Wogonoside: anti-human colon cancer activities and survey of HMG-CoA reductase inhibition properties with molecular modeling

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Abstract

Introduction: The biological activities and interactions of wogonoside in the presence of HMG-COA reductase were investigated using a molecular docking study as a versatile theoretical approach. Wogonoside showed a considerable binding affinity to the enzyme with a docking score of –7.582 kcal/mol. The results indicated that the compound makes hydrophobic contacts with essential residues of the catalytic domain of the enzyme. Therefore, wogonoside could be considered as a potential inhibitor for HMG-COA reductase.

Material and methods: The *in vitro* cytotoxic and anti-colon carcinoma effects of biologically synthesized wogonoside against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines were assessed. The IC_{50} of wogonoside were 105, 198, 173, 382, 71, and 183 µg/ml against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines.

Results: The anti-colon carcinoma properties of wogonoside could significantly remove GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines in a time- and concentration-dependent manner by MTT assay. It appears that the anti-human colon carcinoma effect of recent nanoparticles is due to their antioxidant effects. We obtained results for the HMG-CoA reductase enzyme at the micromolar level. In our study, the inhibition result for HMG-CoA reductase showed a lower micromolar value of 28.70±4.73.

Conclusions: The results showed that Wogonoside has a good affinity with hmG-COA reductase binding site, and it is a promising HMG-COA reductase inhibitor, which has a certain potential in the treatment of colon carcinoma in humans in clinical patients.

Key words: wogonoside, HMG-COA reductase, colon cancer, molecular docking, enzyme activity.

Introduction

Cancer, one of the main causes of death in the world, kills more than 7.5 million people annually. Genetic factors and environmental factors are among the most important factors involved in the development of cancer. On the other hand, environmental factors such as diet, lifestyle, geographical conditions, stressors, age and obesity are involved in the development of this disease. Clinicians use a variety of methods to diagnose and treat a variety of cancers, the most important of which are chemotherapy, radiation therapy, surgery, and hormone therapy, but the most

important way to fight cancer is chemotherapy, which causes many side effects for the patient, including fatigue, nausea, hair loss, etc. [1–4]. These complications can have a great effect on the patient's life quality, because many of the chemical drugs used to treat cancer cause gastrointestinal disorders, kidney damage, etc. Scientists are looking for drugs with fewer side effects than chemical materials, in which natural compounds and molecules have received much attention [4–7]. Natural compounds and molecules have fewer side effects than chemical materials due to the combination of other compounds with a specific drug effect. Many natural compounds and molecules contain anti-cancer agents that can exert their effects at different stages of the onset and growth of cancer cells. The main goal in preventing cancer with natural or chemical substances is to slow down or inhibit the carcinogenic process. This approach focuses purposefully on abnormal intracellular pathways that lead to abnormal cellular function [5–9].

The gradual maturation of biotechnology has been considered not only for treating cancer but also for a wide variety of applications, especially for drug delivery and diagnostic and imaging cases. There are many types of molecules available and choosing the right carriers according to demand is a key issue [7–12]. Molecules are very similar in size to biological molecules and can easily penetrate into the cell; for this reason, one of the goals of biotechnology is to mount molecules and drugs on molecules and transfer them to the target cell [7–13]. It is also possible to create different surface properties for molecules by attaching protective ligands to increase the molecules' resistance to the immune system and increase their presence in the bloodstream, and even binding ligands to specifically bind the molecules to the target tissue [11–15].

Hypercholesterolemia, known as high cholesterol, is a common disease in which excess fat and fatty acids accumulate in the blood. Hypercholesterolemia is one of the major risk factors for atherosclerosis and coronary heart diseases. Especially increased low-density lipoprotein (LDL) and triglyceride levels, which cause hypercholesterolemia, also lead to diseases such as obesity, diabetes and cancer. HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase enzyme catalyzes the reaction in which HMG-CoA is converted to mevalonate through the mevalonate pathway in cholesterol biosynthesis. HMG-CoA reductase is the rate-limiting enzyme that regulates cholesterol synthesis in the body [16–18].

Theoretical studies such as molecular docking are beneficial methods that can provide a reliable insight into the interactions and biological activities of various compounds in the presence of biomolecules [19]. The outcomes of such studies can help biologists to identify the mechanisms that the chemical inhibitors would have in the vicinity of related enzymes [20]. In general, the theoretical approaches provide practical complementary information that can support the results of experimental studies.

In the present study, the properties of wogonoside against common colon carcinoma cell lines GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM were evaluated.

