

Article

Application of Biphasic Numerical Model for the Prediction of Colorectal Carcinoma Cell Response to Co-Treatments

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Abstract

Modern computational biology is increasingly applied in preclinical studies, and mathematical models can provide valuable insights into biological system behavior. Numerical modeling tools can significantly and rapidly help in predicting the cellular response to different treatments, numerous newly synthesized compounds tested on different model systems. This study is devoted to the application of a biphasic numerical model to explain and predict the behavior of colorectal carcinoma cell lines in investigated co-treatments. The model was used to estimate parameters related to cell proliferation and death and to predict cellular behavior through the determination of treatment efficiency and effectiveness. This study showed that the experimental results can be mathematically confirmed, and the data for the most effective treatment can be obtained. The most efficient co-treatment concentration was identified by the model as the condition associated with the lowest proliferation-related parameter and the greatest reduction in cell viability. The model indicated that the most efficient concentration does not appear to induce a rapid adaptive cellular response and may therefore represent a suitable candidate for subsequent treatment cycles. The model suggests that the investigated treatments may have limited therapeutic potential in both cell lines due to the sustained viability of rapidly proliferating cells and evidence of continued de-differentiation.

Keywords: platinum(IV) complexes; *Phellinus linteus*; apoptosis; cell behavior; Frizzled-7 receptor



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1. Introduction

Mathematical models can be used to provide necessary explanations of data obtained by biological experiments in terms of mechanisms regarding tumor growth, as a response of cancer cells to treatments with potential applications in preclinical studies [1–4]. The use of mathematical modeling tools in predicting the cellular response to applied treatments has been increasingly used in preclinical studies in which a large number of newly synthesized compounds are tested on different model systems [4,5]. Avascular tumor growth has been widely studied using numerical models, and the resulting models are becoming increasingly sophisticated. In the present study, one of the proposed models was applied to analyze the behavior of two different colorectal carcinoma (CRC) cell lines, SW-480 and HCT-116,

attached to substrates in 2D systems and basically representing an avascular tumor. The biphasic model of avascular tumor growth that is used in the present study considers the cells and tested treatments at different concentrations by applying the physical laws regarding cancer cells and extracellular fluid, as it was stated in [6], where this model was initially presented. This model represents the properties of an avascular tumor by modeling it using a two-phase model: cells as a viscous, dense phase, and intercellular space filled with a nutrient medium as a liquid phase. The cellular phase implies cell formation and death, with the characteristic viscosity of cancer cells, which is related to the degree of their differentiation. Breward et al. [6] indicate that an important physical property of cancer cells is viscosity, according to which they behave differently in terms of proliferation. Namely, well-differentiated cells will proliferate more slowly because of higher viscosity of the cell population. In contrast to well-differentiated cells, cell populations with poorly differentiated cells have a lower viscosity because of loosened intercellular connections [7], which will contribute to their faster proliferation [8]. On the other hand, the tension of the cell membrane is directly related to cell viability. Therefore, living cells are characterized by a tense membrane, while dead cells (apoptotic cells) are characterized by lower membrane tension [9]. Furthermore, the viscosity of extracellular fluid increases with the increasing number of dead cells, therefore its movement through intercellular spaces decreases, followed by a decreased supply of food needed for cell proliferation. SW-480 cell line represents cells isolated from the early II CRC stage, described as well-differentiated [10]. HCT-116 cells are representative of an advanced form of CRC, isolated from stage IV CRC, poorly differentiated, and representative of an aggressive form of CRC cells [11,12]. It is known that these two cell lines differ in terms of growth mode, population compactness, proliferation rate, and response to treatments, as well as aggressiveness, migratory/invasive potential, and EMT stage [13]. The antiproliferative potential of *P. linteus* polysaccharides has been recently linked to the suppression of the Wnt/ β -catenin pathway in colorectal cancer cells [14–16]. Combined treatments, which were used to test cytotoxicity and pro-apoptotic effects, were designed to treat the cells with a different concentration of *P. linteus* mushroom extract in combination with small non-cytotoxic concentrations of various chemically synthesized cytostatics. This kind of therapy enables simultaneous targeting of various signaling pathways in cancer, with different mechanisms of action of components that constitute combined therapy. The experimentally obtained results of cytotoxicity and type of induced cell death in this study were analyzed using the mentioned mathematical model with the aim of predicting cell behavior after the application of co-treatment. Also, the present work aimed to determine the most effective combination of the investigated co-treatments by analyzing the biological base of the obtained experimental results and model parameters in the context of biological/physical phenomena. Based on the parameters of the model, a conclusion was reached regarding the most efficient and most effective concentration. Also, the model parameters indicate why the most efficient concentration was not the most effective, even if the most effective dose kills the greatest number of cells primarily via apoptosis.

Additionally, the present study uses the parameters of the mathematical model to indicate the differences in cell response to tested treatments in relation to the biological properties of SW-480 and HCT-116 cells. All this was additionally experimentally confirmed at the level of expression of protein markers related to cell differentiation and proliferation (Frizzled-7 receptor and nuclear β -catenin). Furthermore, the prediction of the cellular response in terms of proliferation to the tested co-treatments, in relation to the biological characteristics of the cells, was made.

2. Materials and Methods

2.1. Preparation of Drug Solutions

The procedure for the preparation of *P. linteus* methanol extract was described earlier [17]. Synthesis of Pt(IV) complexes, [PtCl₄(dbu-S,S-eddp)] (C1), [PtCl₄(dpe-S,S-eddp)] (C2), and their appropriate ligands, (L1) and (L2), with data concerning their purity were previously described in detail [18,19]. Stock solutions of the *P. linteus* methanol extract, cisplatin (Cispt), Pt(IV) complexes, and ligands were made in dimethyl sulfoxide (DMSO) and Dulbecco's modified Eagle medium (DMEM) at the concentration of 1 mg/mL and filtered before use through a 0.22 mm Millipore filter (Merck KGaA, Burlington, and SAD). Stocks were diluted further by a nutrient medium to various working concentrations. The concentration of DMSO in the most concentrated working solutions was 0.1% (v/v).

2.2. Cell Preparation and Culturing

Colorectal cancer cell lines SW-480 and HCT-116 were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were propagated in DMEM supplemented with 10% FBS and antibiotics (100 units/mL penicillin and 100 µg/mL streptomycin). Cells were cultured in a humidified atmosphere with 5% CO₂ at 37 °C. All studies were done with cells at 70 to 80% confluence.

2.3. Co-Treatments

Investigation of potential synergistic effects of *P. linteus* extract in co-treatment with cisplatin (Cispt) or Pt(IV) complexes (C1 and C2), or their ligands (L1 and L2), was performed as described: cells were treated with 50 µL of *P. linteus* methanol extract in concentrations in the range of 20, 50, 100, and 500 µg/mL, after which 50 µL of 20 µM Pt-based drugs or their ligands was applied so that their final concentration in the medium was 10 µM. The concentrations of extract in final volume were 10, 25, 50, and 250 µg/mL. Untreated cells served as a negative control, while cisplatin-treated cells were used as a positive control. All studies were done in triplicates in three independent experiments.

2.4. Cell Viability Assay (MTT Assay)

For the determination of cell viability, the MTT assay was performed according to the protocol described earlier [17]. Estimation of cytotoxic effects was presented as IC₅₀ values (doses that induce the inhibition of 50% of cells), calculated in relation to the *P. linteus* extract concentration.

2.5. Fluorescence Microscopic Analysis of Cell Death (AO/EB) Double Staining

Type of cell death induced by co-treatments was analyzed using the acridin orange/ethidium bromide (AO/EB) method, as already reported [17]. Analysis was performed 24 and 72 h after initial treatment with mushroom extract. According to the fluorescence emission and the morphological aspect of chromatin condensation in the stained nuclei, four cell types could be distinguished: viable, apoptotic (early and late), and necrotic cells. Cells were examined using fluorescence microscopy using a Nikon Eclipse Ti microscope (Nikon Instruments Inc., Melville, NY, USA) at 400× magnification, where a minimum of 300 cells was counted in every sample.

2.6. Determination of Protein Expression by Immunofluorescence Staining

Protein analysis of Frizzled-7 receptor and nuclear β-catenin expression was assessed by following the protocol previously described [17]. The fixation of cells after the incubation treatment (24 h) was done on the coverslip using 4% paraformaldehyde. The permeabilization step was done with cold methanol (−20 °C). The blockage of nonspecific antigens

was performed with 1% BSA for 1 h, followed by the addition of primary antibody anti-Frizzled-7 (concentration 5 µg/mL in PBS). After the 1 h incubation, secondary antibody conjugated with NorthernLights™ NL557 (1:100 dilution in PBS) was added for a 1 h incubation period.

For the purpose of localization and quantification of nuclear β-catenin, cells were incubated for 1 h with a solution of primary-antibody anti-nuclear β-catenin (1 µg/mL concentration in PBS). The same procedure was repeated for incubation with secondary antibody conjugated with Alexa 488 (diluted in PBS at a ratio of 1:200). For nuclei staining, DAPI dye was applied. Investigated proteins were visualized using a Nikon Ti-Eclipse inverted fluorescent microscope (Nikon Instruments Inc., Melville, NY, USA) at 600× magnification. Relative fluorescent intensity per cell (%) was quantified on obtained micrographs using the ImageJ software package [20] (Version 1.54g; National Institutes of Health, Bethesda, MD, USA).

2.7. Numerical Model

The behavior of cancer cells was simulated numerically in order to analyze the effect of different treatments on the parameters of the model. The numerical model used in this study was presented by [6]. As it was mentioned in the Introduction Section, this model considers the domain as a two-phase material that consists of cancer cells and extracellular material, and over time, the phase changes due to cell birth and death. In other words, some cells die and extracellular material occupies its place. Oxygen diffuses through the observed domain and is consumed by cancer cells, while cell death occurs due to low oxygen in that particular point of the domain.

