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Different cellular responses to Artemisinin in cancer and normal cells: evaluation of cytotoxic, genotoxic and antioxidant effects with *in silico* molecular docking

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Artemisinin is a natural compound that exhibits cytotoxic effects, particularly on cancer cells. This study investigated the effects of Artemisinin on the cytotoxic, genotoxic, and antioxidant enzyme activities of the human breast cancer cell line (MDA-MB-231) and the normal fibroblast cell line (L929) with molecular docking. Cells were treated with Artemisinin at concentrations of 12.5 µM, 25 µM, and 50 µM. Cell viability was assessed using the MTT assay, DNA damage using the comet assay, and antioxidant enzyme activities (SOD, CAT, GPx) using the ELISA method. MTT analysis revealed a dose-dependent decrease in viability in MDA-MB-231 cells, with an LD_{50} value determined to be 25 µM. In L929 cells, viability was preserved up to 25 µM, but a decrease was observed at 50 µM. Comet assay results showed that DNA damage increased in a dose-dependent manner in MDA-MB-231 cells, while in L929 cells, significant damage occurred only at 50 µM. Antioxidant enzyme analyses revealed significant decreases in SOD, CAT, and GPx activities in MDA-MB-231 cells, while no changes were observed in L929 cells at 25 µM, and enzyme activities decreased at 50 µM. Artemisinin produces cytotoxic and genotoxic effects in cancer cells with showed molecular docking parameter, while exhibiting lower toxicity at low concentrations in normal cells.

Keywords: Artemisinin, Breast Cancer, L929 cell line, Molecular docking, MTT Test, Antioxidant Test

Biogenic compounds derived from natural sources play an important role in drug development studies due to their chemical diversity and biological activities. In particular, the emergence of drug resistance and side effects in tumour chemotherapy has accelerated research into natural compounds. Artemisinin, a sesquiterpene lactone isolated from the *Artemisia annua* plant, initially attracted attention for its potent antimalarial effects. Today, there is evidence that it is effective against many different infectious diseases and various types of cancer[1,2]. Artemisinin and its derivatives also demonstrate therapeutic efficacy against drug-resistant Plasmodium strains, and potential antitumour activity has been reported in malignant cell lines such as HIV, hepatitis B, leishmaniasis and breast cancer[3]. However, the low yield of artemisinin obtained from the *A. annua* plant (< 1%) is one of the key factors limiting production despite increasing global demand. Therefore, elucidating the biosynthetic pathway of artemisinin and identifying high-yield chemotypes is of great importance for both microbial engineering studies and cell-based therapeutic applications[4].

Cancer is a complex disease resulting from disorders in the regulation of the cell cycle, leading to uncontrolled cell proliferation, and is influenced by both genetic and environmental factors[5,6]. Breast cancer is the most common malignancy in women, with an increasing incidence worldwide, and its metastatic forms are particularly associated with high mortality[7,8]. In this context, the MDA-MB-231 cell line is widely used in metastatic breast cancer models due to its estrogen receptor negativity and high aggressiveness[9,10]. In contrast, the L929 fibroblast cell line is a model considered representative of normal cells in various toxicity and cell viability studies[11,12]. Therefore, the comparative analysis of both cell lines constitutes an important research approach for evaluating the selective efficacy of anticancer compounds.

Oxidative stress arises when the production of reactive oxygen species (ROS) exceeds the cell's antioxidant defence capacity and can lead to cell death by causing damage to biomolecules at high levels[13]. However, ROS play a regulatory role in many fundamental biological processes at physiological levels, such as cell signalling, immune

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response, and maintenance of redox homeostasis. When the balance between pro-oxidant and antioxidant systems is slightly disrupted, cells develop adaptive responses, but a serious disruption of this balance leads to irreversible molecular damage and apoptosis. Excessive ROS production originating from cellular mechanisms such as NAD(P)H oxidase and the mitochondrial electron transport chain has been reported to play a role in the development of various pathological conditions, including cancer, atherosclerosis, diabetes mellitus, neurodegenerative diseases, and the ageing process[14].