Material and methods

Enzyme assay

HMG-CoA reductase activity according to the method described by Takahashi *et al*.; DLHMG-CoA + 2 NADPH + 2H + $\rightarrow$ (R) -mevalonate + 2 NADP + was measured spectrophotometrically by using the absorbance change of NADPH at 340 nm in accordance with the reaction. 3. NaN_3: 2 M; 4. phosphate buffer: pH: 7.2; 50 mM: Experiment Conducting Spectrophotometer. After zero adjustment against distilled water at 340 nm, phosphate buffer was added to the test tubes separately as a blank and sample for each sample, NaN_3, NADPH and the sample were added and mixed thoroughly and the absorbance change was followed for 1 min [21]. Then HMG-CoA was added to the cuvette and the absorbance change was monitored for 3 minutes. HMG-CoA activity was calculated using the coefficient of NADPH. (ε: 6.22×10^3 l/mol × cm) is given as IU/l, activities are measured, then we started the IC_{50} study after the control value was found. For the IC_{50} study, we used six different inhibitor concentrations and wrote down the activities and then plotted the IC_{50}. After obtaining the concentration-percent activity equation, we calculated the IC_{50} value. We repeated this task 3 times and calculated the standard deviation and found that our work was correct [22].

Molecular docking study

Molecular docking was used for the assessment of biological activities of wogonoside against the HMG-COA reductase activity. The enzyme structure 2.22 Å and X-ray diffraction method were obtained from the PDB database (http://www.rcsb.org/pdb) (PDB ID: 1HWK) [23]. The enzyme was subjected to some preparation steps before molecular docking calculations. At the first step, the addition of hydrogen molecules was performed. After that, the water molecules were removed from the structure, and a network of H-bonds was created using the optimization step. Finally, the minimization step was applied to the structure utilizing the OPLS3e force field. This

preparation process was performed with the protein preparation module of the Schrödinger suite [24]. The prepared protein was then assessed for the prediction of the binding site, and the active sites of the protein were predicted using SiteMap of Schrödinger [25]. The structure of wogonoside as an SDF file was downloaded from the PubChem database and then prepared with the LigPrep module of Schrödinger [26] to produce the correct molecular geometric and suitable protonation states. A grid box of 20 × 20 × 20 (Å3) was generated around the first active site, and the molecular docking was conducted using Glide of the Schrödinger suite.

Anti-human colon carcinoma properties of wogonoside

The process of the controlled culture of prokaryotic or eukaryotic cells in a filtered or unfiltered flask or cell culture plate by a suitable culture medium. This term is mostly used for culturing multicellular cells. Special culture media are used to culture cells. The cells are usually cultured at 37°C in equipment such as CO_2 incubators. Cell culture should be performed under aseptic (disinfected) conditions because the growth of these cells is much slower than the growth of bacteria and yeasts and there is a possibility of contamination of the culture medium. Antibiotics such as penicillin, streptomycin, or gentamicin are sometimes used to stop the growth of bacteria. In order for cells to proliferate well in culture medium, their density in culture medium must be low. For this purpose, the cells should be passed to the fresh culture medium from time to time. One of the goals of cell culture is to study cells in terms of how they grow, their nutritional needs, and the reasons they stop growing, each of which can have a profound effect on the morphology of the cells we see under a microscope. Therefore, to study the cell growth cycle, develop methods to control the growth of cancer cells and modulate the expression of genes, it is necessary to cultivate these cells in the external environment [27]. With the help of cell culture, cells can be prepared that are in different stages of differentiation and can be differentiated into other cells with the help of hormones and growth factors. With the help of cell culture, homogeneous cells can be prepared and intracellular activities such as DNA replication, DNA transcription synthesis, RNA and protein synthesis and other details related to metabolism can be studied. It is also possible to examine the subsequent events and intracellular currents, such as the displacement of these complexes, the type of intracellular messages, and how the messages are transmitted, after connecting different molecules to the corresponding membrane receptor. The cultured cells can be stored frozen at very low temperatures. Such conditions will maintain the growth rate or genetic composition of these cells and they can be thawed and used again at the appropriate time. This prevents the aging of cells, while it is currently not possible to prevent the aging of animals. When working with laboratory animals, systemic changes due to the effect of the animal's natural homeostasis or the stress of the experiments on the results should be considered, while the use of cell culture eliminates this problem. In addition, standardizing laboratory tests is easier and more practical than tests on living organisms. In laboratory environments, it is much easier to control the physical and chemical factors in the living environment of cells, including acidity, heat, osmotic pressure, and the pressure of gases such as oxygen and carbon dioxide. Cells that are taken directly from the individual are known as primer cells and have a limited lifespan. Most cells have a limited lifespan, except for those taken from a tumor. An immortal cell line can proliferate indefinitely by creating a random or targeted mutation (such as artificial gene expression) and be established as a representative of specific cell types [27].