The change in concentration of cancer cells over time (denoted by α) is defined as:

$$\frac{\partial \alpha}{\partial t} = \frac{(1 + S_1)\alpha(1 - \alpha)C}{1 + S_1C} - \frac{(S_2 + S_3C)\alpha}{1 + S_4C} \quad (1)$$

where C denotes oxygen tension, and S_1 , S_2 , S_3 , and S_4 are parameters of the numerical model. The first term on the right-hand side of Equation (1) represents cell growth, while the second term represents cell death. Therefore, the parameter S_1 is related to cell proliferation, while parameters S_2 , S_3 , and S_4 are related to cell death.

The oxygen consumption rate throughout the domain is defined as:

$$\frac{\partial^2 C}{\partial x^2} = \frac{Q\alpha C}{1 + Q_1C} \quad (2)$$

where the coefficients Q and Q_1 are parameters related to the oxygen consumption rate.

Since the cells were fixed within the plate during experiments performed in this study, this is also reflected in the numerical model, by keeping the cells fixed and neglecting the movement of cells that were originally a part of the model proposed by [6].

The nondimensionalization of equations of the numerical model was performed in the literature [6] and a similar approach was used in this study, but for a two-dimensional domain. This is appropriate for the current study, since the main goal of this study is not to measure the exact values of parameters, but to analyze the influence of different treatments on these parameters and subsequently on the behavior of cells.

Equations (1) and (2) were transformed into an incremental-iterative form and solved numerically using the finite element method [21]. The time-dependent two-dimensional simulations were performed using the PAK finite element software package [22]. Since the experiments were performed in a time-span of 72 h, the time step in simulations was equal to 1 h and the overall time was equal to 72 h. PAK is a general-purpose finite element program developed at the Faculty of Mechanical Engineering, University of Kragujevac,

capable of linear and non-linear structural and coupled field analyses [21,22]. The spatial domain was discretized using a structured mesh of bilinear quadrilateral finite elements. A mesh convergence study was performed using 40×40 , 60×60 , and 80×80 element grids; the differences in predicted viable cell percentages between the two finest meshes were below 0.5%, confirming mesh convergence. Consequently, all production simulations were performed using the 60×60 mesh. The temporal discretization employed an implicit (backward) Euler scheme, which was unconditionally stable for the selected time step of 1 h.

The initial cell concentrations at $t = 0$, denoted by α_0 , are defined using an experimental image of the control cell lines. The zero flux of cell concentration is prescribed on the boundary of the observed domain, and the oxygen tension is prescribed to be equal to zero, while the oxygen tension outside the cancer cell is constant.

The model contains six parameters (S_1 – S_4 , Q and Q_1). Parameters Q and Q_1 were estimated from the control cell lines and subsequently fixed, whereas parameters S_1 – S_4 were estimated separately for each treatment. These parameters were estimated for each considered treatment using experimental data. The estimation procedure was carried out in MATLAB (R2024a, The MathWorks, Inc., Natick, MA, USA). The area of the global minimum was determined using the sequential quadratic programming (SQP) method [23,24] and then the exact value of the global minimum was determined using the Nelder–Mead simplex algorithm [25,26]. The minimization function that was used during the estimation of parameters is given by:

$$SE = (V_{24}^e - V_{24}^s)^2 + (V_{72}^e - V_{72}^s)^2 \quad (3)$$

where the upper index e denotes experimentally measured percentage of viable cells and upper index s denotes the percentage of viable cells obtained in numerical simulations. Since the concentrations were experimentally measured at two moments in time (after 24 and 72 h), these two values were used in the simulation also, to compare the results and minimize the simulation error within the estimation procedure.

In order to analyze the influence of individual parameters on the percentage of viable cells, the sensitivity analysis was performed for one particular case—for the control HCT—116 cell line. First the values of all 6 parameters were estimated for this case and then each was varied individually, while the others were kept constant. The results of the sensitivity analysis are shown in Figure 1.

Additionally, all graphs in Figure 1 use the same scale to facilitate comparison and highlight the relative influence of the parameters. For the same parameter variation interval (25–175% of the estimated value), the range of variation in the percentage of viable cells after 24 h was 20.69% for parameter S_1 , 62.0% for S_2 , 212.4% for S_3 , 105.5% for S_4 , 2.16% for Q , and 2.41% for Q_1 . Similarly, after 72 h, the corresponding ranges of variation were 39.58% for S_1 , 290.83% for S_2 , 294.17% for S_3 , 3.73% for Q , and 4.57% for Q_1 . It is obvious that the parameters related to cell death have the greatest influence on the percentage of viable cells. In the experiments performed in this study, there was no limitation in the oxygen consumption of cells, i.e., the assumption of aerobic conditions was valid. Similarly, within numerical simulations, it was considered that an unlimited supply of oxygen was available. That is the reason why the sensitivity analysis shows that the parameters related to the oxygen consumption rate have almost no influence on the percentage of viable cells. It is acknowledged that the sensitivity analysis presented here is a local (one-at-a-time) analysis, in which each parameter is varied individually while the others are held constant at their estimated values. This approach clearly demonstrates the relative importance of each parameter and is sufficient for the purposes of the present study. A variance-based global sensitivity analysis (e.g.,

Sobol indices) would provide additional information on parameter interactions and is identified as a direction for future work.

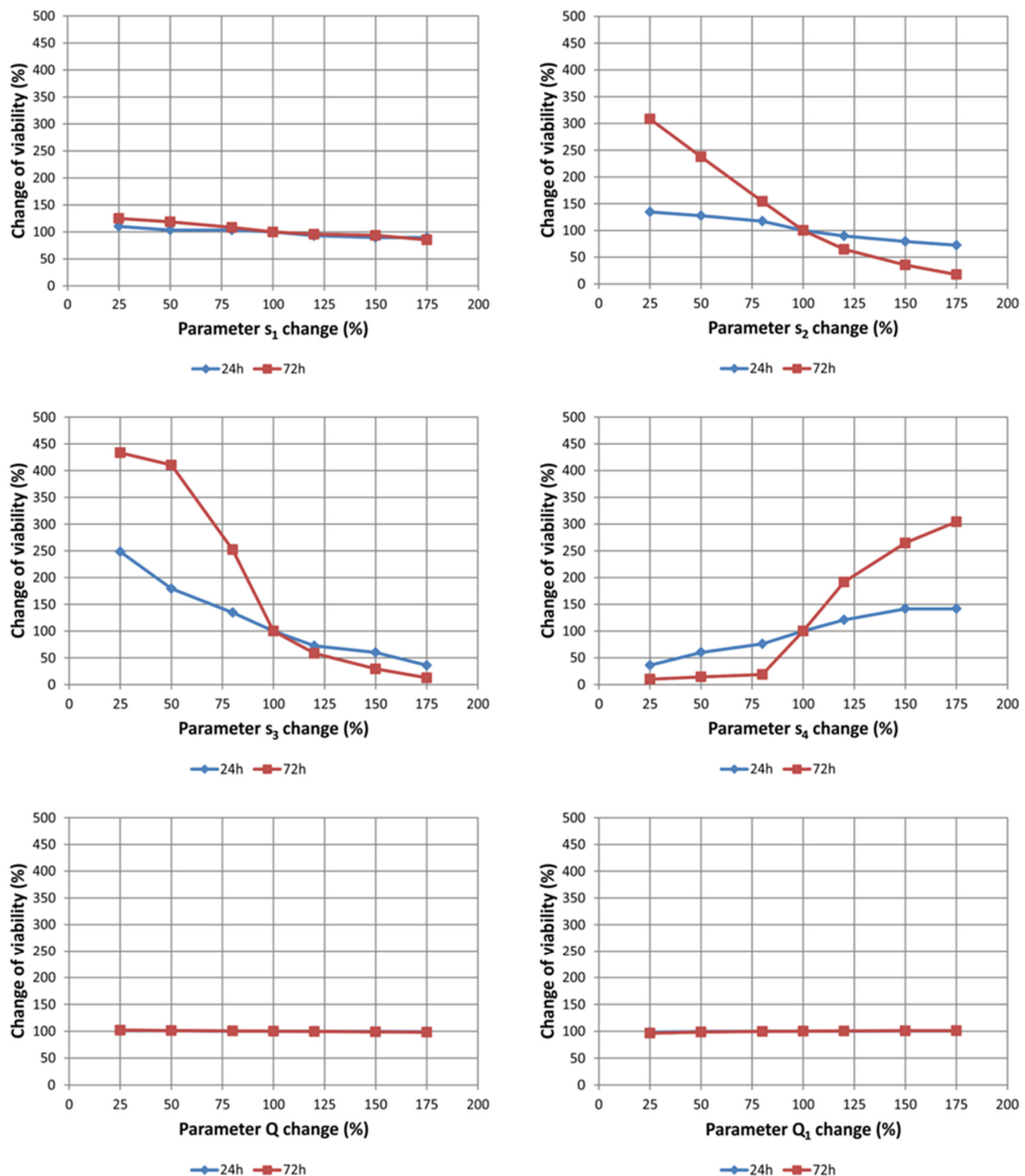


Figure 1. Sensitivity analysis showing the influence of change in parameter values on the percentage of viable cells for the control HCT-116 cell line.

Due to the small influence of the parameters related to the oxygen consumption rate on the result, it was concluded that we should exclude these parameters from the analysis of possible influence of different treatments. Hence, these two parameters were only estimated for control cell lines and considered fixed for all other considered cases. Estimated values of parameters Q and Q_1 for the HCT-116 cell line are equal to 6.05 and 5.14, respectively, while these parameters for the SW-480 cell line are equal to 6.0 and 6.75.