Organisms possess advanced antioxidant defence systems against the harmful effects of free radicals. These systems reduce oxidative damage by either directly capturing reactive oxygen species (ROS) or eliminating chain initiators. Antioxidant molecules maintain cellular redox balance through various mechanisms, such as electron transfer, metal ion chelation, and regulation of gene expression[15,16]. ROS, produced in low amounts at physiological levels, are essential for signal transduction and cell proliferation, but excessive production causes serious damage to nucleic acids, lipids, and proteins[17]. Therefore, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) play a fundamental protective role against oxidative stress by working harmoniously in intracellular and extracellular environments[18].

Single-cell gel electrophoresis (comet assay), first described by Ostling and Johanson (1984) and later refined by Singh and Olive, is a widely used sensitive method for assessing DNA damage caused by genotoxic agents. In this test, each cell forms a comet-like image that is divided into two sections after electrophoresis: the 'head' and the 'tail'. Undamaged, high molecular weight DNA remains in the cell head, while damaged, low molecular weight DNA fragments migrate towards the tail. The degree of genotoxicity is quantitatively determined by parameters such as tail length, tail DNA percentage, and tail moment (Tail Moment = tail length × tail DNA percentage)[19].

Materials and Methods

Cell culture and MTT assay

The MDA-MB-231 and L929 cell lines were obtained from the American Type Culture Collection (ATCC, Global Bioresource Center). MDA-MB-231 cells were cultured in DMEM-F12 medium, while L929 cells were maintained in RPMI medium. Both cell lines were incubated under standard culture conditions at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

Cells were seeded into 96-well plates at a density of 1×10^5 cells per well and incubated for 24 h. Once the cell confluence reached approximately 50–60%, artemisinin was applied at concentrations of 12.5, 25, and 50 μM prepared in culture medium. The control group received only the culture medium. After 24 h of treatment, 50 μL of MTT solution was added to each well and incubated for an additional 4 h. Subsequently, 100 μL of PBS was added and left for 30 min, after which absorbance was measured at 570 nm to determine the LD_{50} value of artemisinin, which was calculated as 25 μM.

DNA damage analysis with the comet assay

MDA-MB-231 and L929 cells were detached from the surface using trypsin-EDTA and seeded into 6-well plates at a density of 2×10^5 cells per well. The cells were incubated for 24 hours, after which Artemisinin was applied at concentrations of 25 μM and 50 μM. After 24 hours of incubation, the cells were diluted with PBS and mixed with low-melting-point agarose (LMA). The mixture was spread onto pre-coated slides with high-melting-point agarose (NMA) and incubated at +4 °C. After the agarose solidified, the slides were removed, treated with a lysis solution, and subjected to electrophoresis at 200 V in an electrophoresis tank. The prepared samples were stored in distilled water, dried, and stained with 0.5% EtBr. DNA damage, tail length, % DNA, and tail moment parameters were evaluated under a fluorescence microscope.

Determination of SOD, CAT and GPx enzyme activity

Confluent MDA-MB-231 and L929 cells were detached from the surface with trypsin-EDTA, counted and distributed equally into new flasks. The cells were separated into control and Artemisinin groups at 25 μM and 50 μM and left to incubate for 24 hours. After washing the cells with PBS, they were centrifuged and SOD, CAT, and GPx activities were measured using an ELISA kit. Standards and samples were added to the plate wells, incubated with the relevant antibodies and reagents, washed, and absorbance values were determined at 450 nm using a spectrophotometer.

Molecular docking analysis

AutoDock Vina was used for molecular docking simulations to study the binding profile of artemisinin

ligand with the JAK2 protein. This protein has been identified as an important target in the MDA-MB-231 human breast cancer cell line.

An increase in the JAK2 protein plays a role in the proliferation of cancer cells. This is because increased JAK2 levels lead to increased PD-L1 levels. The increase in PD-L1 cells allows cancer cells to protect themselves from the immune system. Consequently, increased JAK2 and PD-L1 levels allow cancer cells to protect themselves from the immune system. When JAK2 is inhibited, the increase in PD-L1 is completely blocked. Therefore, JAK2 protein is an important therapeutic target for triple-negative breast cancer[20].

Heteroatoms were removed prior to the binding simulations. Following the removal of heteroatoms, polar hydrogens were added to the three-dimensional model of the 3KRR protein, and the active site residues were identified as ALA880, ARG980, ASN981, ASP994, GLU930, GLU987, GLY856, GLY858, GLY935, GLY993, LEU855, LEU932, LEU983, MET929, PRO933, TYR931 and TYR934.