In this research, we used human umbilical vein endothelial cells (HUVECs) to determine the cytotoxic potential of wogonoside using MTT. Also, the *in vitro* anti-colon carcinoma effects of biologically synthesized wogonoside against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines were evaluated.

In the present study, the cells were cultured in medium (RPMI1640 = Roswell Park MemoryL Institute1640) with 10% FBS combined with penicillin and streptomycin antibiotics in an incubator containing 5% CO_2 in a flask (T25). After three passages for purification, the cells were used to perform the next steps. Cell count and the number of viable cells were performed with a hemocytometer slide using trypan blue. Evaluation of the cytotoxic effect of wogonoside was performed by the modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric test. In this method, MTT, which is yellow, is converted to insoluble and formazan purple dye by the dehydrogenase enzymes in the mitochondria of active cells. The adsorption of this compound can be measured after dissolving at 570–540 nm. After 2 days and covering the flask bottom with cells, the cell layer adhering to the flask bottom was isolated enzymatically using trypsin-EDTA (5%) (tetraacetic acid ethylenediamine), and after transfer to sterile test tubes, it was centrifuged at 2000 rpm for 10 min. The cells were then resuspended in a fresh culture medium with the help of a Pasteur pipette and a cell suspension (10^6 ml/µg) was prepared from them. 40 µl of this cell sus-

pension (equivalent to $10^4 \times 4$ cells) was poured into 96-well plate flat-bottomed wells (for cell culture). Then the final volume of each well with 10% FBS medium reached 200 µl. The first row containing the cell suspension was considered as a negative control (control). After incubation for 18–24 h to remove cells from the stress caused by trypsinization, the supernatant was removed slowly and carefully. A new medium was added to all rows with different concentrations of wogonoside (only a new medium was added to the negative and positive control rows), so that the diluted wogonoside with concentrations of 1–1000 µg/ml was added to the third to sixth rows, respectively, then the plate was incubated in CO_2 for 48, 24 and 72 h. After the incubation time, the plate was taken out of the incubator and 20 µl of MTT (Sigma) was added to all wells, and incubated for 3 h. The supernatant was then gently removed and 100 µl of DMSO was added to the wells and pipetted to dissolve the formazan crystals. The amount of light absorption (OD) according to the intensity of the blue color of formazan at 540 nm was read by an ELISA reader. To convert OD to the percentage of living cells, the following formula was used and the percentage of living cells at each concentration was calculated after 48, 24 and 72 h [27]: Cell viability (%) = (Sample A/Control A) × 100.

The concentration of the tested compounds that reduced the percentage of living cells by half was considered as the IC_{50} (the half maximal inhibitory concentration) [27].

Statistical analysis

At least three independent replications were performed for each data set and the result was presented as mean ± SD. Statistical analysis of data was done with SPSS software version 22 and one-way ANOVA and Duncan tests. Significance was considered at the level of $p \leq 0.05$.

Results and discussion

Enzyme result

HMG-CoA reductase inhibitor compounds are mostly prescribed for lipid lowering to treat hypercholesterolemia. Additionally, they are well tolerated; their pharmacological interactions with other drugs can give rise to some inverse clinical consequences [28, 29]. The two main classes of these enzyme inhibitors are competitive and oxysterol inhibitors [30]. Oxysterol molecules, which are oxidative factors of cholesterol, can play an important role in the regulation of enzyme activity in mammalian cells. Additionally, oxysterols exhibit a diversity of effects independent of the inhibition of this important enzyme. In our study, the inhibition result of wogonoside on HMG-CoA reductase showed a lower value of 28.70 ±4.73.