It should be noted that, while the nominal model contains 6 parameters, the effective number of free parameters estimated per treatment is reduced to 4 (S_1 , S_2 , S_3 , S_4), since Q and Q_1 are fixed at the values determined from the control cell lines. This reduction significantly improves the conditioning of the estimation problem relative to the two available experimental observations (viable cell percentages at 24 h and 72 h). Furthermore, the use of a two-stage optimization combining the global SQP method with the local Nelder–Mead simplex algorithm reduces the risk of convergence to local minima. To assess practical identifiability, profile likelihood analysis was performed for the four free parameters (S_1 , S_2 , S_3 , S_4) using the control HCT-116 dataset. In this approach, each parameter was fixed at a range of values while the remaining parameters were re-optimized, and the resulting change in the log-likelihood ($2\Delta LL$) was compared against the $\chi^2(1)$ threshold of 3.84 corresponding to the 95% confidence level. The results are shown in Supplementary Figure S1. Parameters S_2 , S_3 , and S_4 show bounded 95% confidence intervals within the physically meaningful parameter space, indicating practical identifiability. Parameter S_1 shows a flat profile ($2\Delta LL$ remains below the threshold across the explored range), indicating practical non-identifiability given only two experimental observations. This behavior is expected because the proliferation parameter S_1 affects the viability trajectory gradually over time, whereas only two observation points (24 h and 72 h) are available to constrain the estimation; additional time points would be required to resolve this parameter uniquely. This result is explicitly acknowledged as a constraint of this study's design. A summary of identifiability results is provided in Table S5. The inclusion of an intermediate 48 h measurement in future studies would substantially improve practical identifiability and uncertainty quantification.

2.8. Statistical Analysis

Obtained experimental data are expressed as means $\pm$ standard errors (SEs). The magnitude of correlation between variables was calculated using the SPSS statistical software package (version 17.0, SPSS Inc., Chicago, IL, USA). The effect of each treatment was expressed by IC50 values calculated from the dose curves by the computer program CalcuSyn (v2.1, Biosoft, Cambridge, UK).

3. Results

3.1. Effects of Treatments and Co-Treatments on Cell Viability

Cytotoxic effects of *P. linteus* extract on SW-480 and HCT-116 cells were previously published in [17]. When it comes to SW-480 cells, co-treatments generally showed weaker effects on cell viability after 24 h, when compared to the effects exerted on HCT-116 cells. However, after 72 h of treatment, slightly stronger effects were observed for *P. linteus* + Cispt and *P. linteus* + C1 co-treatments (Table 1). The most significant cytotoxicity was exerted by *P. linteus* + C2 co-treatment after 72 h with the lowest IC50 value (28.32 ± 0.91 $\mu\text{g/mL}$), especially when compared to the weaker effect of this co-treatment on the HCT-116 cell line (Table 1). Among all tested co-treatments, *P. linteus* + C2 caused the strongest cytotoxicity effect on HCT-116 cells with $\text{IC}_{50} = 63.38 \pm 2.09$ $\mu\text{g/mL}$ being observed after 72 h, while the co-treatments with ligands showed the weakest effects (Table 1).

Table 1. Cytotoxic effects (IC₅₀ values) and SI values of co-treatments on colorectal cancer HCT-116 and SW-480 cell lines.

Cell Line	<i>Phellinus linteus</i> + 10 µM	IC ₅₀ (µg/mL)	
		24 h	72 h
SW-480	Cispt	155.07 ± 0.03	81.69 ± 0.85
	C1	202.99 ± 0.63	83.13 ± 2.11
	L1	>250	>250
	C2	212.25 ± 2.12	28.32 ± 0.91
	L2	>250	176.67 ± 3.03
HCT-116	Cispt	178.38 ± 0.57	131.60 ± 0.09
	C1	160.03 ± 0.45	100.45 ± 0.60
	L1	>250	131.25 ± 1.03
	C2	97.57 ± 5.11	63.38 ± 2.09
	L2	>250	>250

Results are presented as the mean from 3 independent experiments ± SE.

3.2. Effects of Co-Treatments on Type of Cell Death

Considering that tested compounds and mushroom extract showed cytotoxic effects on SW-480 and HCT-116 cells after 24 and 72 h, we further determined the type of cell death by using the AO/EB microscopic method. Combined treatments created morphological features in these two cell lines, characteristic for apoptosis and necrosis. Typical cellular changes during apoptotic process are: rounding up of the cell, membrane blebbing and loss of its integrity, reduction in cellular volume, retraction of pseudopods, and formation of apoptotic bodies, altogether accompanied by the condensation of chromatin and nuclear fragmentation. Cells in apoptosis are colored green, while necrotic cells, which have a condensed morphology, are distinguished as uniformly colored red.

According to our results, all co-treatments had a weaker effect on the SW-480 cell line, whereas, generally, the highest percentage of cells was found to be in early apoptosis. The strongest apoptotic effects were caused by co-treatment that consisted of *P. linteus* + Cispt and *P. linteus* + C1, after both 24 h and 72 h of treatment. In these co-treatments, the highest percentage of cells in late apoptosis was observed after 72 h of treatment (60% for *P. linteus* + Cispt and 61% for *P. linteus* + C1). A stronger necrotic effect was observed after longer exposure to co-treatments and obviously only in the highest applied concentration of extract (22% for *P. linteus* + C2 and 18% for *P. linteus* + L1). Generally, the weakest pro-apoptotic effects were induced by the combination with ligands (Figures 2(1a,1b) and 3A).

On the contrary, among two tested colorectal cancer cell lines, HCT-116 cells were more sensitive to all applied treatments than the SW-480 cell line. Chemical compounds created pro-apoptotic and necrotic effects on HCT-116 cells, with apoptosis as the dominant type of cell death. However, necrosis was present at significant percentages, even up to 11% after 24 h and 23% after 72 h of treatment. It is obvious that the highest concentration of mushroom extract created the highest percentage of necrotic cells in all applied treatments. However, the combination of *P. linteus* + Cispt induced the lowest percentage of necrotic HCT-116 cells. Meanwhile, the best pro-apoptotic effects were caused by *P. linteus* + C2, whereas the highest percentage of cells was observed in late apoptosis after 72 h (60% for *P. linteus* + C2). Therefore, cytotoxicity that the applied treatments showed was evidently the result of their pro-apoptotic effects on HCT-116 cells (Figures 2(2a,2b) and 3B).

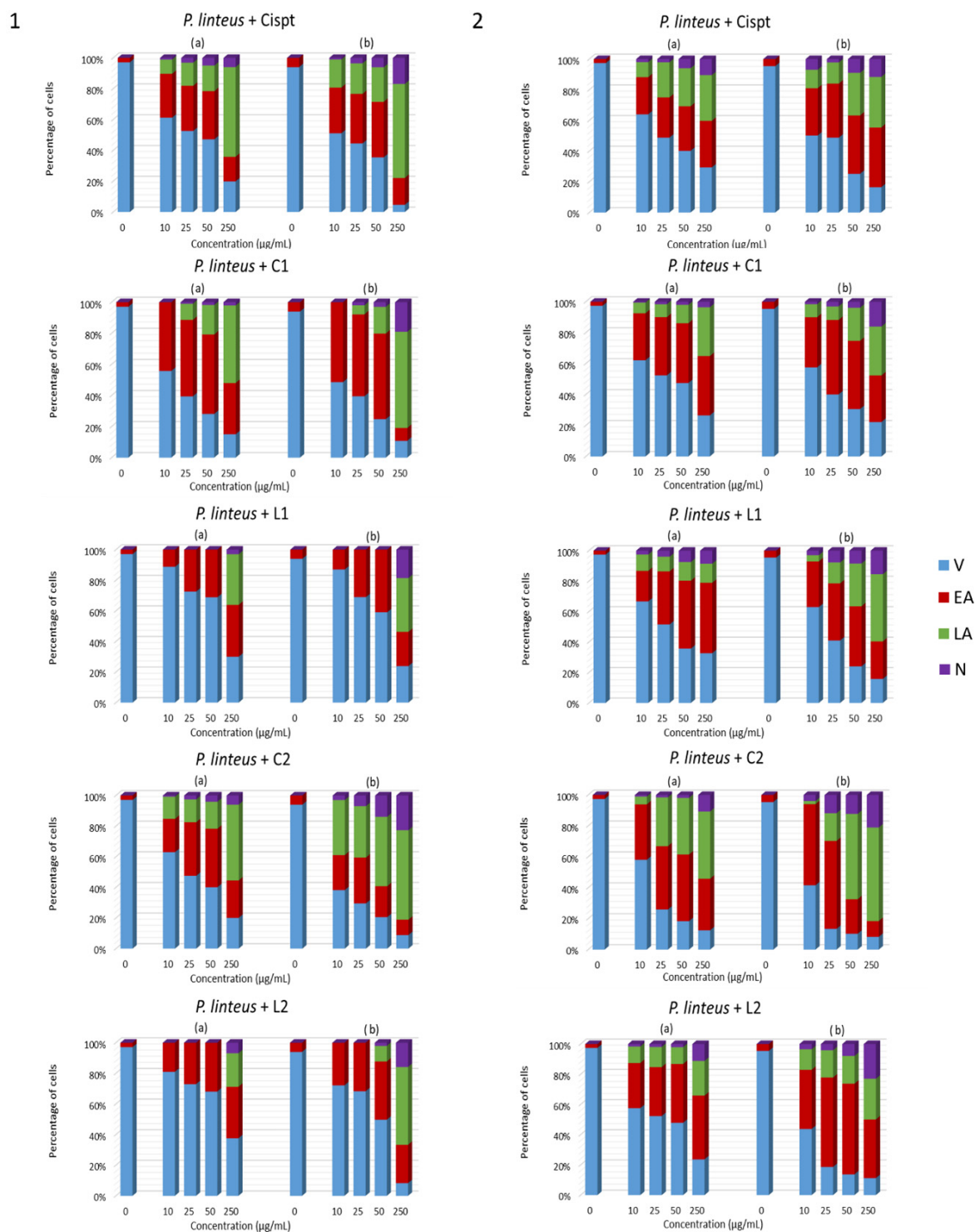


Figure 2. Effects of co-treatments on SW-480 (1) and HCT-116 (2) cell lines; (a) 24 h after treatment and (b) 72 h after treatment. V: viable cells; EA: early apoptotic cells; LA: late apoptotic cells; N: necrotic cells.