The docking simulations used a grid box with dimensions of 40 × 40 × 40 $Å^3$, centered at x = 13.247, y = 7.640 and z = 4.445. The spacing between the grid points was set to 0.375 Å.

Ligand preparation

The three-dimensional ligand structure was first optimised using Avogadro2 software to ensure appropriate geometry and energy minimisation. The ligand was then converted into PDB format to make it compatible with molecular docking studies. To verify molecular quality and visually inspect the structures, both the ligands and receptor proteins were visualised using Discovery Studio Visualizer 2025 prior to the docking simulations to ensure structural integrity[21].

Results

MTT test analysis results

Artemisinin was found to reduce cell viability in MDA-MB-231 cells at increasing doses, while preserving cell viability in L929 Fibroblast cells at doses of 12.5 µM and 25 µM. However, at a dose of 50 µM, it was found to reduce cell viability by half. The LD_{50} value of MDA-MB-231 cells, i.e. the concentration at which 50% of the cells died, was determined to be 25 µM. The averages of the absorbance values obtained were subtracted from the blank values and normalized (Fig. 1 and Fig. 2).

Comet Assay Analysis Results

Three different concentrations of Artemisinin (0, 25 and 50 µM) were applied to preparations obtained from MDA-MB-231 and L929 cell lines, and the comet assay method was used for genotoxicity measurements. The mean values of DNA damage parameters, such as % DNA, tail length, and tail moment, were determined for cells in preparations at each concentration. The comet results obtained for the concentrations studied are presented in Table 1 for the MDA-MB-231 and L929 cell lines. These results are

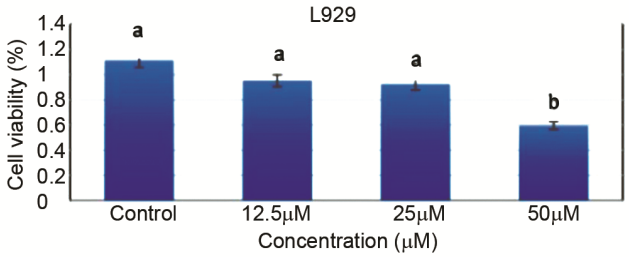

Fig. 1 — Effects of artemisinin on L929 cell viability.

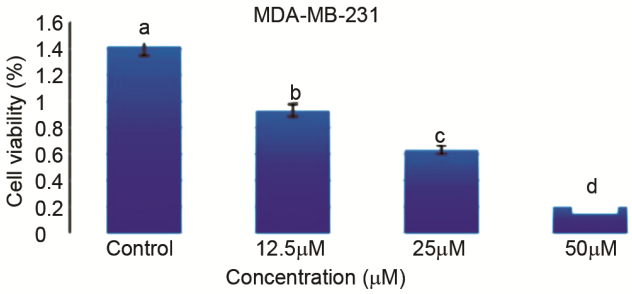

Fig. 2 — Effects of artemisinin on MDA-MB 231 cell viability.

Table 1 — Average values of DNA damage (±SD) percentage, tail length and tail moment in control and treatment groups in MDA-MB-231 cells.

Groups	Tail DNA % (±SD)	Tail Length (±SD)	Tail Moment (±SD)
MDA-MB-231 control	12.57±2.36	2.011±0.02	0.25±0.004
MDA-MB-231 25 µM	38.13±6.45	14.344±3.22	5.46±0.20
MDA-MB-231 50 µM	98.88±10.93	47.421±1.32	46.88±0.14
L929 control	11.7±2.4	2.04±0.03	0.23±0.00072
L929 25 µM	17.11±5.11	9.029±1.65	1.544±0.08
L929 50 µM	39.23±5.89	22.1±2.8	8.66±0.16

shown in Fig. 3, together with images obtained under a fluorescence microscope. Compared to the control group, it was determined that DNA damage increased with increasing concentration in MDA-MB-231 cells. In L929 cells, no significant change was observed in the group treated with 25 µM Artemisinin, while an increase in DNA damage was observed in the group treated with 50 µM Artemisinin.

Evaluation of SOD enzyme activity

Compared to the L929 cell line, significant changes were observed in the MDA-MB-231 cell line in terms of SOD enzyme activity between the control group and the groups administered increasing doses of Artemisinin, and a decrease was detected in the enzyme activity examined. In L929, no statistically significant change was detected in the 25 µM application group, but a decrease in enzymatic activity was determined in the group administered 50 µM Artemisinin (Fig. 4).

