

***IN VITRO* CYTOPHYSIOLOGICAL RESPONSE INDUCED BY THREE FOOD ADDITIVES ON SOME MAMMALIAN CELL LINE MODELS**

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Abstract

This research is focused on cellular response to sodium metabisulfite (E223), sodium benzoate (E211), and sodium nitrite (E250) application on two normal mammalian cell lines, namely MCF-12A (ATCC CRL-3598) and Vero (ATCC CCL-81). The monitored parameters were cell viability (MTT assay), cell morphology (optical microscopy) and cell survival (clonogenic assay). The treatment was applied in doses of 12.5; 25; 50 and 100 µg/mL. According to all applied tests, our results proved a dose-response relationship, the MCF-12A cell line showing the highest sensitivity.

Keywords: sodium metabisulfite (SMB), sodium benzoate (SB), sodium nitrite (SN), citotoxicity, clonogenic assay

Introduction

The current increasingly intensive use of food additives, as well as the presence of several additives in the same food product, lead to acute and negative effects. The long-term consumption produces cumulative effects, increasing the risk of developing some forms of cancer, cardiovascular diseases, and other degenerative diseases caused by chronic toxicity phenomena (Fiolet et al. 2018, Srour et al. 2019, Sellem et al. 2024). More than 3000 different additives, intentionally added to foods, are used as antioxidants and preservatives (Abdulummeen et al. 2012, U.S. Food and Drug Administration). Nowadays over 10,000 chemicals and additives are allowed in food in the United States, and approximately 99% of these were introduced after 2000, being approved only by the food and chemical industry, but not by the Food and Drug Administration that ensures the safety human food supply. On the other hand, the European Commission (EC) published Commission Regulation (EU) 2023/2108, which sets new lower limits for nitrite and nitrate food additives, as a measure of the European Plan to fight cancer (OJ L 2108, 2023). Adding of these additives is an accepted practice, but it is not without controversy. The categories of the food additives frequently used are antioxidants, preservatives, colorants, flavor enhancers, sweeteners, acidulants, anti-caking agents, anti-foaming agents, bulking agents, etc. (Inetianbor et al. 2015).

Most studies performed in Romania have been focused on biochemical identification of food additives and legislation issues (Dumitrescu and Milu 1990, Banu 2008) and less on cytotoxicity studies on human and animal cell lines (Pop et al. 2014, Pop et al. 2016, Baciuc et al. 2020). On a global level, especially recently, there has been an intensification of concerns regarding the possible negative effects of food additives on humans and animal organisms, the aim being to establish their cyto- and genotoxic potential (Yilmaz et al. 2014, Ataseven et al.

2016, Dosay-Akbulut 2020, Altunkaynak et al. 2021, Yoosuf and Shah 2021, Yilmaz et al. 2023).

Since preservatives, coded E200-E299, are used more intensively in the food industry, as a result of a lifestyle specific to large urban centers, the quantities of processed food have increased. Three representative compounds of this additive category are studied here as food additives, namely sodium metabisulfite (E223), sodium benzoate (E211) and sodium nitrite (E250). The purpose of these studies is to obtain new scientific data regarding the detection of the effects of food additives on the cyto-physiological processes of an animal and a human cell line, particularly important aspects for clarifying their mechanisms of action, a fact that will facilitate the development of effective protection and treatment methodologies for diseases related to exposure to additives.

Materials and Methods

Tested compounds. The products included in the study are part of the food additives group, namely, the sodium metabisulfite (SMB), sodium benzoate (SB), and sodium nitrite (SN), which were *in vitro* tested on normal cell lines, in different doses and time intervals. All three food preservatives were purchased from Sigma-Aldrich, Darmstadt, Germany.

The **biological material** used in the *in vitro* tests, in order to evaluate and quantify the cytotoxic effects of the studied food additives, was represented by healthy cell cultures, respectively kidney cells from the African monkey *Cercopithecus aethiops*, Vero (ATCC CCL-81, ATCC, Rockville, MD, USA) and epithelial cell lines from the human mammary gland, MCF-12A (ATCC CRL-3598, ATCC, Rockville, MD, USA). These cells were cultured in Dulbecco's modified growth medium, DMEM (Dulbecco's Modified Growth Medium, PAN-Biotech GmbH, Aidenbach, Germany) supplemented with fetal bovine serum (FBS, Euroclone S.p.A., Milano, Italy) 2% and 10%, respectively, and 1% antibiotic solution, Penicillin 100 µg/mL and Streptomycin 100 IU/mL (Capricorn Scientific GmbH, Ebsdorfergrund, Germany), at a temperature of 37°C, in a humidified environment, with 5% CO₂ (Vunjak-Novakovic and Freshney 2006).

MTT assay. Estimation of the food additives impact on cell viability was carried out by the colorimetric method with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT - Merck KGaA, Darmstadt, Germany), adapted from Mosmann (1983), Laville (Laville et al. 2004) and van Meerloo (van Meerloo et al. 2011). The method is based on mitochondrial dehydrogenases potential of living cells to convert the yellow water-soluble substrate (MTT) into the water-insoluble dark blue formazan. The amount of produced formazan is directly proportional to the number of living cells (Stockert et al. 2012). Briefly, the cells were detached with trypsin/EDTA (Biowest, Nuaille, France), then counted and resuspended in 96-well microplates (7x10³ cells/well for the Vero cell line and 6x10³ cells/well for the MCF-12A cell line), and placed under the same temperature and humidity conditions. After reaching monolayer (24 hours), the cells were treated for 24 and 48 hours with the compounds added to the final culture medium of 300 µL/well, in which the doses were: 12.5; 25; 50 and 100 µg/mL. As a control, cells grown only in complete culture medium were used. Cell viability was assessed after 24 h and 48 h of application of each additive, respectively. The cells were processed according to the MTT protocol, absorbance was measured at 570 nm with the Biochrom EZ Read 400 automated microplate reader, and the cell viability was calculated referring to the following formula:

$$\% \text{ Cell viability} = A_{\text{sample}} / A_{\text{control}} \times 100$$

where A = Absorbance

Cell morphology test. The interaction of different food additives with cellular structures was also highlighted through the morphological changes generated by them, both after 24 and after 48 hours of treatment. Cell morphology was visualized with a Nikon Eclipse TS100 inverted microscope equipped with a MshOt MS60 digital camera (Nikon, Tokyo, Japan).

Clonogenesis assay. The colony formation assay is an *in vitro* test that allows the assessment of the degree of cell survival related to the ability of a single adherent cell to develop into a colony, this being defined as consisting of at least 50 cells. This test provides analysis of each cell in a population for its ability to achieve "unlimited" divisions (Franken et al. 2007, Backer et al. 2011, Rafehi et al. 2011), and just only a fraction of the cultured cell population maintains the capacity to produce colonies. In present study, both normal cell lines were seeded in 12-well plates (100 cells/well) and allowed to grow for one day, when the 24-hour treatment with the tested additives was applied. After treatment, the growth medium was replaced with fresh medium, the procedure being repeated every 2-3 days until the end of the experiment. The assay was stopped after colonies with more than 50 cells/colony were formed in the control group. Well-plates were washed with phosphate-buffered saline (PBS - Biological Industries, Beit Haemek, Israel), colonies were fixed with ethanol and stained with 0.5% Crystal violet. Colonies were counted and analyzed by ImageJ software (Collins 2007, Guzmán et al. 2014).

Statistical analysis. Results of *in vitro* tests were expressed as mean $\pm$ standard error (ES). The difference between mean values for each parameter was expressed by Student's t test (Cann 2002). Statistical significance was set at $p < 0.05$ and each sample was performed in triplicate. The IC_{50} values of the tested food additives were estimated by polynomial dose-response curve plots for each concentration of the MTT assay.

Results and discussions

In vitro research, on normal cell cultures, aimed to investigate the toxicity degree of some food additives, using a wide range of doses, ranging from 12.5 to 100 $\mu\text{g/mL}$, the tested products being **sodium metabisulfite** (SMB), **sodium benzoate** (SB) and **sodium nitrite** (SN). The cell lines used were Vero monkey kidney and MCF-12A human mammary gland epithelial cell lines.