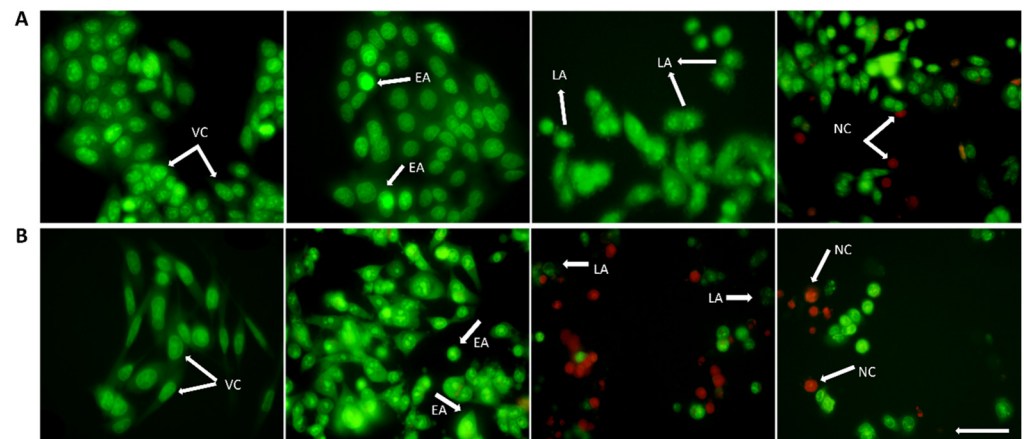


Figure 3. Representative micrographs of SW-480 (A) and HCT-116 (B) cells in different stages of cell death after co-treatments. VC: viable cells; EA: early apoptotic cells; LA: late apoptotic cells; NC: necrotic cells.

3.3. Results of the Numerical Simulations

Results of parameter estimation are shown in Figures 4 and 5. The maximum estimation error across all considered cases was 0.14 for SW-480 and 0.16 for HCT-116, indicating that the estimation errors are consistently small and that the model captures the experimental trends without systematic bias. The agreement between model-predicted and experimentally observed viable cell percentages across all 40 treatment cases and both cell lines is presented in Supplementary Figure S2A–C. Panel C shows an observed vs. predicted scatter plot for all cases; the overall RMSE is 1.27% and $R^2 > 0.99$, confirming excellent model fit without systematic bias. Panels A and B show the estimated proliferation parameter S_1 as a function of *P. linteus* concentration for each co-treatment in SW-480 and HCT-116 cells, respectively, demonstrating consistent concentration-dependent trends across all treatments. Since the main goal of this study is to analyze the influence of different treatments on the cell viability and on the parameters of the numerical model, the graphs in this section show relative values of the parameters in comparison with the parameters of the control cell lines, rather than the exact values of parameters. As can be concluded from these graphs, a general trend can be observed that the increased concentration of *P. linteus* causes a reduction in the parameter S_1 , related to the cell proliferation. This is logical since the higher concentration of *P. linteus* also causes a reduction in cell viability. Similarly, the parameters S_2 and S_3 that are related to cell death and whose increase causes a decrease in cell viability are also decreased for higher concentrations of *P. linteus*. The parameter S_4 is also related to cell death, but its increase causes an increase in cell viability, and this parameter decreases for higher concentrations of *P. linteus*.

A Pearson product moment correlation test was performed to determine whether there was a correlation between cell viability and varied concentration of *P. linteus* when applied as co-treatment with the considered complexes. The analysis was also performed for the values of estimated parameters. The results are listed in Table 2 for the both the SW-480 and HCT-116 cell line. The fields marked with an asterisk (*) indicate that there is a significant correlation (with $p < 0.05$). As it can be seen, it was found that for all considered combinations for the SW-480 cell line there was a significant correlation between increased dosage and smaller percentage of viable cells, while for the HCT-116 cell line, a significant correlation was found for some considered combinations. Also, for almost all considered cases, there is a strong correlation between increased concentration of *P. linteus* and parameter S_3 , which is related to cell death. As was concluded in the sensitivity analysis (Figure 1), this parameter has the highest influence on cell viability and therefore it

is logical that this parameter has the strongest correlation with the increased concentration of *P. linteus*. It should be noted that the Pearson correlation analysis was applied to four concentration levels per treatment, which limits the statistical power of this test. The results should therefore be interpreted as indicative of trends rather than a definitive statistical confirmation, and are presented in conjunction with the mechanistic evidence provided by the numerical model parameters.

The change in cell viability over time for one considered treatment with the C1 complex for the SW-480 cell line is shown in Figure 6. The dots on the graph show the experimentally measured values, while solid lines represent the results obtained in numerical simulations. Results of the simulation agree well with experimental data. Also, as can be observed, the higher concentration of *P. linteus* in co-treatment with the C1 complex causes a higher decrease in cell viability within the first 24 h and a slower increase in cell viability over time.

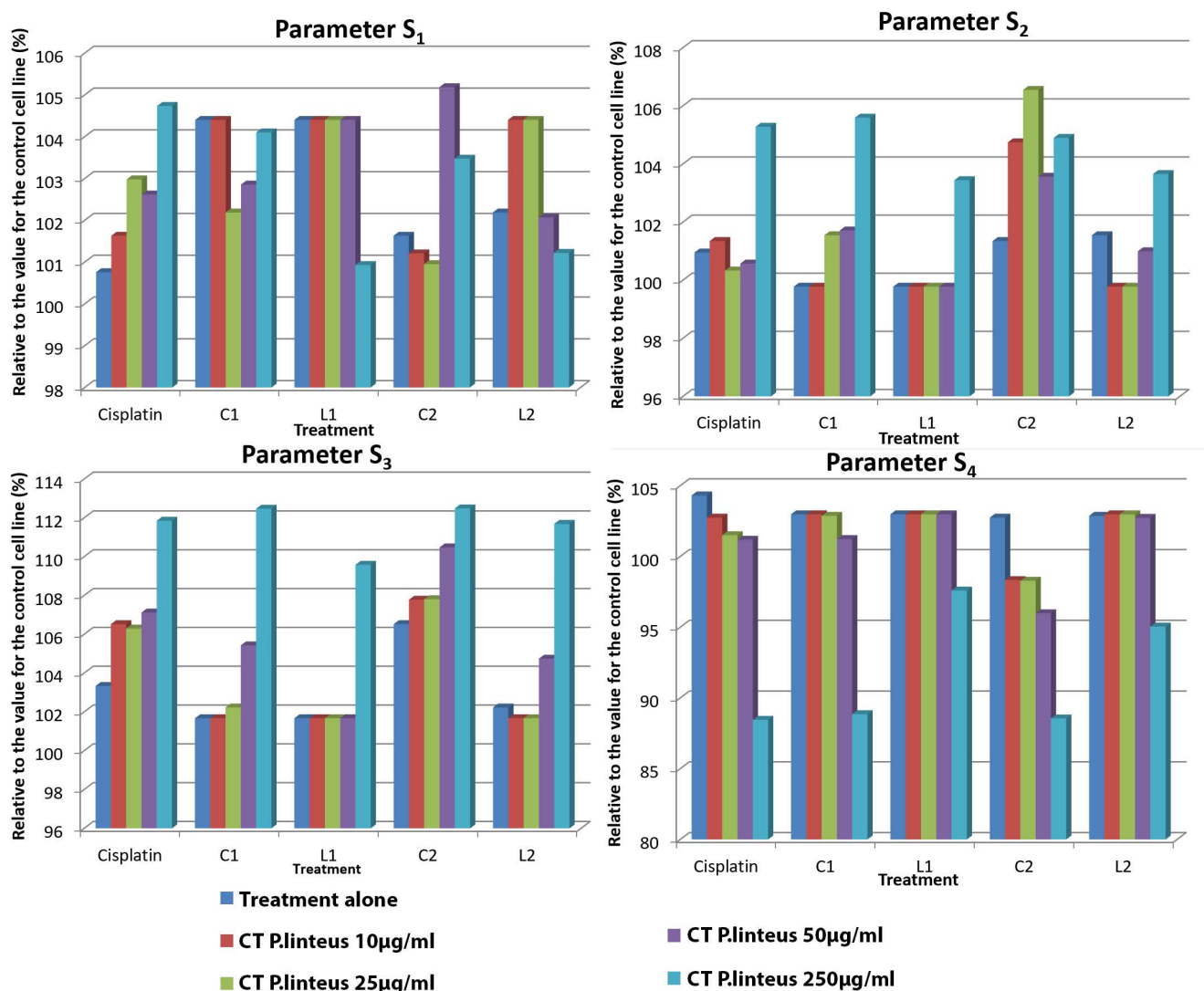


Figure 4. Results of the estimation procedure for the SW-480 cell line for different considered treatments.