Evaluation of CAT enzyme activity

Compared to the L929 cell line, a significant decrease in enzymatic activity was detected in the MDA-MB-231 cell line treated with increasing doses of Artemisinin in terms of CAT enzyme activity. In L929, no statistically significant change was detected in the 25 µM application group, but a decrease in enzymatic activity was determined in the group treated with 50 µM Artemisinin (Fig. 5).

Evaluation of GPx enzyme activity

When evaluating GPx enzyme activity in L929 cells with increasing doses of Artemisinin, no statistically significant difference was found between the control and the 25 µM application group. However, a decrease in enzymatic activity was determined in the group treated with 50 µM Artemisinin. In MDA-MB-231 cells, a significant decrease was observed with increasing doses (Fig. 6).

Molecular Docking Study

Developing and discovering new drugs remains a challenging task for researchers in the pharmaceutical industry, requiring a significant amount of time and substantial financial investment. When it comes to developing new cancer therapeutics using traditional pharmaceutical approaches, this problem is particularly problematic because it results in a significant waste of time and resources. Therefore, utilising modern computational methods and novel technologies could facilitate the discovery of new molecules with significant therapeutic potential, potentially transforming the treatment paradigm for numerous diseases.

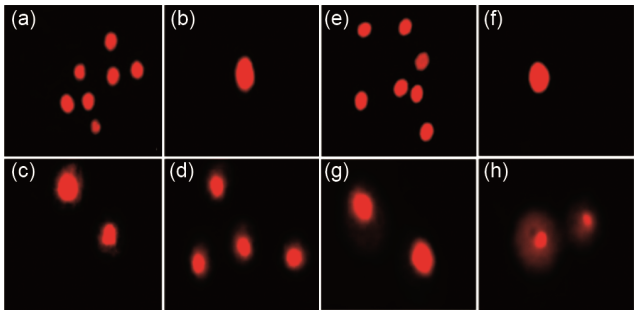

Fig. 3 — Comet images captured under a fluorescence microscope. L929 cell line control group (a, b), L929 cell line group treated with 25 µM Artemisinin (c), L929 cell line group treated with 50 µM Artemisinin (d). MDA-MB-231 cell line control group (e, f), MDA-MB-231 cell line treated with 25 µM Artemisinin group (g), MDA-MB-231 cell line treated with 50 µM Artemisinin group (h).

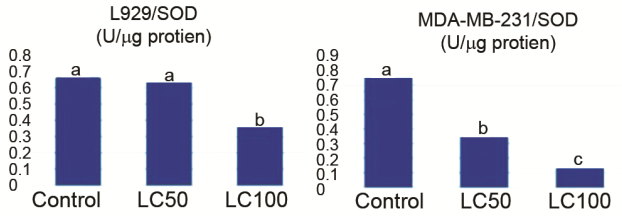

Fig. 4 — Comparison of SOD enzyme activities in MDA-MB 231 and L929 cell line control and treatment groups.

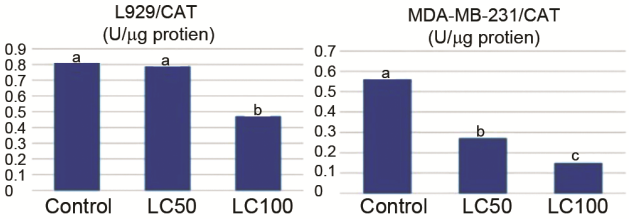

Fig. 5 — Comparison of CAT enzyme activities in MDA-MB 231 and L929 cell line control and treatment groups.

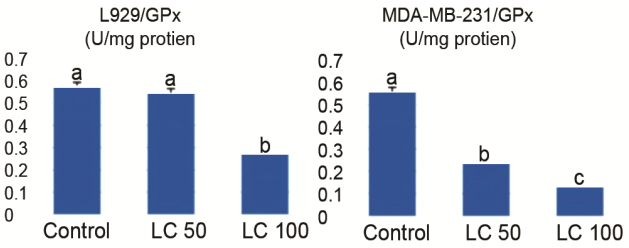

Fig. 6 — Comparison of GPx enzyme activities in MDA-MB 231 and L929 cell line control and treatment groups.

