. However, this also highlights to the need for careful consideration when designing combination therapies [64].

While resveratrol in the present study exhibited an antagonistic effect on cell viability when combined with tamoxifen, oxyresveratrol in another study by [65] showed the synergistic effects of when combined with anticancer agents doxorubicin and melphalan. They reported that oxyresveratrol not only enhanced cytotoxicity but also altered cell morphology and induced apoptosis without generating cardiotoxic metabolites [65]. The differing effects of resveratrol and oxyresveratrol provide valuable thoughts relating to the potential for developing innovative therapeutic strategies that influence the strengths of both related compounds and decreasing their weaknesses.

.Incorporating conjugation techniques and nanoparticle delivery methods, could be also

explored to improve the delivery and efficacy of compounds like resveratrol in cancer treatments [66-69]. This approach may further optimize therapeutic outcomes by enhancing target specificity and reducing off-target effects, a concept aligned with our findings on the selective inhibition of hGST P1-1 by resveratrol.Therefore, the current study highlights the importance of careful consideration when combining therapeutic agents, as interactions can vary significantly depending on the specific drugs and involved mechanisms.

4. Conclusion

This study aimed to show the effects of resveratrol on the catalytic activity of hGST P1-1, as well as its efficacy on MCF-7 breast cells. This effect was used individually and in combination with tamoxifen. Additionally, the combined treatment of resveratrol and tamoxifen was studied to investigate its antagonistic effect on cell viability. The results of the present study provide new insights into the drug design efforts targeting GSTs and elucidate the structure-function relationship of resveratrol and tamoxifen through molecular docking, providing a deeper understanding of their interactions with hGST P1-1. Resveratrol exhibited a mixed type of inhibition, which adds to the complexity of its interaction with tamoxifen. The outcome of the study may contribute to understanding of drug interactions at the molecular level and highlight the necessity for careful consideration when combining therapeutic agents. Future studies should focus on elucidating the precise molecular mechanisms underlying the antagonistic effects observed in the current study. Additionally, in vivo studies and clinical trials are necessary to evaluate the therapeutic potential and safety of combining resveratrol with tamoxifen in breast cancer treatment.

5. Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

6. Formatting of funding sources

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