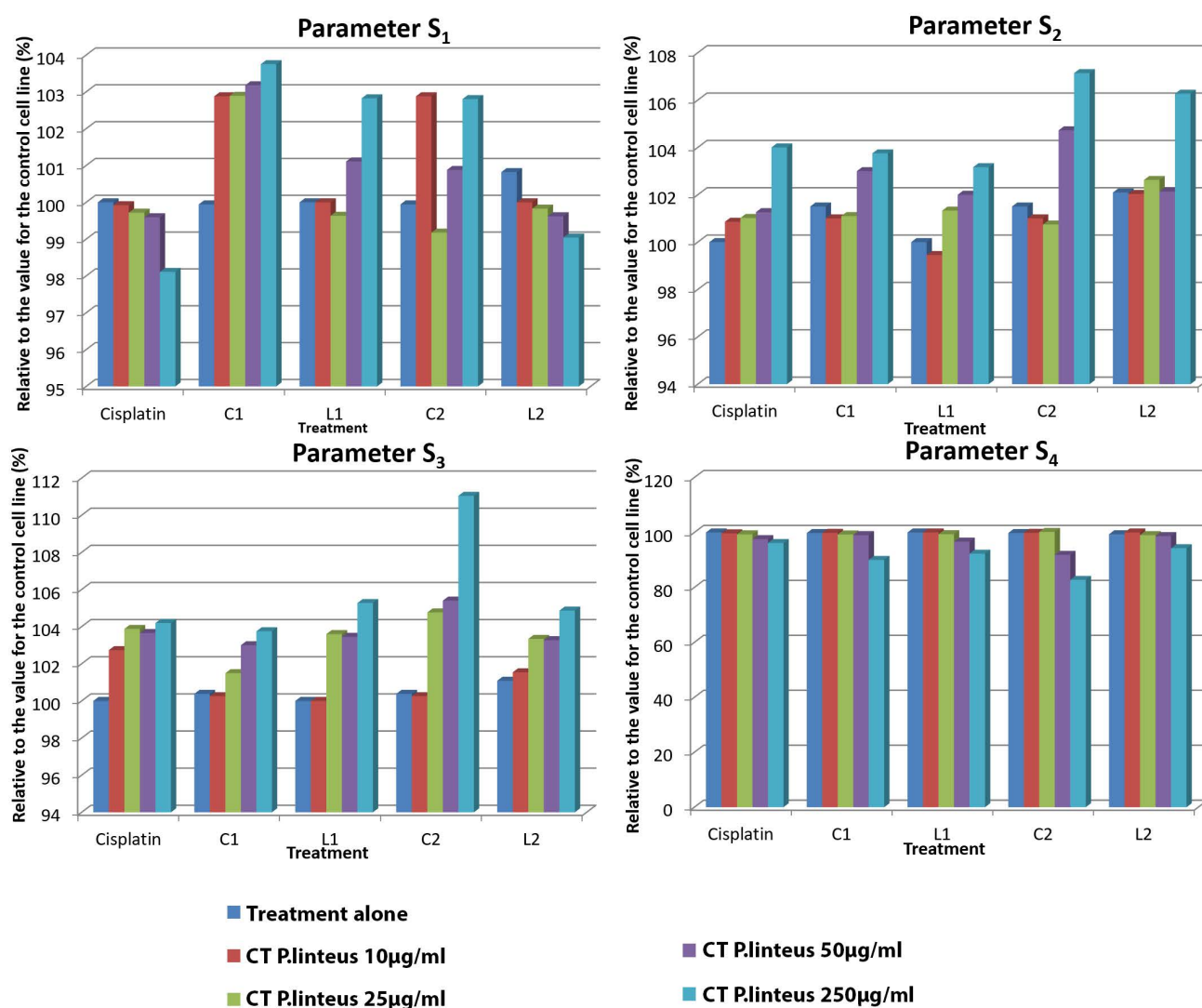


Figure 5. Results of the estimation procedure for the HCT-116 cell line for different considered treatments.

Table 2. Significant correlation ($p < 0.05$) between increased concentration of *P. linteus* in co-treatment and cell viability and change in parameters for SW-480 and HCT-116 cell lines.

Treatment	Viability After 24 h	Viability After 72 h	S ₁	S ₂	S ₃	S ₄
SW-480						
Cispt	*	*	-	-	*	-
C1	*	*	*	-	*	*
L1	*	*	*	-	*	*
C2	*	*	-	-	-	-
L2	*	*	-	*	*	-
HCT-116						
Cispt	*	-	-	-	*	-
C1	*	*	*	-	*	*
L1	-	*	*	-	*	*
C2	-	-	-	-	-	-
L2	-	*	-	*	*	-

* Significant correlation ($p < 0.05$); - no significant correlation ($p \geq 0.05$).

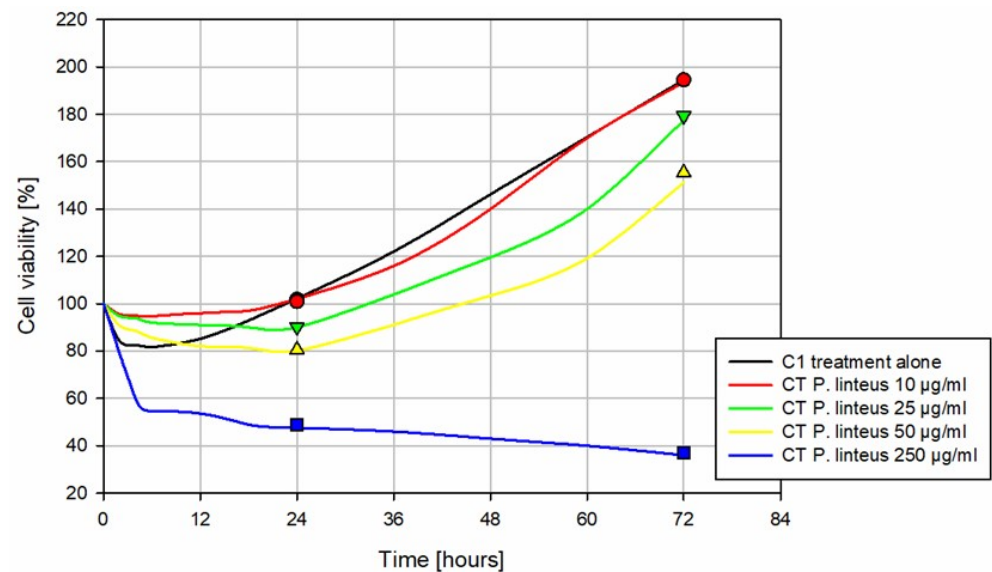


Figure 6. Change in cell viability over time for SW-480 cell line for the co-treatment consisting of the C1 complex and *P. linteus* in diverse concentrations. Solid lines represent results of numerical simulations, while dots represent experimentally measured values.

3.4. Frizzled-7 and Nuclear β -Catenin Protein Expression

3.4.1. Frizzled-7 Expression

All tested co-treatments significantly reduced the expression of the Frizzled-7 receptor in the tested SW-480 cell line, when the effects are compared to control (untreated) cells (Figure 7), with a membrane and the cytoplasmic location of this protein. Treatments cause a reduction in this marker's expression on protein level, and resulted in the following trend: *P. linteus* + C1 and C2 > *P. linteus* + L1 and L2 > *P. linteus* + Cispt. The Frizzled-7 receptor was found localized mainly in the cytoplasm in areas close to the nucleus in all applied treatments (Figure 7).

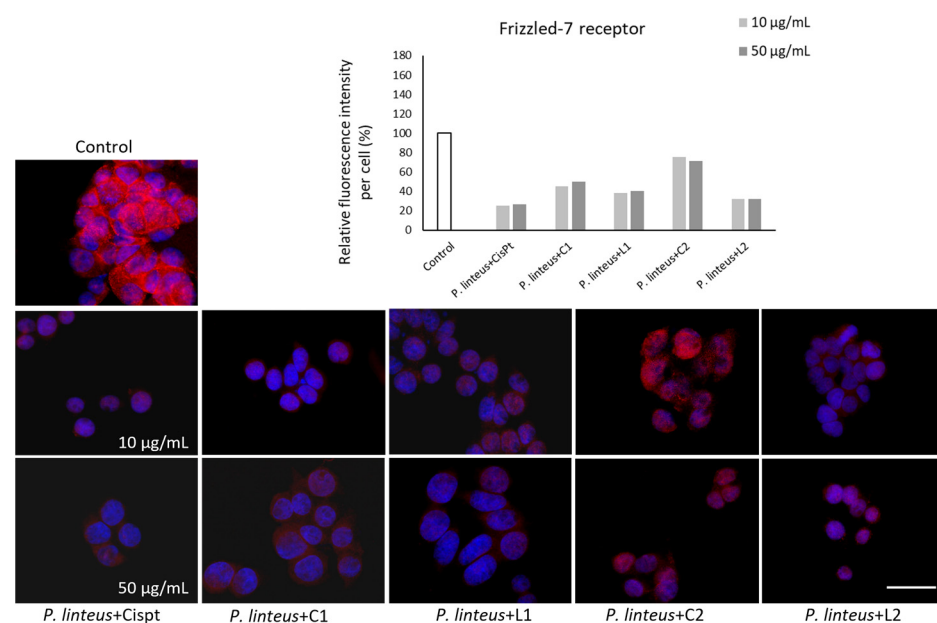


Figure 7. Representative micrographs showing the protein expression of the targeted markers in SW-480 cells 24 h after treatments. Cell nuclei were stained blue (DAPI); Frizzled-7 receptors were stained red (secondary antibody conjugated with NorthernLights™ NL557). Scale bar: 40 µm. Fluorescence intensity of analyzed proteins presented as changes relative to control cells in %.

Tested combined treatments induced the elevation of Frizzled-7 receptor expression in HCT-116 cells (Figure 8). The localization of this protein was mainly in cytoplasmic and membrane areas of cytoplasm. It can be observed that when a higher *P. linteus* concentration was applied, the elevation of this protein was greater. The highest Frizzled-7 receptor expression was detected in the *P. linteus* + L1 treatment, while the weakest effect was caused by *P. linteus* + Cispt and *P. linteus* + C2, which can be observed in representative micrographs also.