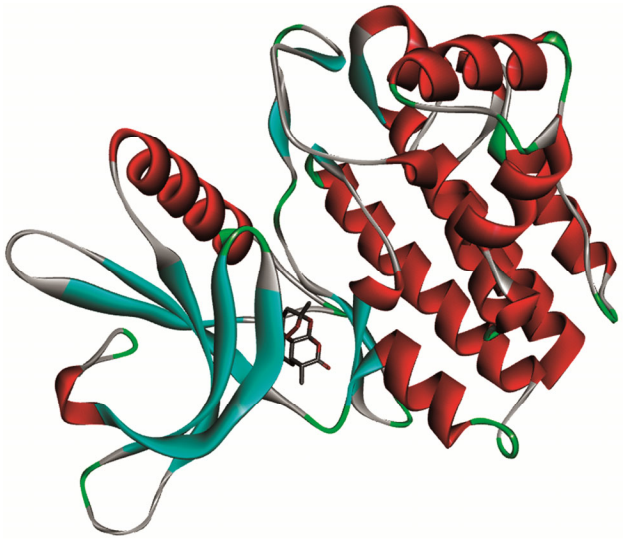

Fig. 7 — The binding position of Artemisinin within the active site of the JAK2 protein (PDB ID: 3KRR).

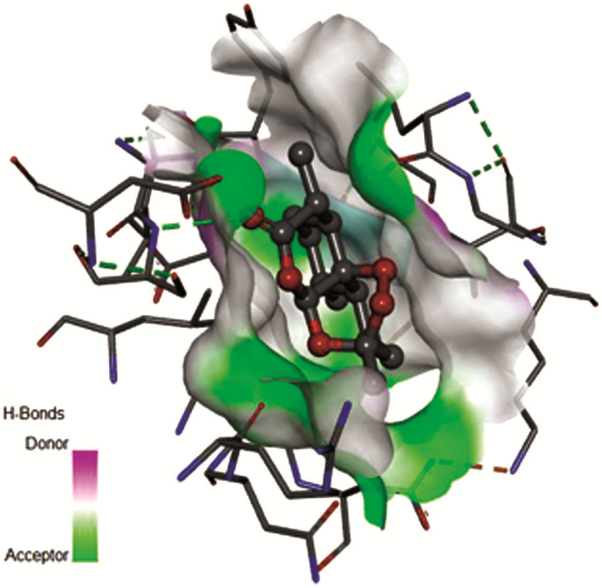

Fig. 8 — Three-dimensional docking of artemisinin and the JAK2 protein (3KRR and A chain).

The novel computational approach of molecular docking enables the prediction of the most stable binding conformations and the highest affinities between biological targets and their respective ligands. This approach extends beyond modeling complex systems, such as ligand-receptor binding, to define receptor conformational pliability and variations within and between receptor binding pockets.

The best binding energy calculated for Artemisinin was determined to be -8.8 kcal/mol. The number of hydrogen bonds was found to be 1. The inhibition value (Ki value) was found to be 0.35 mM. The

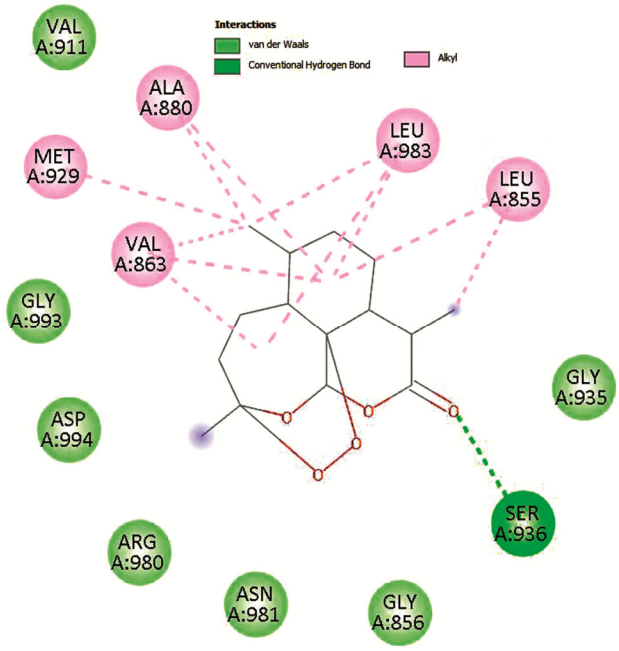

Fig. 9 — Two-dimensional docking of artemisinin and the JAK2 protein (3KRR and A chain).

binding site of the ligand in the protein is shown in Fig 7. Three-dimensional and two-dimensional structures of the interactions of the Artemisinin ligand with the JAK2 protein are shown in Fig 8 and 9. The artemisinin molecule, examined as a ligand, was evaluated to successfully inhibit JAK2, a protein in the MDA-MB231 cancer cell line. These findings suggest that Artemisinin may play a significant role in preventing or slowing breast cancer progression by inhibiting the proliferation of the MDA-MB231 cell line through JAK2 inhibition (Fig 7,8,9).