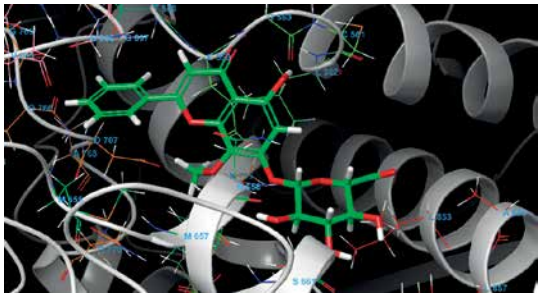

Figure 1. Docking pose of wogonoside among HMG-COA reductase residues

Docking results

The biological activities of wogonoside in the proximity of HMG-COA reductase were investigated using a molecular docking study. The docking pose of wogonoside among the residues of the enzyme is shown in Figure 1, and Figure 2 shows the interactions between this chemical compound and HMG-COA reductase. It is apparent that the ligand has created several hydrophobic contacts and hydrogen bonds with the enzyme residues. The residues with hydrogen bonds are Gly560 and Arg590. For the hydrogen bond of Gly560, this residue is a hydrogen bond donor, while in the case of Arg590, the amino acid is a hydrogen bond acceptor. It shows that wogonoside can undergo interactions with biomolecules in both a hydrogen bond acceptor and a hydrogen bond donor manner. There are also thirteen hydrophobic contacts between wogonoside and the enzyme, which could lead to adequate inhibitory activity for the compound. One of the residues with a hydrophobic contact is Glu559, which has previously been reported to be one of the crucial members of catalytic residues of the enzyme [31]. Another residue with a hydrophobic contact is Asp767, and like Glu559, this amino acid is among the catalytic residues of the enzyme [32]. These hydrophobic contacts can increase the inhibitory activity of wogonoside against HMG-COA reductase. Various parameters obtained from the docking calculations are presented in Table I. As can be seen, the compound has a remarkable binding affinity to the enzyme with a docking score of –7.582 kcal/mol. The other parameters show the characteristics of the ligand-enzyme complex. For instance, Glide Ligand Efficiency shows the binding energy between the molecule and the binding partner. Glide Evdw and Glide Ecoul present the Van der Waals and Coulomb energy, respectively. The value of interaction pose is numerically calculated with Glide Emodel. The position of the first active site in the enzyme structure is presented in Figure 3. Two essential parameters for the evaluation of an