Estimation of the studied food additives impact on cell viability

In the case of 24-hour treatment with the sodium metabisulfite of normal Vero cell line, there was a dose-dependent effect on cell viability, with values ranging from 91.15% (12.5 $\mu\text{g/mL}$) to 76.56% (100 $\mu\text{g/mL}$), compared to the untreated control (100%). Significant decrease in Vero kidney cell line viability was reported after 48 h treatment with sodium metabisulfite, with values of 77.64% at the lowest dose tested (12.5 $\mu\text{g/mL}$) and 52.63% at the highest dose (100 $\mu\text{g/mL}$), as illustrated in Figure 1.

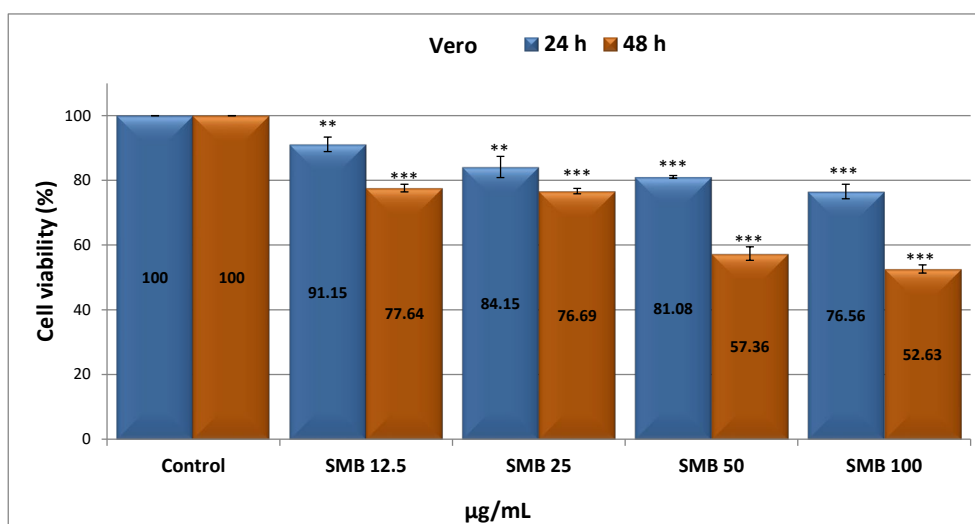


Figure 1. Effect of 24- and 48-hours treatment with different concentrations (µg/mL) of sodium metabisulfite (SMB) on the viability of Vero monkey kidney cell line. **p<0.01, ***p<0.001

Sodium metabisulfite was also tested on human mammary epithelial cell line, MCF-12A. The 24-hours treatment with this food additive was associated with an insignificant impact on cell viability, with values between 95.01% at the minimum concentration used (12.5 µg/mL) and 85.57% at the maximum one (100 µg/mL), corresponding to weak cytotoxic effects of 4.99% and 14.43%, respectively, as seen in Figure 2. The 48-h treatment with SMB at the same doses resulted in a more evident decline in cell viability. Thus, at the dose of 12.5 µg/mL, a cell viability value of 69.48% was found, and at the maximum tested dose of 100 µg/mL, the value of 52.97% was recorded, representing notable cytotoxic effects, of 30.52% and 47.03%, respectively. It is noteworthy that significant and similar in amplitude cytotoxic effects were found in both cell lines.

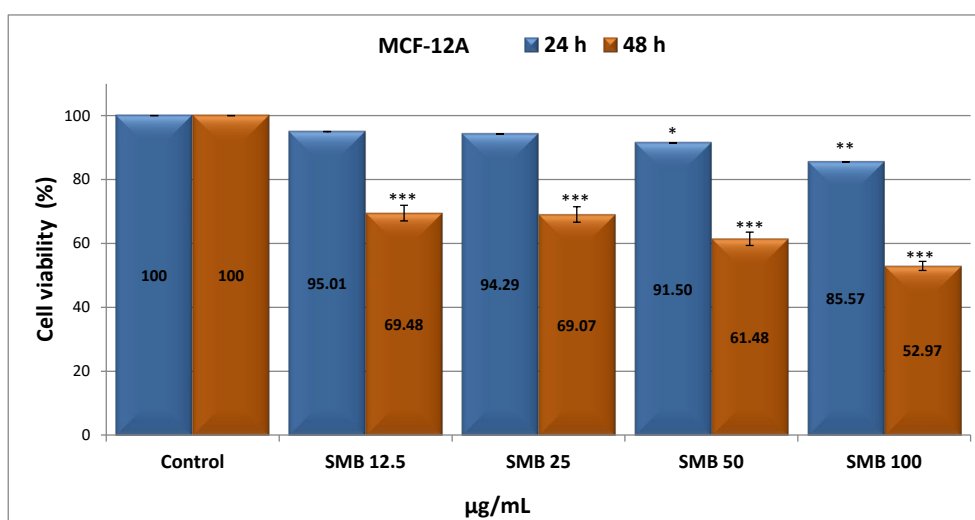


Figure 2. Effect of 24- and 48-hour treatment with different concentrations (µg/mL) of sodium metabisulfite (SMB) on the viability of MCF-12A epithelial cell line. *p<0.05, **p<0.01, ***p<0.001

The *in vitro* screening included the testing of another food additive, namely sodium benzoate (SB), on the same normal cell lines, monkey kidney (Vero) and mammary gland epithelial cell lines (MCF-12A), respectively. As can be seen in Figure 3, the treatment for 24 hours with SB of Vero cell line caused, at the minimum tested dose (12.5 µg/mL), a slight effect on cell

viability, the value being 94.23%, and at the maximum used dose (100 $\mu\text{g/mL}$), the impact on cell viability was greater, with a value of 77.65. The 48-hour treatment with SB determined at the minimum dose (12.5 $\mu\text{g/mL}$) a moderate reduction of the cell viability, being 81.58%, but at the maximum dose (100 $\mu\text{g/mL}$) this parameter decreased, reaching of 67.62%, these values revealed cytotoxic responses of 18.42% and 32.38%, respectively.

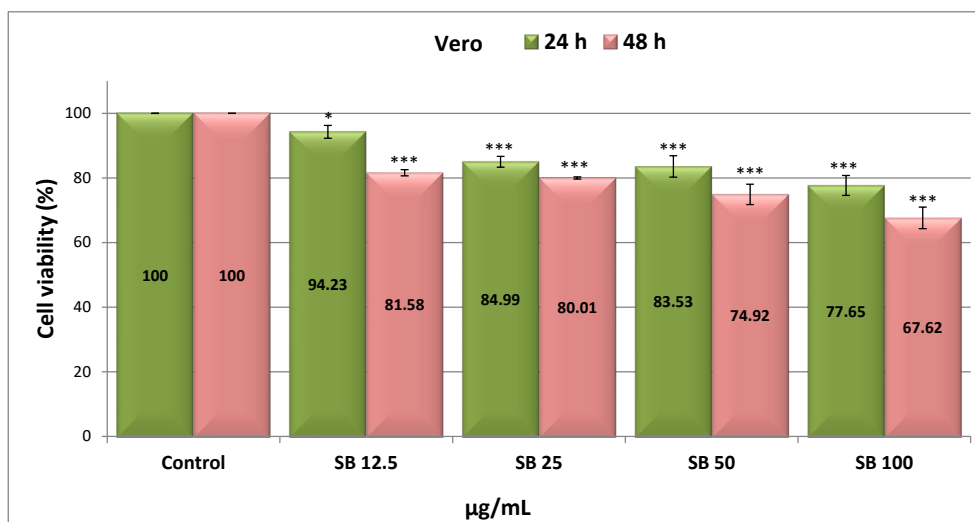


Figure 3. Effect of 24- and 48-hour treatment with different concentrations ($\mu\text{g/mL}$) of sodium benzoate (SB) on the viability of Vero monkey kidney cell line. * $p < 0.05$, *** $p < 0.001$

A particular situation was observed in MCF-12A epithelial cell line versus Vero kidney cell line (Figure 4). Thus, after the 24-hour treatment, a minor interference with cell cytophysiology was found, the cell viability being between 95.71% (12.5 $\mu\text{g/mL}$) and 90.98% (100 $\mu\text{g/mL}$). However, extending SB treatment to 48 h led to a more intense cytotoxic effect at all four doses tested, the cell viability decreasing up to 51.24% (100 $\mu\text{g/mL}$), compared to the untreated control.