By comparing the results obtained for all tested treatments on both cell lines, it can be observed that HCT-116 cells have a significantly higher basal protein expression of the Frizzled-7 receptor. Also, the co-treatments had stronger effects on this marker's expression in HCT-116 compared to SW-480 cells.

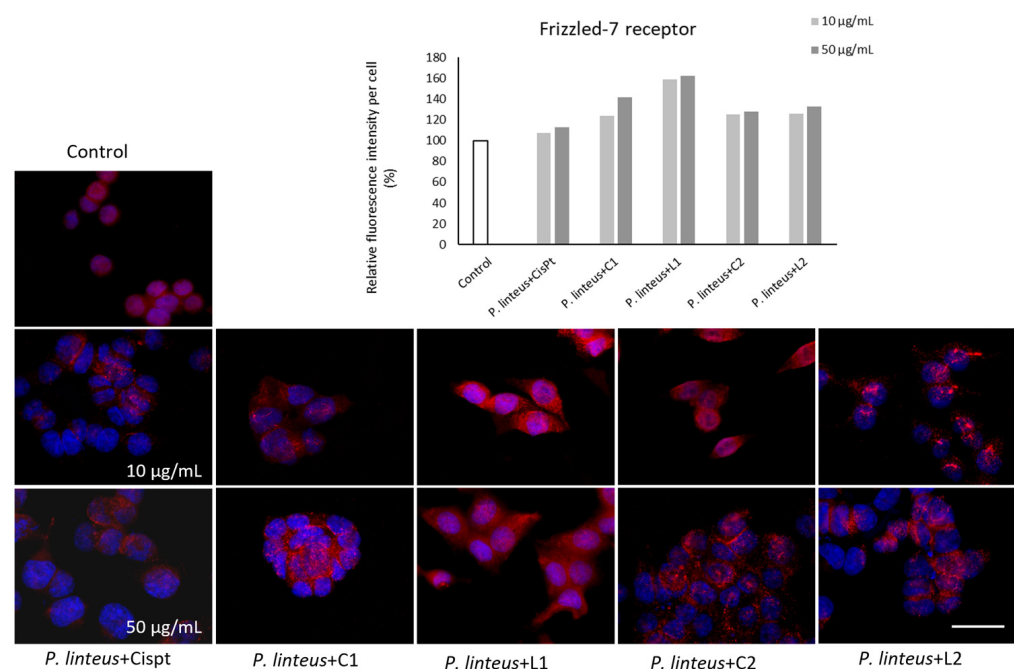


Figure 8. Representative micrographs showing the protein expression of the targeted markers in HCT-116 cells 24 h after treatments. Cell nuclei were stained blue (DAPI); Frizzled-7 receptors were stained red (secondary antibody conjugated with NorthernLights™ NL557). Scale bar: 40 µm. Fluorescence intensity of analyzed proteins presented as changes relative to control cells in %.

3.4.2. Nuclear β -Catenin Expression

High concentrations of co-treatments increased nuclear β -catenin protein expression (except *P. linteus* + L2) in SW-480 cells, compared to the control, with the presence of nuclear β -catenin mainly detected in the cell cytoplasm (Figure 9). *P. linteus* + L1, *P. linteus* + C2 and *P. linteus* + L2 reduced nuclear β -catenin protein expression, while only lower concentrations of *P. linteus* + L1 co-treatment caused nuclear β -catenin localization mainly in the nuclei area (Figure 9). Co-treatment with Cispt caused the highest expression of this marker, while *P. linteus* + L1 was the most effective for nuclear β -catenin reduction (Figure 9).

Nuclear β -catenin was detected in the cytoplasm and nucleus of untreated (control) HCT-116 cells, while co-treatments caused a decrease in the expression of this protein after 24 h. Under the influence of *P. linteus* + Cispt and *P. linteus* + L1 co-treatments, this examined marker was observed in the cytoplasm and nucleus of HCT-116 cells, while in other co-treatments this protein was located only in the cell nuclei area (Figure 10). The strongest suppressive effect was shown by *P. linteus* + L1 and *P. linteus* + L2, while the weakest effects were shown by *P. linteus* + Cispt and *P. linteus* + C2 (Figure 10).

Co-treatments showed a stronger suppressive effect in the HCT-116 cell line than in SW-480 cells. The most intense reduction in nuclear β -catenin expression is shown by *P. linteus* + L1 in both cell lines (lower concentrations in SW-480 and higher in HCT-116 cell lines).

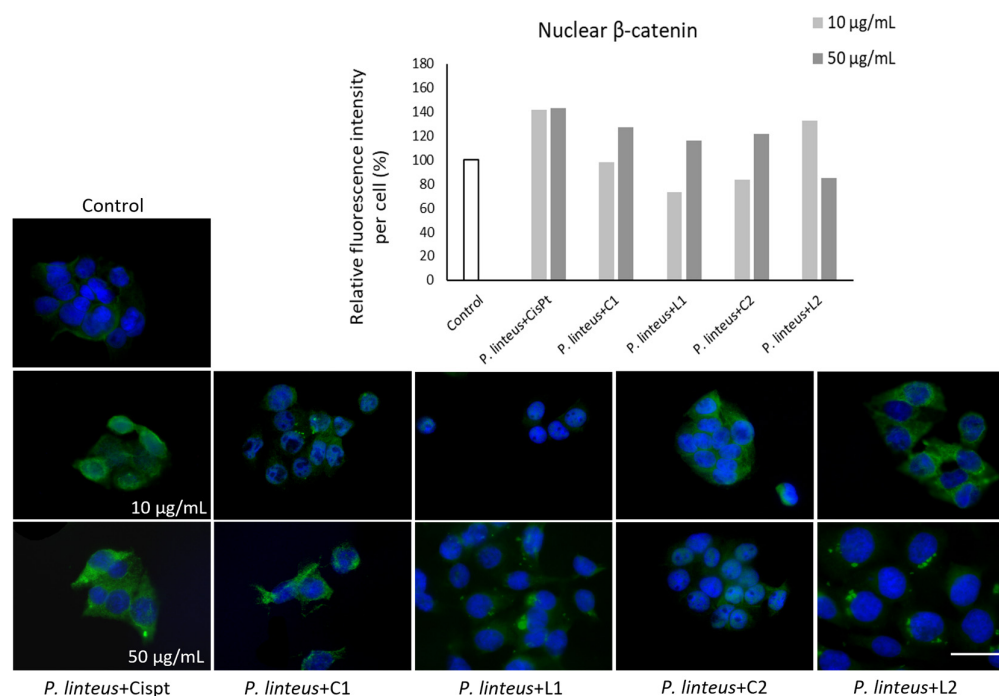


Figure 9. Representative micrographs showing the protein expression of the targeted markers in SW-480 cells 24 h after treatments. Cell nuclei were stained blue (DAPI); nuclear β -catenin proteins were stained green (secondary antibody conjugated with Alexa488). Scale bar: 40 μm . Fluorescence intensity of analyzed proteins presented as changes relative to control cells in %.

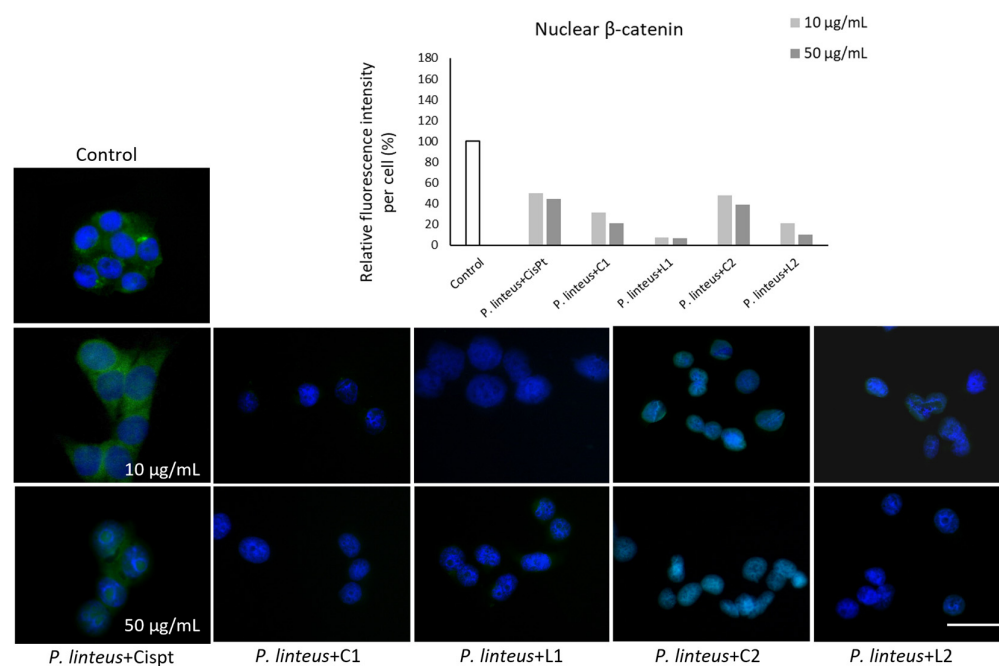


Figure 10. Representative micrographs showing the protein expression of the targeted markers in HCT-116 cells 24 h after treatments. Cell nuclei were stained blue (DAPI); nuclear β -catenin proteins were stained green (secondary antibody conjugated with Alexa488). Scale bar: 40 μm . Fluorescence intensity of analyzed proteins presented as changes relative to control cells in %.