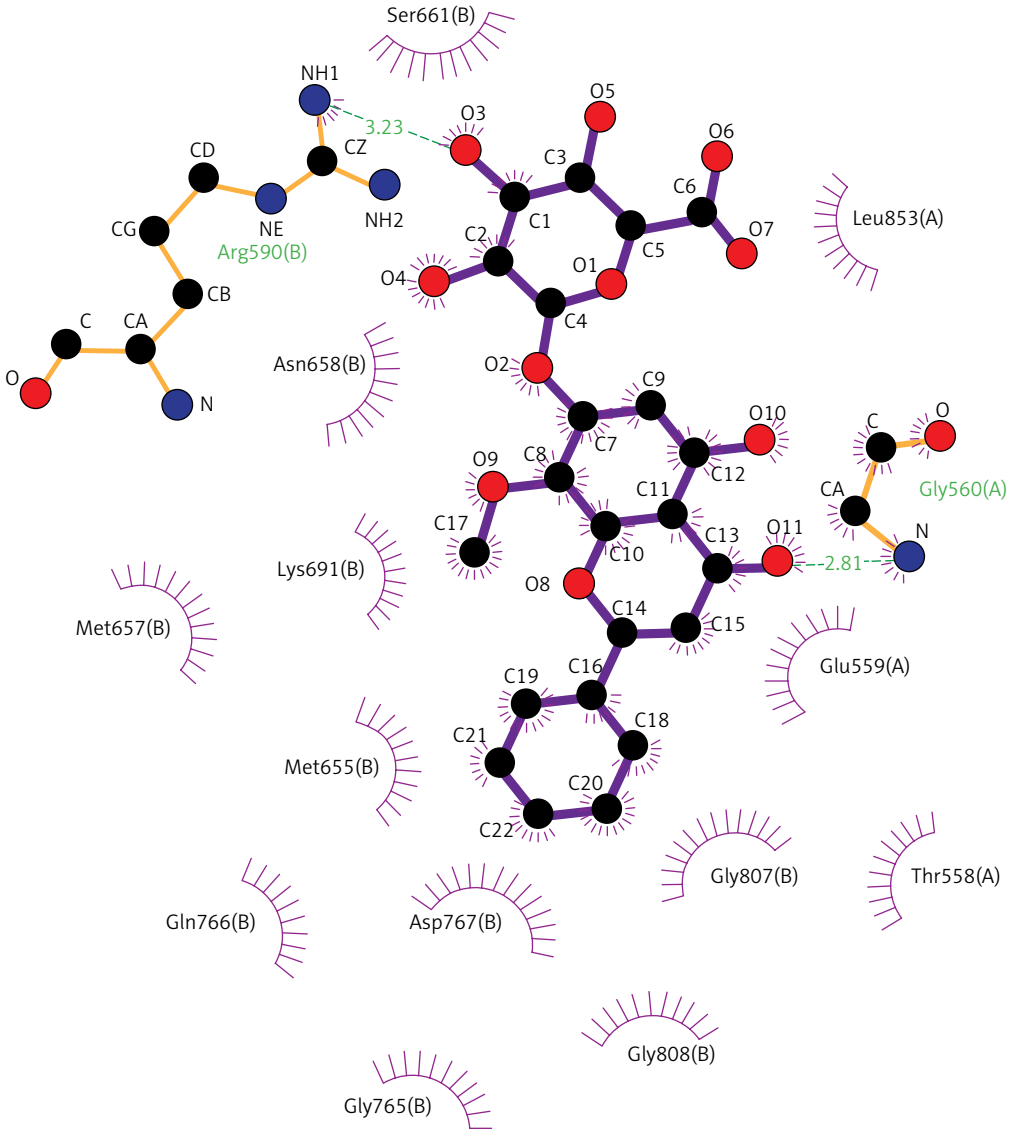

Figure 2. Interactions of wogonoside and HMG-COA reductase. Green dashed lines indicate hydrogen bonds, and semicircles show hydrophobic contacts

active site are Dscore and site score. Dscore for the first active site is 1.040, and the site score is 1.072. Both of the scores are greater than one, which shows the reliability of the predicted active site. These scores also indicate the suitable drugability of this site. This drugability proves that this site is able to bind to the drug-like molecules tightly. In Figure 3, the blue color shows the hydrogen bond donor regions and the red color indicates the hydrogen bond acceptor areas. The hydrophobic regions are presented with yellow color. The residues that are engaged in the first active site are presented in Table II. In general, wogonoside is able to create several hydrophobic contacts and hydrogen bonds with various amino acids of the HMG-COA reductase in both hydrogen bond donor areas and hydrogen bond acceptor regions. In addition to the experimental studies, these results can also prove that wogonoside could be considered as a potential inhibitor for this enzyme.

Table I. Parameters obtained from the molecular docking calculations

Parameters	HMG-CoA reductase
IC_{50} [mM]	28.70
Docking score [kcal/mol]	–7.582
Glide ligand efficiency [kcal/mol]	–0.230
Glide Ecoul [kcal/mol]	–8.768
Glide Evdw [kcal/mol]	–38.848
Glide Emodel [kcal/mol]	–59.335
Glide energy [kcal/mol]	–47.615

Anti-cancer part

Cancer is recognized as one of the leading

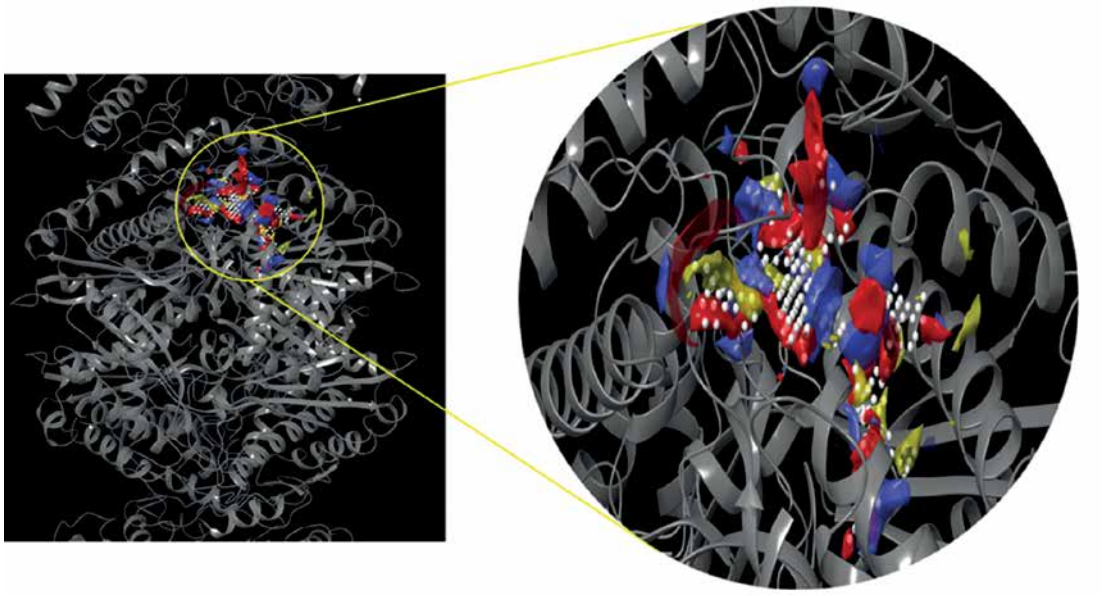

Figure 3. Position of the first active site of HMG-COA reductase

Table II. Residues of the first active site with Dscore of 1.040 and site score of 1.072