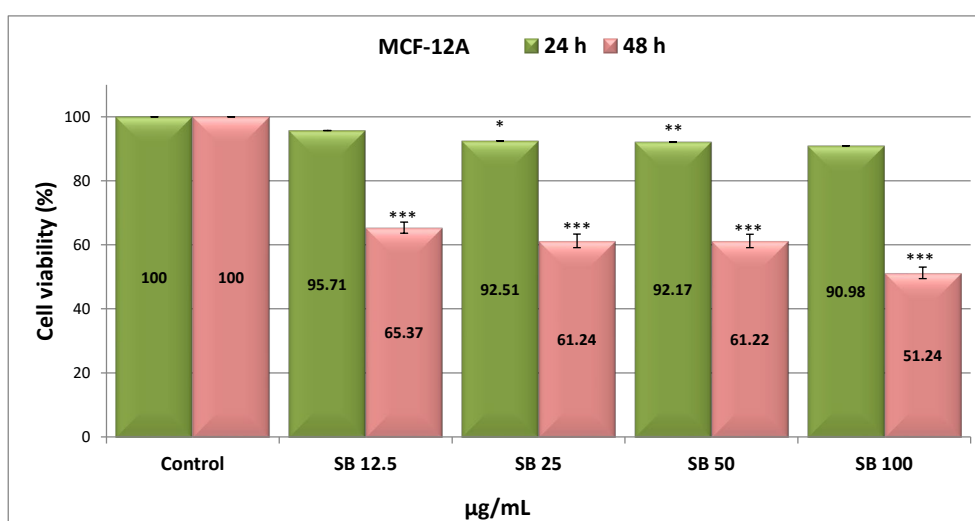


Figure 4. Effect of 24- and 48- hour treatment with different concentrations ($\mu\text{g/mL}$) of sodium benzoate (SB) on the viability of MCF-12A epithelial cell line. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

In vitro studies included investigation of a third food additive, sodium nitrite (SN), using the same normal mammalian cell lines. As presented in Figure 5, the first three doses of SN applied

on Vero kidney cell line resulted in a similar pattern of cell viability both after 24 and 48 hours of treatment. Only at 100 $\mu\text{g/mL}$ of SN, the cytotoxic effect was approximately 10% more intense after 48 hours of treatment compared to the value reported at 24 hours, indicating cell viability values of 76.36% (after 24 h), respectively 66.46% (after 48 h).

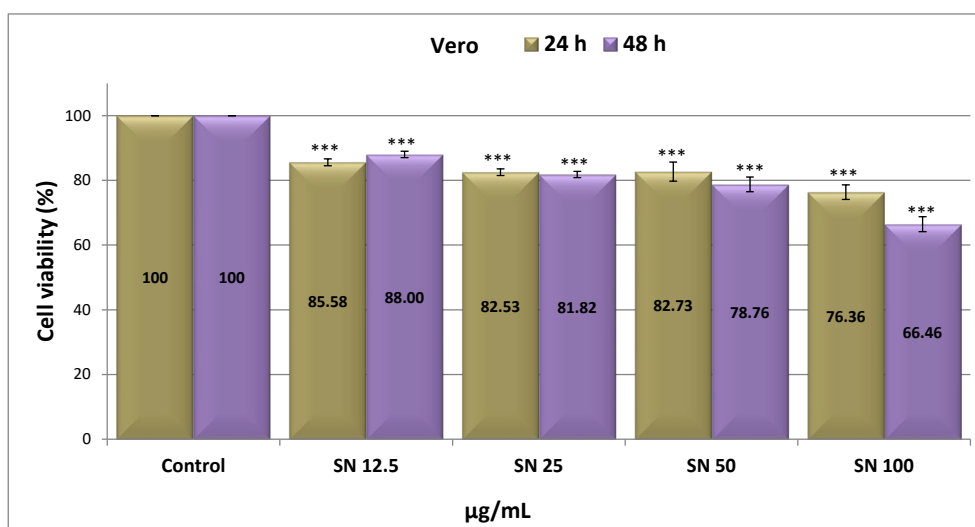


Figure 5. Effect of 24- and 48-hour treatment with different concentrations ($\mu\text{g/mL}$) of sodium nitrite (SN) on the viability of Vero monkey kidney cell line. *** $p < 0.001$

As in the case of SB application, the response of MCF-12A cell line to the 24-hour treatment of (SN) was less intense than that observed in Vero kidney cell line, the viability being insignificantly lower than the untreated control, with values of 95.05% (12.5 $\mu\text{g/mL}$), and 85.45% (100 $\mu\text{g/mL}$). Instead, the 48-hour treatment with SN led to a stronger interaction with cell viability, registered values of 79.98% (12.5 $\mu\text{g/mL}$) and 57.48% (100 $\mu\text{g/mL}$) (Figure 6).

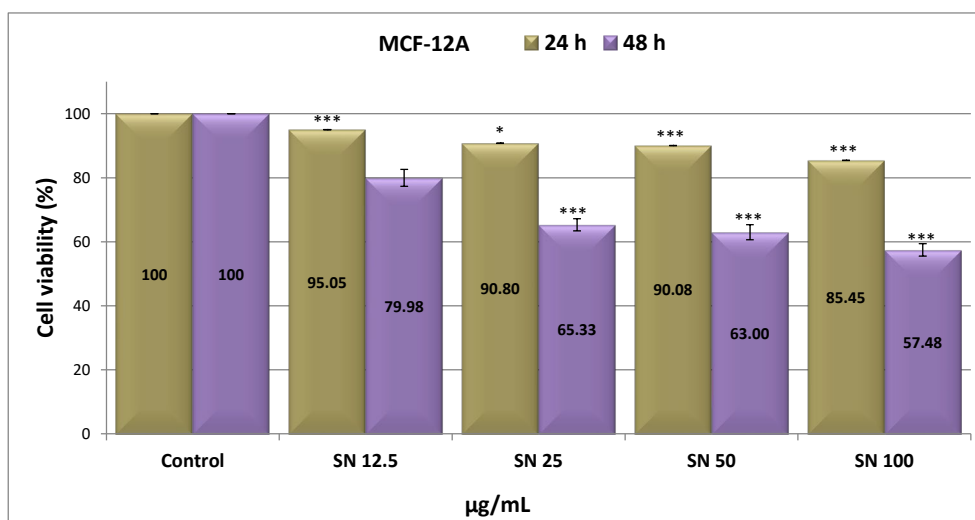


Figure 6. Effect of 24- and 48- hour treatment with different concentrations ($\mu\text{g/mL}$) of sodium nitrite (SN) on the viability of MCF-12A epithelial cell line. * $p < 0.05$, *** $p < 0.001$

The evaluation of the impact of the studied food additives on cell viability through the MTT test confirmed their cytotoxic effect, the amplitude of which varied depending on the compound, the *in vitro* dose applied - the existence of the dose-effect relationship being demonstrated - and the type of cell culture. Thus, in the both cell lines, the 48-hour treatment with sodium metabisulfite generates a notable cytotoxic impact even at the minimum dose used

(12.5 µg/mL), of greater amplitude in MCF-12A cell line, which is accentuated at the maximum dose (100 µg/mL), reaching values close to the minimum inhibition threshold of 50% imposed by international standards. In addition, the MCF-12A mammary epithelial cell line shows a higher susceptibility to the toxic action of the studied food additives, compared to Vero kidney cell line.