4. Discussion

Phellinus linteus (Berkeley & Curtis) Teng is a very potent mushroom species when it comes to the prevention and remediation of various diseases, among which are gastrointestinal disorders including cancer [27,28]. *P. linteus* methanol extract already showed synergistic, antiproliferative effects when used in combination with some standard anti-cancer drugs (such as cetuximab or gemcitabine), applied for the treatment of a variety of human malignancies, especially against colon cancer cells in vitro and in vivo [28,29].

Platinum derivatives are important chemotherapeutic agents used to treat many epithelial malignancies, including CRC [30]. Moreover, platinum(IV) compounds have proven to be more efficient than cisplatin or oxaliplatin because of their lower toxicity, and they better affect malignant cells that are resistant to cisplatin [31]. This is why the interest in Pt(IV) complexes is growing, especially when it is known that cisplatin can create synergies with other drugs or natural compounds in the treatment of CRC [32,33]. In this context, there is enormous interest in improved combinations of more effective Pt(IV) complexes with some natural sources such as mushroom species, or their active compounds, which have already shown anticancer potential [34]. This was accomplished by the synergism of cytostatic effects that both compounds induce, with minimal toxicity on healthy cells. The mechanistic basis of the observed synergy between *P. linteus* and Pt(IV) complexes likely involves the convergent targeting of multiple cellular pathways. Pt(IV) complexes exert cytotoxicity primarily through DNA crosslink formation and the induction of mitochondria-mediated apoptosis [31], while bioactive polysaccharides and triterpenoids present in *P. linteus* extracts have been reported to modulate immune signaling, downregulate anti-apoptotic Bcl-2 family members, and suppress Wnt/ β -catenin pathway activity [27,28]. The co-treatment approach thus simultaneously targets DNA integrity and pro-survival signaling cascades, which is consistent with the reduction in Frizzled-7 receptor expression and nuclear β -catenin levels observed in the present study. This multi-target mechanism reduces the likelihood of single-pathway resistance emerging under treatment pressure.

In addition to chemotherapy, natural products that have no cytotoxicity showed an ability to amplify the cytotoxic effects of chemotherapeutics on cancer cells, as well as to reduce the side effects of chemotherapy, which can improve standard anticancer therapy [34]. Co-treatments are able to sensitize cancer cells and reduce their resistance to chemotherapeutics [35], because this combined approach in CRC treatment affects more than one target molecule in the cell and increases the potential of chemotherapy, i.e., chemosensitization [36,37].

Our data confirm that applied co-treatments show significant and potent synergistic cytotoxicity, considering that by the addition of low concentrations of tested substances (10 μ M), Cispt and C2 were able to significantly reduce the IC₅₀ values for *P. linteus* when applied as single treatment, as already investigated earlier [17]. These results demonstrate that compounds present in methanol extract of *P. linteus* have synergistic cytotoxic effects with tested Pt-based drugs.

In summary, our results indicate that combinations of methanol extract from the fruiting body of *P. linteus* with Pt(IV) complexes exert a better effect than the expected inhibition evaluated when assessing the effects of treatment alone on SW/480 and HCT-116 cells. The synergistic effect of *P. linteus* was more prominent with Cispt or C2 in a subcytotoxic concentration, which was confirmed by AO/EB staining. Results of fluorescence showed that all co-treatments triggered apoptosis at a significantly high level with characteristic changes such as membrane blabbing and the condensation of nuclear material [38,39]. Co-treatment induced the highest apoptosis ratio with a significant percentage of cells observed in late apoptosis. The obtained results indicate that increasing the concentration of *P. linteus* extract leads to an increase in the number of necrotic cells.

It is well known that available oxygen and nutrients are key factors for cell growth [40]. Considering that, in this experiment, the effects of the treatment were observed after 24 and 72 h, we can conclude that the oxygen flow is constant; therefore, the effect of this environmental factor on cell death can be taken as negligible. This is also confirmed by the applied mathematical model, and we can attribute cytotoxic effects directly to co-treatment effects.

The applied mathematical model provides information regarding the effect of treatments on four given different parameters, one of which is related to the proliferative capacity of the treated cells, namely S_1 , and three for cell death. However, the S_1 parameter indicates the strength of the antiproliferative effect of the treatment on the examined cells.

As our research shows, the S_1 parameter indicates the dose that suppresses the proliferation of cancer cells the most in the strongest applied treatment. If the S_1 parameter is lower for a given dose, the stronger the antiproliferative effect that dose exerts. On the other hand, the S_4 parameter is related to the effect of the treatment on the maintenance of cell viability, which is irreversible during proliferation, and the higher it is, the stronger the effect of the treatment is on the maintenance of the viability of the examined cell. According to this, the cells develop resistance, and they become ready for proliferation. Based on this parameter of the model, we can predict the behavior of a certain cell line in terms of whether a certain treatment will favorably affect viability, i.e., stimulate the proliferation of cell lines. Therefore, we can conclude that there is a relationship between parameters S_1 and S_4 , with the S_1 parameter indicating greater effectiveness of the treatment, while the S_4 parameter is related to the biological characteristics of the cell line.

The other two parameters, S_2 and S_3 , are related to cell death. Parameter S_2 refers to the most efficient concentration that induces a significant increase in apoptosis, especially early apoptosis during which cells are still attached to the substrate. This parameter also indicates the most effective concentration, and that if we increase (double) the concentration, the cytotoxicity results will stay pretty much the same.

The increase in the S_3 parameter is proportional to the effectiveness of the dose, i.e., effects on the percentage of permanently dead cells (late apoptosis and necrosis). The higher the S_3 parameter, the higher the percentage of permanently dead cells.

The density of the proliferating cells is one of the prominent physical factors that influence the effects of the treatment. In this regard, differences in the cellular response to the investigated co/treatment can be analyzed. The SW-480 cell line is well-differentiated, as indicated by the high level of Frizzled-7 receptor expression and the lower level of nuclear β -catenin, experimentally measured in the control [41], and the cells are tightly connected in clusters. Strong intercellular connections prevent the treatment from reaching each cell easily, which explains the higher number of surviving cells after 24 h and the weaker acute cytotoxic effect of treatments compared to HCT-116 cells.

Co-treatments showed a ten-fold stronger effect on SW-480 cells after 72 h than at 24 h, and as it could be observed that the mortality of these cells increased with time. These data correlate with the experimental results of apoptosis at 24 and 72 h.

P. linteus + C2 and *P. linteus* + Cispt co-treatments showed the highest cytotoxicity with the lowest IC50 values on SW-480 cells, which correlates with the lowest values of the S_1 parameter in the applied mathematical model (Figure 4). Experimental results for apoptosis confirm the strongest cytotoxic effect of these co-treatments. Comparing the results of the mathematical model and experimental data, it can be concluded that a lower S_1 parameter in small treatment concentrations indicates a more effective cytotoxic treatment, and thus a lower experimentally obtained IC50 value. The lowest value of the S_1 parameter can be observed for the 25 $\mu\text{g/mL}$ concentration *P. linteus* + C2 co-treatment, which indicates the strongest antiproliferative effect of this treatment on SW-480 cells.

In addition, the 25 µg/mL concentration *P. linteus* + C2 co-treatment gives the highest values of the S_2 parameter according to the model, so it is the most efficient concentration on the SW-480 cell line. The concentration that gives the highest S_2 parameter indicates the influence of concentration on IC50 values; so, the concentration of 25 µg/mL is closest to the lowest IC50 value of *P. linteus* co-treatment with C2, which correlates with the experimental data for 72 h (Tab 1. IC50 = 28.32 µg/mL for 72 h). We can define this concentration as the most efficient. According to the applied model and literature data, the freed space obtained by detaching a large number of permanently dead cells is a parameter that affects the stimulation of proliferation [6]. On the other hand, by increasing the number of dead cells, the resistance of extracellular fluid increases; therefore, its movement through intercellular space decreases, and thus the supply of nutrients needed for the proliferation of the cells decreases. Additionally, cells that survive receive signals from dying and dead cells to start proliferating [39].

Based on everything stated, we can conclude that the 25 µg/mL concentration *P. linteus* + C2 is the most efficient because it maintains the optimum balance between the number of early apoptotic attached cells and the number of dead cells that are in late apoptosis and necrosis, which was experimentally confirmed regarding SW-480 cells. Based on the model parameters for *P. linteus* + C2 co-treatment, we can predict further cellular behavior, i.e., that low concentrations, and especially the 25 µg/mL concentration *P. linteus* + C2, will not provoke further stimulation of proliferation, which is considered a favorable outcome of the treatment. This is also indicated by experimental data, where the reduction in Frizzled-7 receptor expression in SW-480 cells can be observed. Given that the Frizzled-7 marker indicates the activity of the Wnt pathway involved in aggressiveness and stemness, the examined treatment induces additional differentiation of the tested cell lines, and thus the suppression of proliferation, aggressiveness, and stemness [42]. Nuclear β-catenin is reduced in SW-480 cells, which indicates that the expression of genes for which β-catenin is responsible is downregulated; therefore, it can be predicted that these cells respond well to the low treatment concentrations. A significant contribution of the applied mathematical model to biological research is that the analysis of the obtained parameters of the model for a given treatment indicates the most efficient and the most effective dose.