Chain	The number of predicted residues of the first active site
A	Residues: 536,556,557,558,559,560,561,562,565,735,751,752,755,758,759,762,853,857
B	Residues: 525,526,590,628,652,653,654,655,656,657,658,659,683,684,686,688,689,690, 691,692,735,762,765,766,767,768,769,770,801,802,803,805,806,807,808,809,810,814,826,831

causes of death in today's society, and several drugs have been introduced to treat this disease, but most common cancers are not yet controllable and this disease imposes huge costs on the patient and society. The main factor in the development and progression of cancer has not yet been precisely identified; however, the available data suggest that metabolic disorders in the tissue and immune disorders may be involved in the development and exacerbation of this disease. In addition, metabolic disorders in the production and excretion of oxygen free radicals are important factors affecting cancer cells. Free radicals are destructive compounds that are produced as a by-product by the body's chemical reactions and are destroyed by the body's defense system and enzyme system and antioxidants. However, in cases where the body's metabolic disorders and the production of free radicals are high and they are not destroyed by the neutralizing system, due to their instability, these compounds have a strong tendency to react with a variety of molecules in the body. It is estimated that each cell in the human body is exposed to free radicals 10,000 times a day and DNA strands 5,000 times a day. Damage to cell components includes proteins (genetic disorder), fats (lipid oxidation), and cell membranes (permeability disorder); if the damage is not repaired, it leads to disruption of the chemical reaction and normal proteinization of the cell and the formation of harmful compounds and sometimes cancer cells in the body. It is reported that thousands of cancer cells are produced daily in the human body, which are killed by the body's defense system. In some cases, due to dysfunction of the above systems, cancer cells proliferate and conditions for cancer develop in different tissues. According to the above, antioxidants play a vital role in preventing disorders caused by the effects of free radicals and thus the prevention and treatment of cancer. Antioxidants are a wide range of molecular compounds with complex properties that combine with and neutralize free radicals. More than 60,000 types of molecular antioxidants have been identified so far. Antioxidants can be effective in three known ways to prevent and treat cancer: 1. destruction of free radicals; 2. strengthening the immune system to destroy cancer cells; 3. preventing the adhesion of cancer cells to other cells and preventing their proliferation.

In this study, the cells treated with different concentrations of the wogonoside were assessed by MTT assay for 48 h regarding the cytotoxic properties on normal (HUVEC) and colon malignancy cell lines, i.e. GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM (Figures 4 and 5).

The viability of malignant colon cell lines decreased dose dependently in the presence of wogonoside. The IC_{50} values of wogonoside were 105, 198, 173, 382, 71, and 183 µg/ml against