Our results are in agreement with those recorded in the scientific literature regarding the toxicity of certain food additives. Thus, recent data (Ghasemi et al. 2022) revealed the cytotoxic effect of sodium metabisulfite (SMB) on L-929 normal fibroblast and K-562 human leukemia cell lines, illustrated by a significant dose-dependent reduction of cell viability ($p < 0.01$). SMB over 100 µg/mL promotes significant cytotoxicity in A549 human alveolar epithelial cell line, with membrane damage and lysosome disruption. Also, association of SMB with non-cytotoxic propylene glycol induced the potentiation of SMB toxicity (Yoo et al. 2018). Other studies demonstrated the cytotoxic effect of SMB on HFFF2 human fetal foreskin fibroblasts by inducing apoptosis (Alimohammadi et al. 2021). Regarding sodium benzoate (SB), it was found that at a concentration as low as 0.1%, commonly added to preserve soft drinks, it produced a significant decrease of cell viability in rat cortical neurons and human HeLa tumor cell line (Park et al. 2011). Furthermore, SB, frequently used in cosmetics, exert a very low cytotoxic activity on the CCD1072Sk human newborn fibroblast cell line, compared to other additives, such as methylparaben or imidazolidinyl urea (Spindola et al. 2018). The SB (6.25 mM-50 mM) inhibited cell viability by inducing apoptosis in HCT116 colon cancer cell line and activation of nuclear kappa B (NFkB) factor implied in proliferation and cell survival, the process being more intense especially at the dose of 50 mM (Yilmaz and Karabay 2018). *In vitro* and *in vivo* studies showed significant suppression of splenocyte proliferation at response to 72 and 96 hours of SB treatment, decrease of IL4, IL6, IFNg and IL17 cytokine expression in splenocytes, proving the immunomodulatory potential of this food additive (Yadav et al. 2016). Sodium nitrite (SN) was found to cause a significant negative effect on the proliferation of human gastric adenocarcinoma (GAS) epithelial cell line in a dose- and time-dependent manner (Sun et al. 2006). Other studies showed the toxicity of SN at the dose of 5 mM on isolated rat hepatocytes, cytotoxicity related to the formation of reactive oxygen species (ROS) and lipid peroxidation, with fragmentation of the lysosomal membrane and release of proteases, with mitochondrial damage (Kiani et al. 2017).

Determination of the half-maximal (50%) inhibitory concentration (IC₅₀)

Based on the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test, the IC₅₀ was determined, this parameter being frequently used as a general indicator of the cytotoxicity degree caused by various xenobiotics (Shimamura et al. 2014, Swinney 2015). In this context, the next objective of our studies was the determination of the IC₅₀ value, both after 24-hour and 48 hours of treatment. As can be seen in Table 1, cellular reactivity to the tested additives differs from one line to another, the cell line with renal origin (Vero) being more sensitive after short treatment (24 h), IC₅₀ values being approximately halved compared to those recorded in MCF-12A cell line. Prolonging of the treatment up to 48 hours determined higher IC₅₀ values in Vero cell line compared to MCF-12A ones, which seems the intervention of adaptive phenomena or neutralization mechanisms, processes whose efficiency is more intense after a longer exposure interval to the analyzed additives.

Table 1. IC₅₀ values (µg/mL) of each additive tested after 24 and 48 hours of treatment administrated to normal MCF-12A and Vero cell lines.

Variant	MCF-12A		Vero	
	IC ₅₀ 24 h	IC ₅₀ 48 h	IC ₅₀ 24 h	IC ₅₀ 48 h
SMB	702.95	141.81	338.05	165.23
SB	756.43	127.33	444.02	218.83
SN	600.21	163.01	284.66	269.06

Evaluation of the effect of some food additives on cell morphology

The food additive-cells interaction was also quantified through evaluation of the morphological changes of MCF-12A and Vero cell lines after treatment with the three additives. Analyzing Plates I-VI, a decrease in the adhesion capacity of cells to the substrate (culture flask) can be seen, process realized by identifying some spaces in the cell monolayer, the amplitude of the phenomenon being correlated with the increase in the dose of and the exposure time. In addition, very large cells surrounded by a granular halo or small cells with folded membrane and nucleus with a fragmented appearance were reported suggesting the existence of an apoptotic process (Plate I). The cytomorphological changes provoked by sodium benzoate treatment were more intense compared to sodium metabisulfite, the establishment of intercellular connections being less at the maximum dose of 100 µg/mL (Plate II). The cytotoxic effect of sodium nitrite on MCF-12 cell line was highlighted by loss of substrate adhesion and difficulty in establishing the cell monolayer due to the appearance of abnormal folding or elongation of the cell membrane (Plate III).

In the case of the Vero cell line, the cytotoxic effect of SMB seems to be more significant compared to MCF-12 cells, especially after 24 hours of treatment, the phenomenon being in concordance with lower viability settled by MTT assay (Plate IV). The response of Vero cell line to SB treatment is more intense compared to that of MCF-12A cell line, the weakening of ability to adhere to the substrate being observed not only at the maximum tested concentration, 100 µg/mL, but also at 50 µg/mL (Plate V). The microscopic study of the cells exposed to the SN additive showed that Vero cell line express different degrees of morphological changes, similar to those observed after treatment with the other two additives, namely: loss of cell adhesion, membrane contraction, cell fragmentation, resulting in a reduction of live cells density (Plate VI). In general, low concentrations of food additives triggered to minor changes in cell viability. For example, the use of sodium and calcium lactate to treat umbilical cord endothelial cell line (HUVEC) did not lead to DNA damage, contraction or fragmentation in the nucleus, changes specific to the phenomenon of apoptosis (Javaheri-Ghezeldizaj et al. 2023).

Highlighting the action of some food additives on the clonogenesis process

The clonogenic assay was used to determine the ability of a single cell to proliferate indefinitely, thus retaining its reproductive potential to form a colony or clone, with that cell considered clonogenic (Munshi et al. 2005). To evaluate the impact of the treatment of the three food additives on clonogenesis, three doses were used, namely: the IC₅₀ dose – D2, the halved dose (1/2) – D1 and the double dose (2/1) – D3 respectively, in relation to the dose established as IC₅₀, for each individual additive. As can be seen in Figures 7-9, the clonogenic capacity of the treated cells downgrades compared to the control. It should be noted that sodium nitrite had the lowest and highest surviving fraction (SF) values, varying from 15.97% (Vero, SN-D3) to 114.67% (MCF-12A, SN-D1). Also, for both cell lines, the low concentration induced a higher SF compared with higher concentrations of food additives.

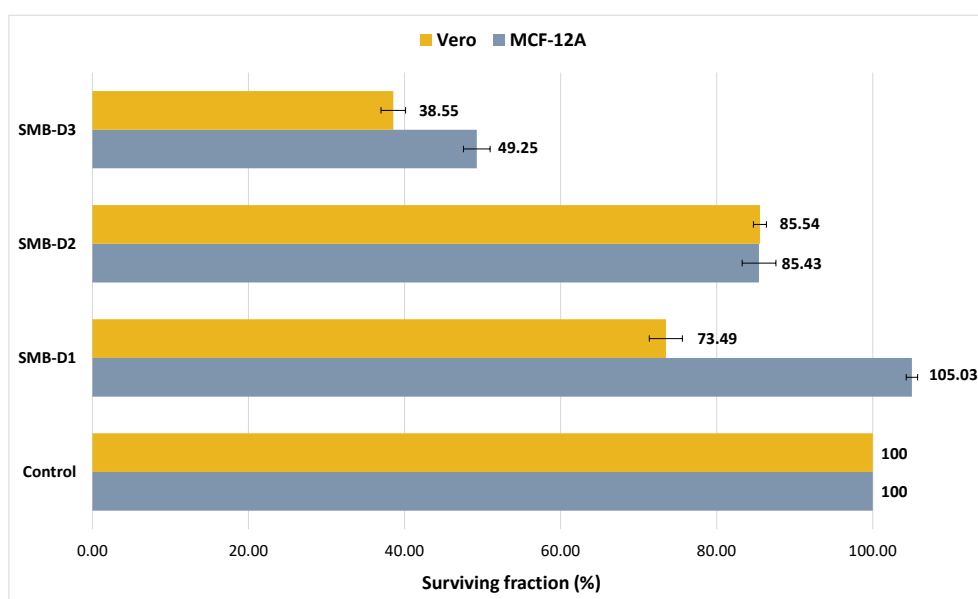


Figure 7. Colony surviving fraction of MCF-12A and Vero cell lines after 24 h with SMB