The most efficient concentration is the one that shows the greatest antiproliferative effect and induces early apoptosis in cells the most with a smaller effect on dead cells and is characterized by low values of the S_1 parameter and high values of the S_2 parameter. The most effective dose is also defined by the fact that further increasing the concentration does not induce better effects. The S_3 parameter of the mathematical model indicates the effect of applied treatment concentrations on permanently dead cells. According to the experimental data, the highest level of this parameter is reciprocal to the measured percentage of dead cells (late apoptotic and necrotic), i.e., indicates the most effective concentration of treatment. According to the model, high concentrations of *P. linteus* + C2 co-treatment induced the greatest increase in the S_3 parameter, which correlates with the experimentally obtained results for the percentage of dead cells regardless of the type of cell death. According to the experimental data, *P. linteus* + C2 co-treatment induced the highest cytotoxicity in all tested concentrations, especially 250 µg/mL, with 8% of live cells, 10% of cells in early apoptosis, and 82% of late apoptotic and necrotic cells, which is expected. A high percentage of permanently dead cells (late apoptotic and necrotic) caused by the treatment with high concentrations (50 and 250 µg/mL) of *P. linteus* + C2 co-treatment, and the weak antiproliferative effect of the 50 µg/mL concentration (high S_1 parameter), may indicate rapid cell proliferation, which is also indicated by the increased level of nuclear β-catenin. Namely, the freed space created by the detachment of almost 80% of the cells enables proliferation, but also the condensation of the liquid extracellular

phase by a large number of dead and detached cells, thus making it difficult for nutrients to pass through and reach live cells. Given that the S_4 parameter is reduced in these two concentrations, and that SW-480 cells are slow-proliferating, it can be expected that proliferation will not occur, as also indicated by the experimentally confirmed reduced expression of the Frizzled-7 receptor in the 50 $\mu\text{g}/\text{mL}$ concentration. This concentration additionally increases cell differentiation through the reduction in this marker, and given that nuclear β -catenin expression is increased, we can expect these cells to develop survival mechanisms in the form of EMT and mechanisms for anti-apoptosis and resistance, which is basically an unfavorable scenario induced by high-concentration treatment.

According to the results of the mathematical model, we can conclude that lower concentrations of non-effective tested *P. linteus* + C1, *P. linteus* + L1, and *P. linteus* + L2 treatments exerted weak cytotoxic effects, and the S_4 parameter was high, thus having a strong influence on maintaining the viability of the tested cells, which correlated with a high S_1 parameter value (Figures 4 and 5).

The highest concentration of 250 $\mu\text{g}/\text{mL}$ of the treatments showed the most significant effect on the reduction in cell viability. In the *P. linteus* + C2 treatment, all tested concentrations showed a significant effect on the reduction in the S_4 parameter and reducing the values of S_1 in lower concentrations, indicating that low concentrations of this co-treatment have the most favorable cytotoxic effect on SW-480 cells.

HCT-116 cells demonstrated poor differentiation, as an aggressive type of CRC with a high expression of the Frizzled-7 receptor and nuclear β -catenin [12]. These cells grow in low density and possess weak intercellular connections, so treatments can more easily reach individual cells and diffuse between them. Therefore, the best acute cytotoxic effect of *P. linteus* + C2 co-treatment ($\text{IC}_{50} = 63.38 \mu\text{g}/\text{mL}$, for 24 h) was as expected. This co-treatment showed a stronger effect on HCT-116 cells compared to the SW-480 cell line for a shorter incubation time ($\text{IC}_{50} = 97.57 \mu\text{g}/\text{mL}$). By prolonging the incubation time, the cytotoxicity decreases by 30%, which is a significantly weaker effect compared to SW-480 cells.

The model showed that a low concentration of *P. linteus* + C2 co-treatment (10 $\mu\text{g}/\text{mL}$) induced high S_1 and S_4 values, indicating that this concentration will further stimulate the population of rapidly proliferating HCT-116 cells to further proliferate. This is confirmed by the stable expression of the nuclear form of β -catenin and an increased level of Frizzled-7 receptors, as well as 58%, i.e., 42% of living cells after 24 h, i.e., 72 h, for the low concentration of *P. linteus* + C2 co-treatment.

The 25 $\mu\text{g}/\text{mL}$ concentration of *P. linteus* + C2 co-treatment with the lowest S_1 parameter and reduction in viability compared to the control by more than 70% in 24 h and more than 85% in 72 h can be defined as the most efficient antiproliferative dose for this co-treatment. However, a high S_4 value for this concentration indicates sustained viability; therefore, it can be assumed that it will either stimulate the proliferation of rapidly proliferating HCT-116 cells or their stemness and thus aggressiveness. This indicates the complexity of stage IV CRC cells and the sustainability of their viability, even under extremely unfavorable conditions. This concentration reduces the number of viable cells the most, and a further increase in concentration does not proportionally reduce the percentage of viable cells.

The high values of S_2 parameter for the 50 $\mu\text{g}/\text{mL}$ concentration *P. linteus* + C2 correlates with the experimentally obtained IC_{50} value ($\text{IC}_{50} = 63.38 \mu\text{g}/\text{mL}$ at 72 h) for HCT-116 cells. Treatment of these cells with this concentration increased the expression of the Frizzled-7 receptor, while nuclear β -catenin expression was reduced. These results indicate that 50 $\mu\text{g}/\text{mL}$ of *P. linteus* + C2 co-treatment induce additional de-differentiation of HCT-116 cells [16]. This result indicates a poor effect of this treatment on HCT-116 cells. For this cell line, the strongest effect of the highest concentration dose (250 $\mu\text{g}/\text{mL}$) for

P. linteus + C2 co-treatment is observed, regarding the increase in S_2 and S_3 parameters' values; hence, this is the most effective concentration for the induction of cell death. The effectiveness was experimentally confirmed with 90% of cells in apoptosis and over 80% of late apoptotic and necrotic cells after 72 h.

When it comes to other co-treatments applied (*P. linteus* + C1, *P. linteus* + L1 and *P. linteus* + L2), high S_2 and S_3 parameters, only for dose 250 $\mu\text{g/mL}$, indicate the obtained IC50 values for these co-treatments, also experimentally confirmed by the strongest cytotoxic effect.

Also, a high S_1 parameter value indicates a weak antiproliferative effect, and a reduced S_4 parameter value for the highest concentration (250 $\mu\text{g/mL}$) of *P. linteus* + C2 co-treatment with 8% viable and poorly differentiated HCT-116 cells may indicate that these cells will develop even stronger aggressiveness and dormancy as a survival option. All this supports the characteristics of HCT-116 cells, and speaks of the aggressiveness of stage IV CRC cells, as well as the generally unfavorable impact of various treatments and high concentrations on this cell line.

Several limitations of the applied mathematical model should be acknowledged. First, the biphasic avascular model was originally developed for three-dimensional tumor spheroids and is applied here to a 2D adherent cell culture system; the biological fidelity of the model is therefore constrained by this geometrical simplification. Second, the model does not explicitly account for the pharmacokinetics of the applied compounds or their intracellular uptake kinetics, so the estimated parameters should be interpreted as effective, phenomenological quantities rather than direct biochemical rate constants. Third, the number of experimental time points (24 h and 72 h) used for parameter estimation is small relative to the number of free parameters; future studies should include intermediate time points (e.g., 48 h) to further constrain the estimation and improve parameter confidence. Despite these limitations, the model successfully captures the experimentally observed trends and provides a useful framework for ranking treatment efficacy and predicting re-treatment potential.

5. Conclusions

The presented study shows that the results of various experimentally obtained biological data can be additionally analyzed and explained by using mathematical models in biological research. Presented results of this study suggest that *P. linteus* could play a role in the treatment of colorectal cancer when combined with other commonly used anticancer agents, especially Pt-based agents. Exceptional cellular selectivity, reflected in different responses to co-treatments, is a significant result of this study, in terms of confirmed anticancer activity.

Based on the presented results, we can conclude that the concentration of 25 $\mu\text{g/mL}$ *P. linteus* + C2 co-treatment can be pointed out as the most efficient concentration on SW-480 cells with the lowest S_1 parameter [43], the highest S_2 parameter, significant effectiveness S_3 , and significant impact on the reduction in viability maintenance S_4 . This most efficient concentration does not provoke the cell to a quick response and adaptation, so it may represent a suitable candidate for re-treatment, which will be the subject of further research. Additionally, low-dose *P. linteus* + C2 co-treatment significantly reduced Frizzled-7 expression as well as the nuclear form of β -catenin, which confirmed the positive effect of this co-treatment on SW-480 cells.

Based on the experimental results and the model parameters, we can conclude that the tested treatments are not recommended for application on the HCT-116 cell line due to the sustained viability of rapidly proliferating cells and additional de-differentiation through increased protein expression of the Frizzled-7 receptor.

Future studies should include an intermediate experimental time point (48 h), which would substantially improve practical identifiability of the proliferation parameter S_1 , and a global (variance-based) sensitivity analysis to further quantify parameter uncertainty. These methodological advances will strengthen the predictive power of the biphasic numerical model as a tool for preclinical treatment screening [44].

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/mca31030109/s1>, Table S1: Effects of co-treatments on SW-480 cells 24 h after treatment. VCs: viable cells; EAs: early apoptotic cells; Las: late apoptotic cells; NCs: necrotic cells; Table S2: Effects of co-treatments on SW-480 cells 72 h after treatment. VCs: viable cells; EAs: early apoptotic cells; Las: late apoptotic cells; NCs: necrotic cells; Table S3: Effects of co-treatments on HCT-116 cells 24 h after treatment. VCs: viable cells; EAs: early apoptotic cells; Las: late apoptotic cells; NCs: necrotic cells; Table S4: Effects of co-treatments on HCT-116 cells 72 h after treatment. VCs: viable cells; EAs: early apoptotic cells; Las: late apoptotic cells; NCs: necrotic cells; Table S5: Summary of practical identifiability results for model parameters S_1 – S_4 (profile likelihood analysis, control HCT-116); Figure S1: Profile likelihood analysis for parameters S_1 – S_4 ; Figure S2: Parameter estimation validation—estimated S_1 by treatment and observed vs. predicted viable cells.

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Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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

Abbreviations

The following abbreviations are used in this manuscript:

AO/EB	Acridine orange/ethidium bromide
<i>P. linteus</i>	<i>Phellinus linteus</i>
Cispt	Cisplatin
Pt(IV)	Platinum(IV) complex
CRC	Colorectal carcinoma

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