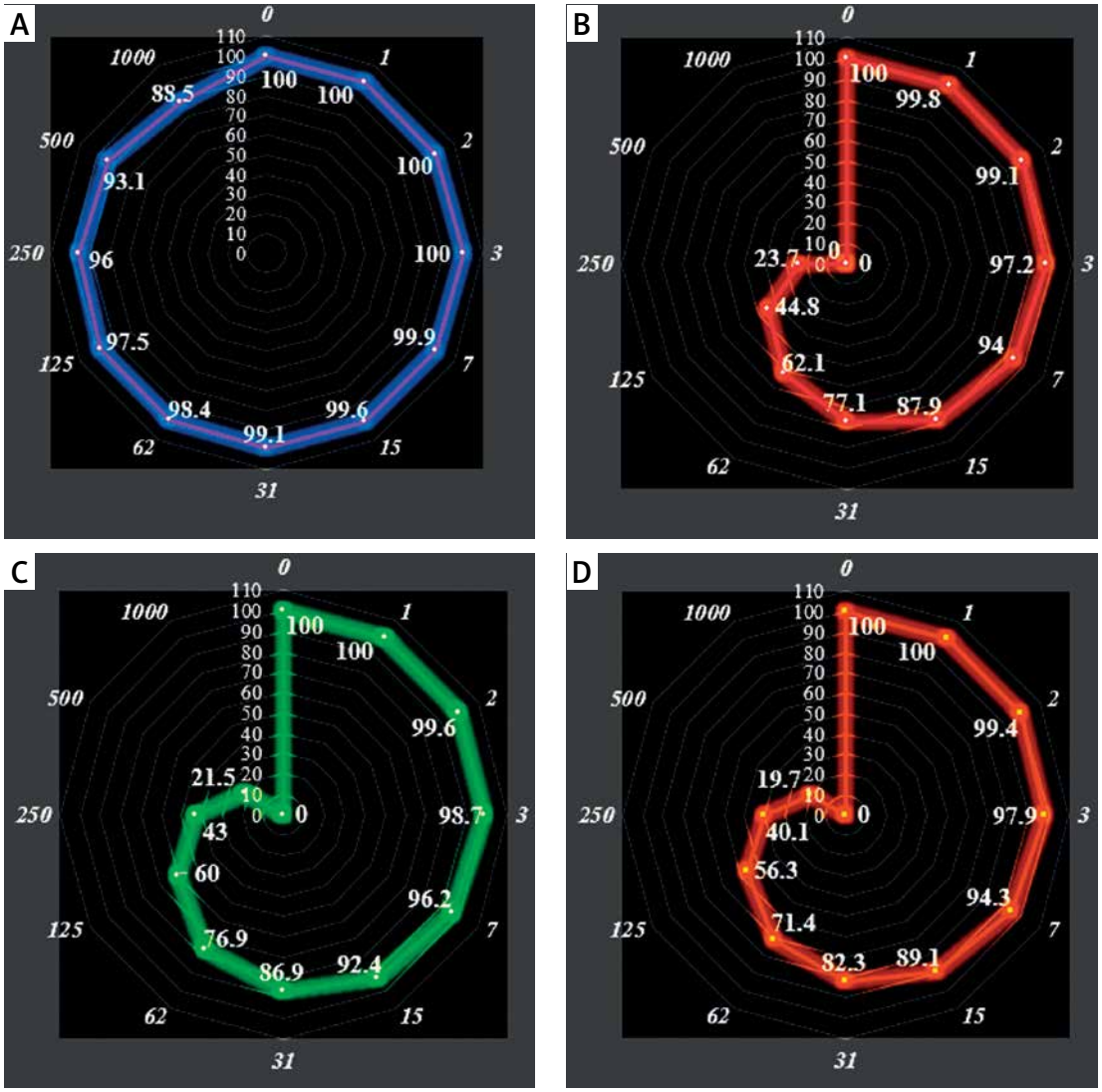

Figure 4. Anti-human colon carcinoma properties (cell viability (%)) of wogonoside (concentrations of 0–1000 µg/ml) against normal (HUVEC: **A**) and human colon carcinoma (GP5d (**B**), MDST8 (**C**), and HCA-46 (**D**)) cell lines. The numbers indicate the percent of cell viability at the concentrations of 0–1000 µg/ml of wogonoside against several human colon carcinoma cell lines

GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cell lines, respectively (Table III).

The absorbance rate was evaluated at 570 nm, which represented viability on the normal cell line (HUVEC) even up to 1000 µg/ml for wogonoside (Table III and Figures 4 and 5).

In conclusion, theoretical approaches can give valuable information about the details of the experimental study. It can help biologists to understand the mechanisms of biological activities. Molecular docking is one of the popular methods among these approaches. In this study, the results of docking calculation revealed that wogonoside can be considered as a promising inhibitor for HMG-COA reductase with a considerable binding affinity to the binding site of the enzyme. Due to the advantages of molecules such as the ability to carry drugs, reduce toxicity, controlled drug release and specific drug delivery to the target tissue, these structures have been able to attract the attention of many researchers. As a result of these features, biotechnology has great potential for cancer treatment that can move from the research laboratory to the patient's bedside. One possible concern that limits the administration of some molecules in treating cancer is their toxicity, which needs further investigation. However, molecule-based cancer therapies will continue to be developed to improve treatment outcomes. The viability of malignant colon cell lines decreased dose dependently in the presence of wogonoside. The IC_{50} values of wogonoside were 105, 198, 173, 382, 71, and 183 µg/ml against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cell lines, respectively. After clinical study, wogonoside can be utilized as an efficient drug in the treatment of colon carcinoma in humans.