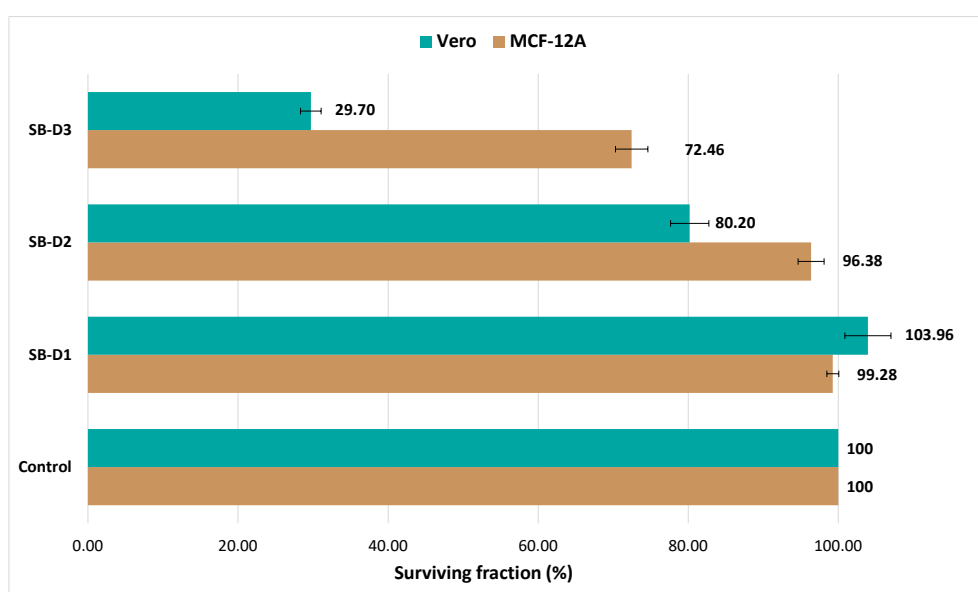


Figure 8. Colony surviving fraction of MCF-12A and Vero cell lines after 24 h with SB

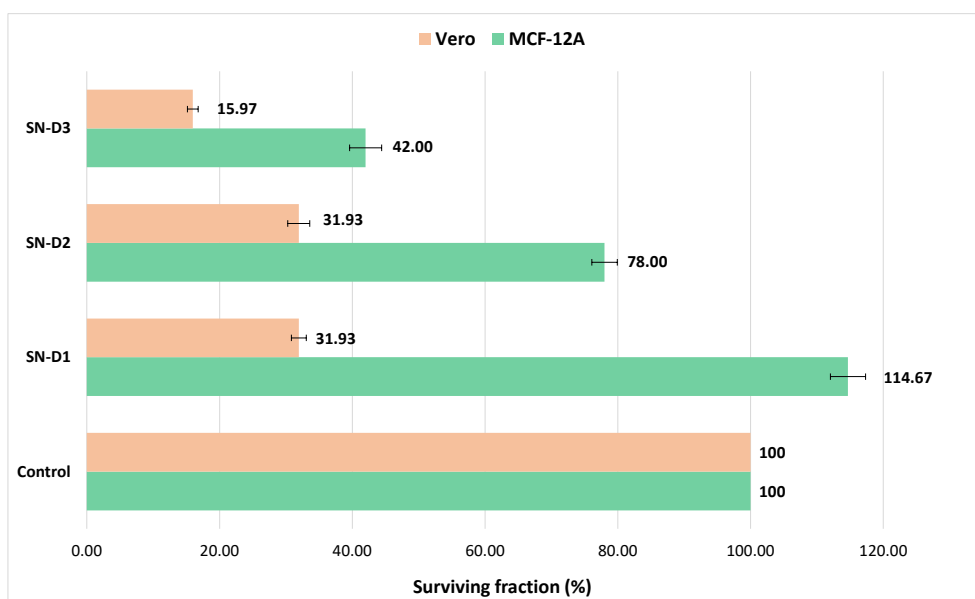


Figure 9. Colony surviving fraction of MCF-12A and Vero cell lines after 24 h with SN

Comparable results, in the sense of a reduced effect at $1/8$ and $1/2$ of IC_{50} doses, were obtained after treating A431 cell line with the dibutyl phthalate (DBP) or methyl paraben (MePB) additives, the changes being more accentuated when the cells are exposed to the binary mixtures of DBP and MePB than when exposed to these substances individually. The authors emphasize the importance of risk assessment for cumulative exposures of additives in order not to underestimate the risk of adverse effects associated with exposure to chemical mixtures (Miranowicz-Dzierżawska et al. 2023).

The macro-morphological aspects of colony formation after different food additives treatment are presented in Figures 10, 11.

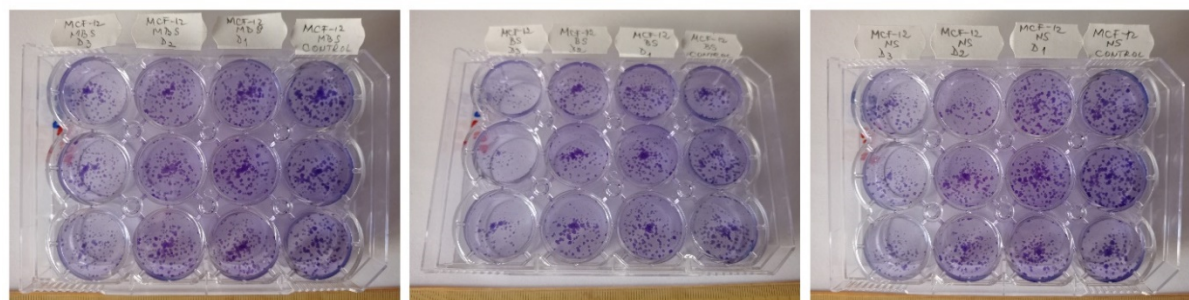


Figure 10. Macroscopic aspects of the clonogenic capacity of MCF-12A cell line after treatment with various food additives doses $1/2$ IC_{50} (D1), IC_{50} (D2) and $2/1$ IC_{50} (D3)

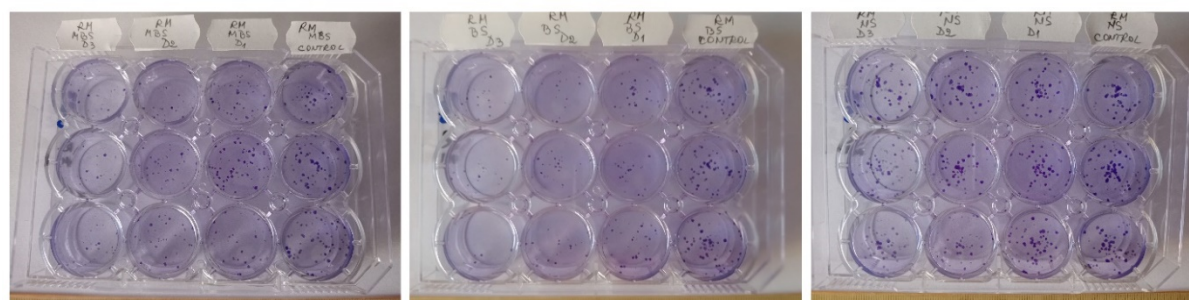


Figure 11. Macroscopic aspects of the clonogenic capacity of Vero cell line after treatment with various food additives doses $1/2$ IC_{50} (D1), IC_{50} (D2) and $2/1$ IC_{50} (D3)

Conclusions

Our results regarding the reactivity of Vero normal kidney and MCF-12A human mammary epithelial cell lines to the action of some food preservative have proven a cytotoxic effect of them, differentiated in amplitude depending on the compound, the dose of the *in vitro* treatment – the existence of the dose-effect relationship being demonstrated – and by the type of cell line culture. Thus, in the case of both cell lines, treatment with sodium metabisulfite determined a notable cytotoxic impact even from the minimum used dose, of greater amplitude in MCF-12A cell line. For both studied cell lines, the cytotoxic response was accentuated at the maximum dose, reaching values near to the threshold of minimum 50% inhibition imposed by international standards. The application of sodium benzoate generated a more marked cytotoxic effect on MCF-12A epithelial cell line at the maximum dose, aspect also reported in the case of sodium nitrite.

It was observed that the clonogenic capacity of cells undergoing treatment was decreased compared to the control, noting that after sodium nitrite application both the lowest (Vero cell line) and the highest (MCF-12A cell line) values of surviving fraction were recorded.

Microscopic observations of cells exposed to the tested food additives revealed different degrees of morphological changes, such as: loss of cell adhesion, membrane contraction, cell fragmentation, resulting in a reduction in the density of living cells.

The obtained results represent the scientific foundation of some future research directions which, on the one hand, will highlight the effect of food additives on other cell lines, normal or tumor, in order to use *in vitro* cell systems as models for rapid testing of the toxicity of food additives and, on the other hand, they will facilitate the development of effective protection and treatment methodologies for diseases induced by exposure to food additives.

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PLATE I

Morphology of MCF-12A cell line after treatment with SMB applied for 24 and 48 h
(magnification of $\times 100$; scale bar $100\ \mu\text{m}$)

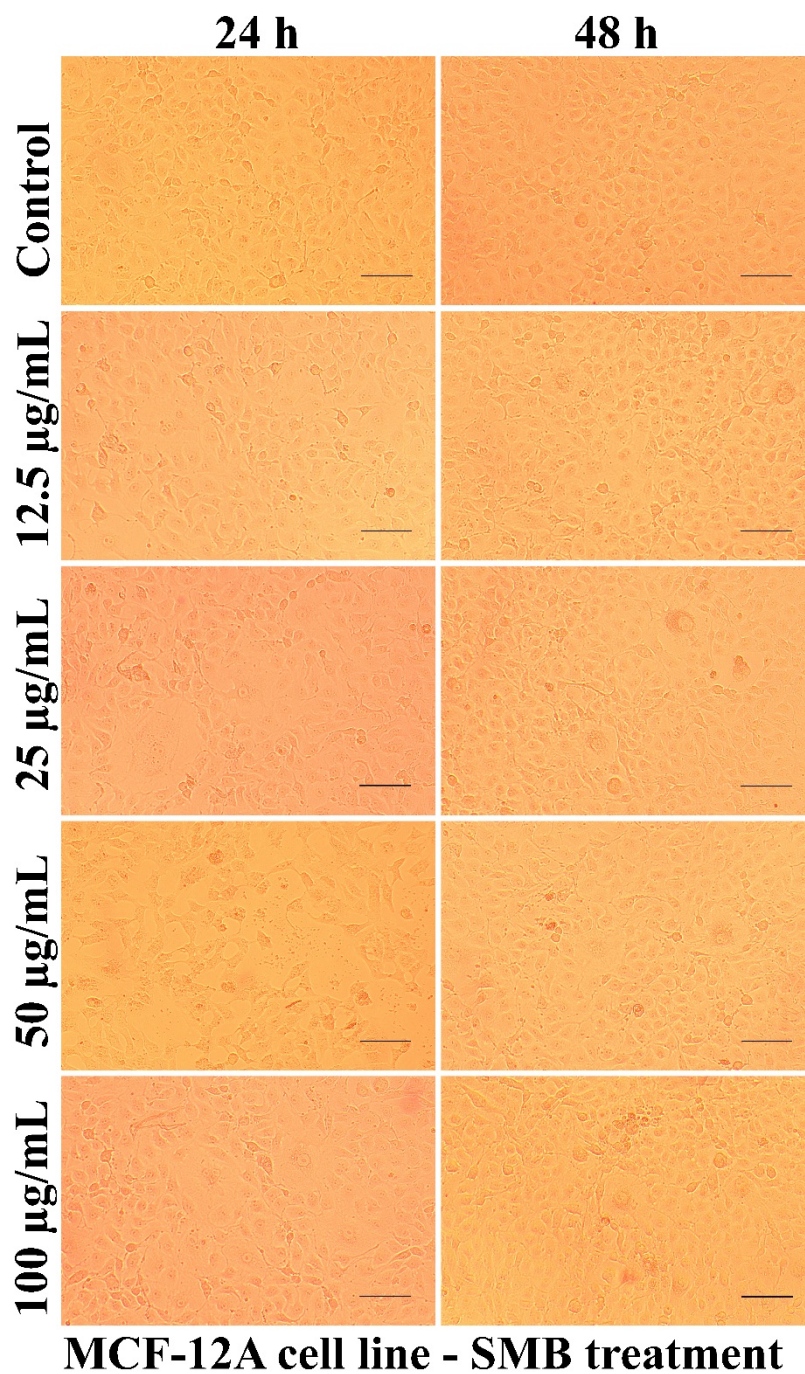
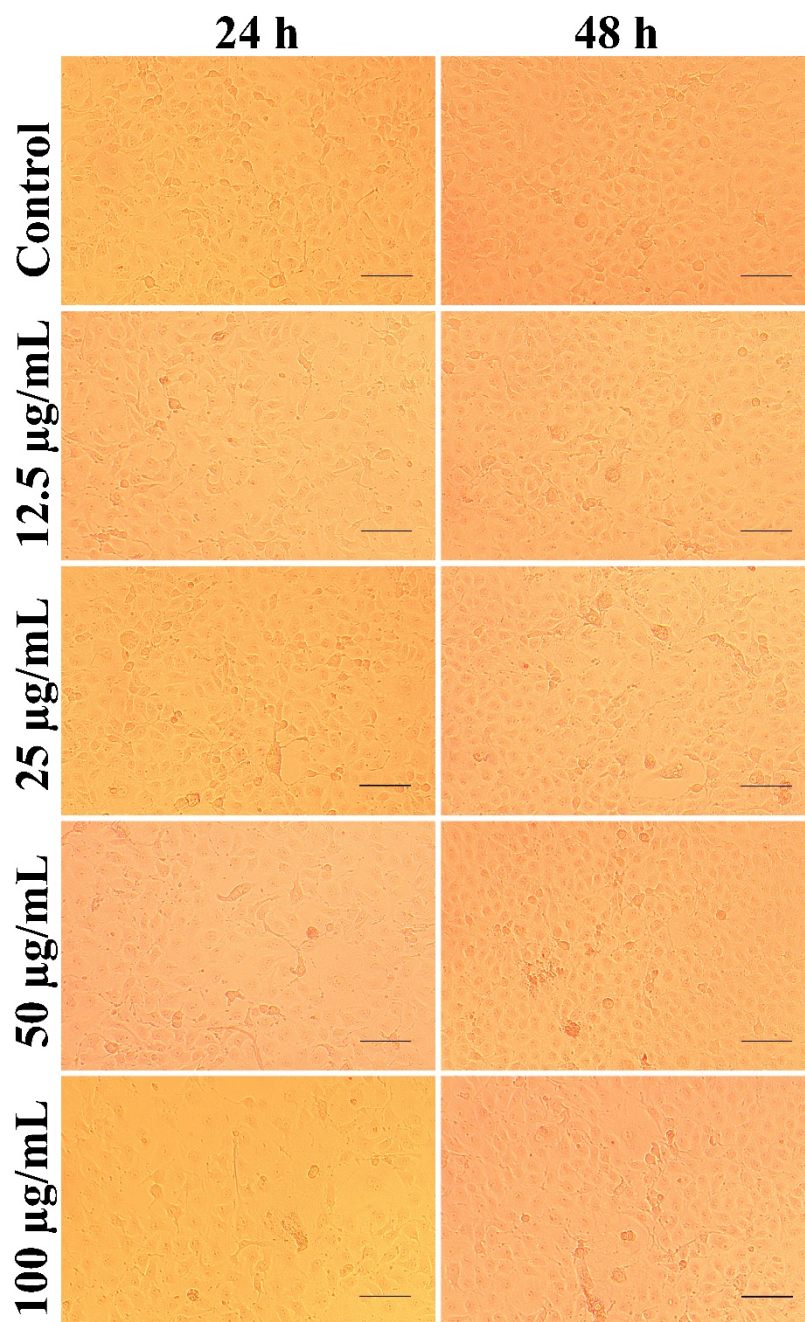