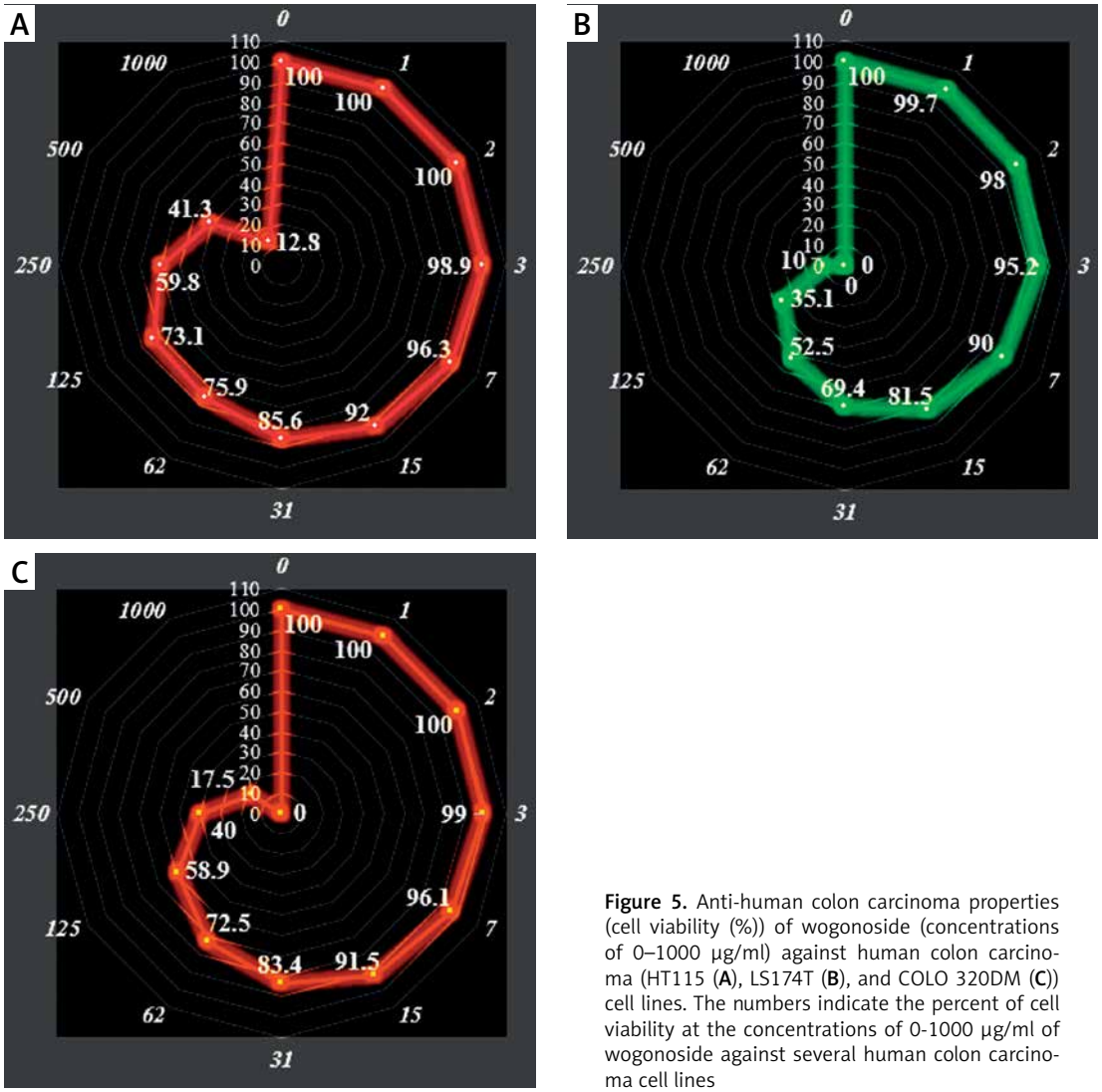

Figure 5. Anti-human colon carcinoma properties (cell viability (%)) of wogonoside (concentrations of 0–1000 µg/ml) against human colon carcinoma (HT115 (**A**), LS174T (**B**), and COLO 320DM (**C**)) cell lines. The numbers indicate the percent of cell viability at the concentrations of 0-1000 µg/ml of wogonoside against several human colon carcinoma cell lines

Table III. IC_{50} of wogonoside in the anti-human colon carcinoma test

	HUVEC	GP5d	MDST8	HCA-46	HT115	LS174T	COLO 320DM
IC_{50} [µg/ml]	–	105 ±0	198 ±0	173 ±0	382 ±0	71 ±0	183 ±0

Conflict of interest

The authors declare no conflict of interest.

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