PLATE II

Morphology of MCF-12A cell line after treatment with SB applied for 24 and 48 h
(magnification of $\times 100$; scale bar $100\ \mu\text{m}$)



MCF-12A cell line - SB treatment

PLATE III

Morphology of MCF-12A cell line after treatment with SN applied for 24 and 48 h
(magnification of $\times 100$; scale bar $100\ \mu\text{m}$)

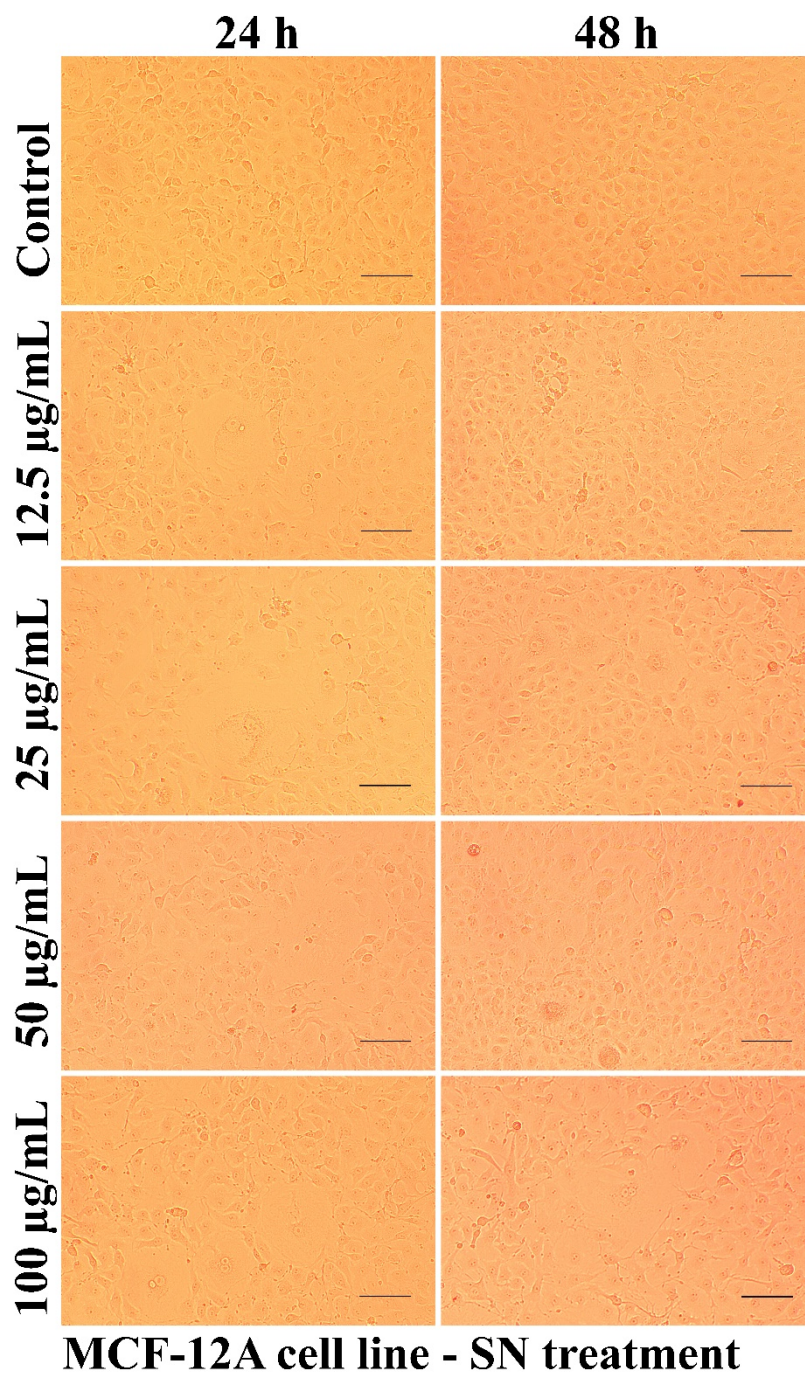
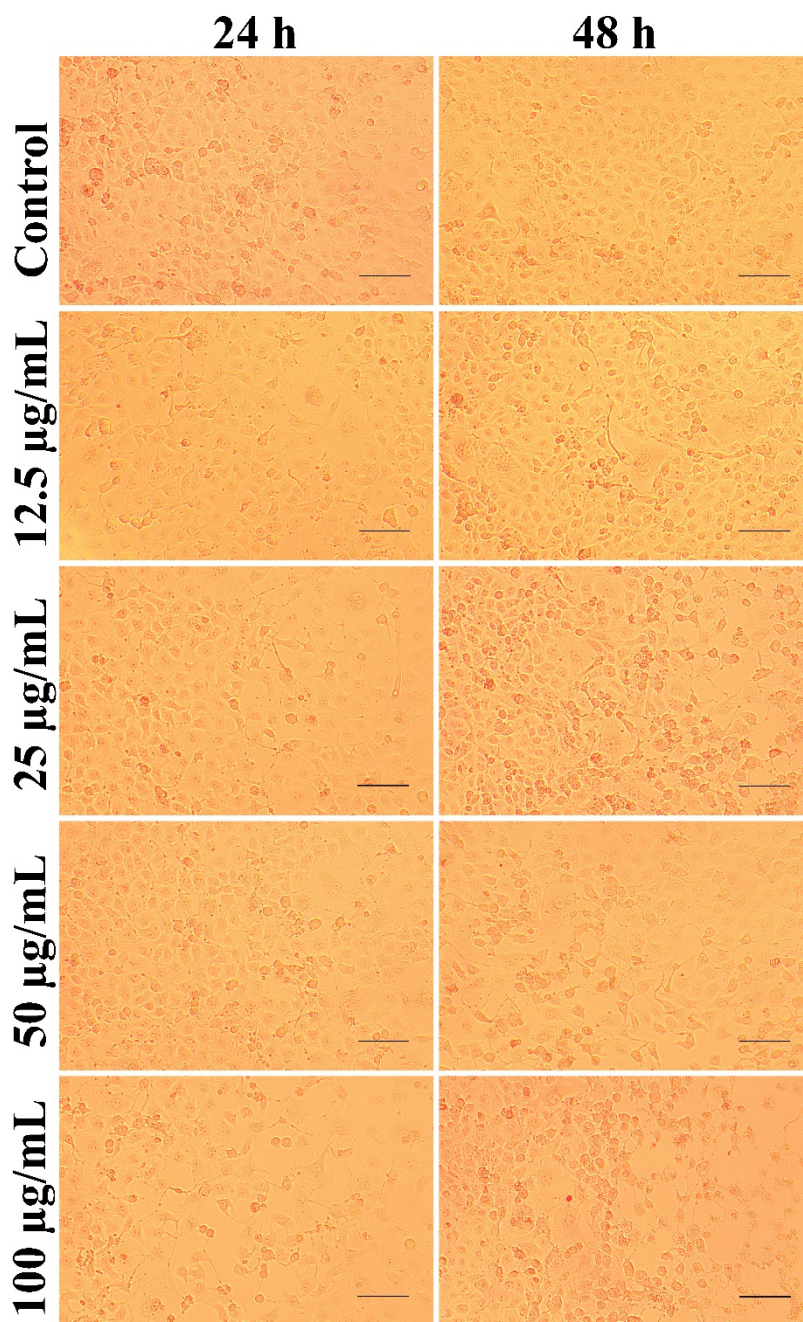


PLATE IV

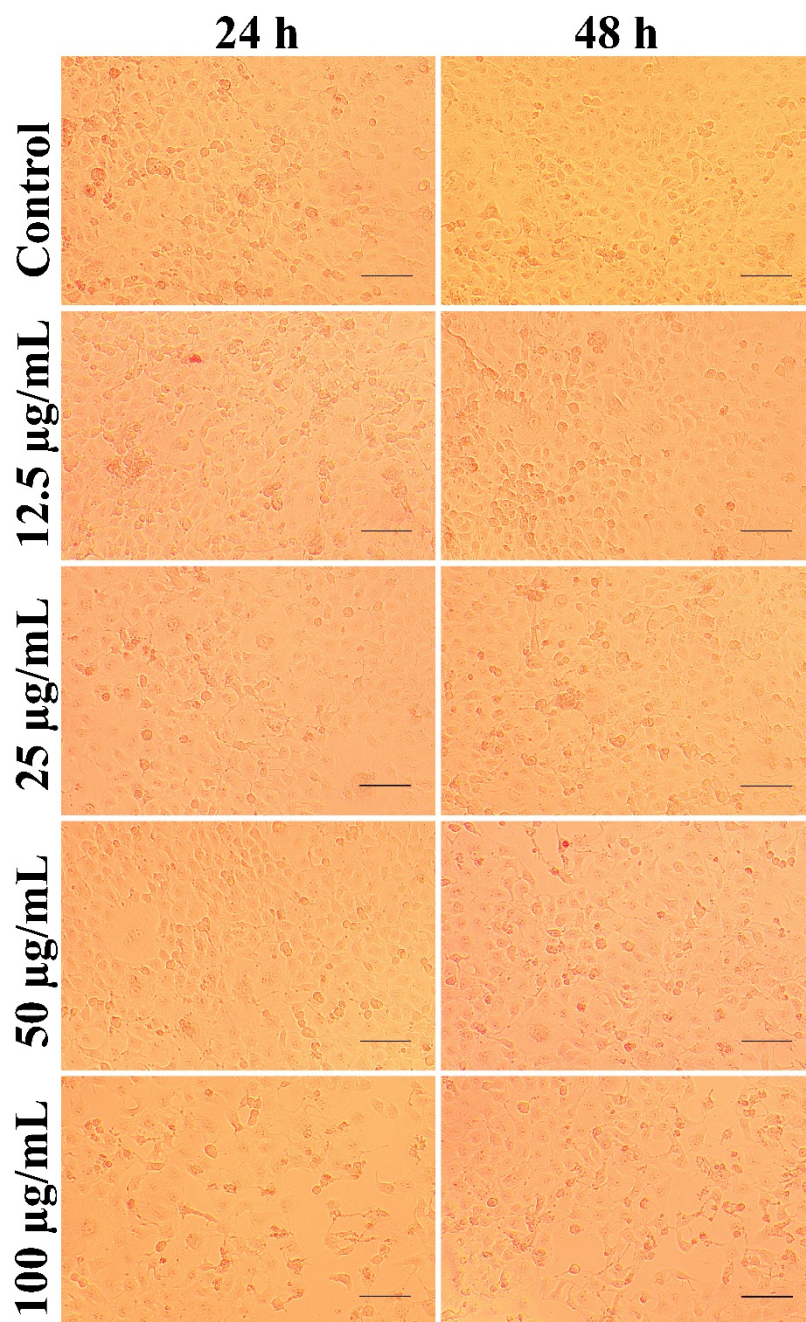
Morphology of Vero cell line after treatment with SMB applied for 24 and 48 h
(magnification of $\times 100$; scale bar $100\ \mu\text{m}$)



Vero cell line - SMB treatment

PLATE V

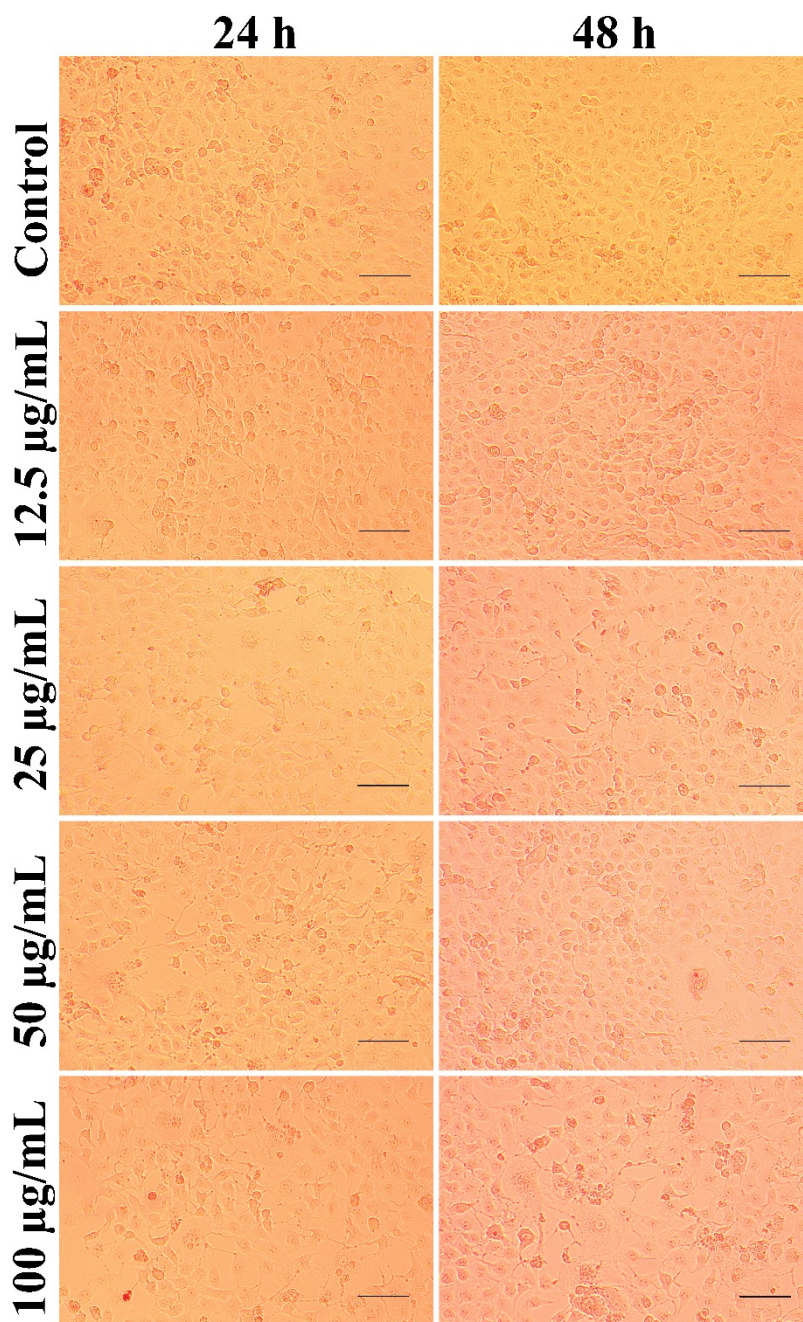
Morphology of Vero cell line after treatment with SB applied for 24 and 48 h
(magnification of $\times 100$; scale bar $100\ \mu\text{m}$)



Vero cell line - SB treatment

PLATE VI

Morphology of Vero cell line after treatment with SN applied for 24 and 48 h
(magnification of $\times 100$; scale bar $100\ \mu\text{m}$)



Vero cell line - SN treatment