Discussion

In an era where scientists are seeking compounds with higher specificity for molecular and cellular targets, awareness of artemisinin is increasing due to its multifunctionality. These compounds are known to have important targets for several different diseases. Conceptually, modern projects in molecular pharmacology aim to increase treatment efficacy and reduce unwanted side effects by developing compounds that attack disease-related target molecules with high affinity. Natural products have developed various chemical mechanisms as chemical weapons in plants to protect against infections from bacteria, viruses, and other microorganisms. Multifunctional molecules are thought to be more versatile and therefore more successful than single-function molecules in protecting plants from

environmental damage. The artemisinin plant has been shown to be effective against cancer, and research supports the role of artemisinin as a protective agent[22].

At this point, artemisinin interacts outside the cell by forming a covalent bond with transferrin in the serum and is taken up into the cell via endocytosis[23]. Artemisinin causes cancer cell death in a very potent and selective manner due to its interaction with iron and the "haem" molecule. Research has shown that the antiproliferative effect of artemisinin in cancer cell cultures stems from its relationship with iron and the "haem" molecule[24]. It has been reported that the endoperoxide bond in the molecular structure of artemisinin can interact with Fe^{+2} and induce ferroptosis (iron-dependent cell death)[25]. A decrease in the activity of glutathione peroxidase 4 enzyme in the mevalonate pathway leads to increased lipid hydroperoxide formation, which in turn causes ferroptosis[26]. The functional group in the endoperoxide structure participates in the alkylation of proteins by binding to highly reactive carbon-centred radicals within the cell and forming reactive oxygen species. This mechanism suggests that intracellular iron plays an important role in the formation of both antiparasitic and anticancer properties[27]. Iron is essential for cell growth and proliferation; however, it also triggers cancer formation, tumour growth, and metastasis[28]. The effect of iron on the development of common cancer types such as non-small cell lung cancer and colorectal cancer has been demonstrated[29]. Cancer cells increase the transfer of ferritin in serum due to high Transferrin Receptor 1 (TfR1) levels, and this can be used as a parameter to distinguish between cells. The high selectivity of artemisinin and its derivatives is promising for research[30]. Studies have demonstrated that artemisinin has the potential to exhibit anticancer effects even in cell cultures resistant to chemotherapy and radiotherapy[31].

In anticancer research using *A. annua*, short-term bicalutamide and long-term *A. annua* tablets may cause regression in metastatic prostate cancer[32]. Artesunate exhibits antimetastatic, antitumour, and antiangiogenic effects in metastatic renal cell carcinoma cell cultures and confers sensitivity to sorafenib[33]. It causes a decrease in mitochondrial membrane potential and regression in tumours in *in vivo* pancreatic cancer xenograft models, and it stops tumour growth in gastric cancer[5]. It can prevent recurrence when used in combination with other chemotherapeutic agents in glioblastoma[34]. It exhibits cytotoxic and synergistic effects with 5-fluorouracil in liver cancers[35]. It can achieve complete remission in sarcomas and reduce recurrence in colorectal cancers. Artemisinin exhibits antiproliferative effects by downregulating cyclin-dependent kinase 2 and 4 receptors in endometrial cancer cell cultures and exhibits apoptotic effects in oral squamous cell carcinomas[36]. Artesunate can be used in combination with futemustine and dacarbazine in uveal melanoma and can halt cancer development. Dihydroartemisinin exhibits apoptotic effects in lung cancer cell cultures through mRNA suppression[37]. Studies have shown that artemisinin and its derivatives interact synergistically with other molecules; for example, they are more effective when used in combination with D3, vitamin C, curcumin, allicin, butyrate, and resveratrol[38].

In this thesis study, Artemisinin was applied to MDA-MB-231 and L929 cell lines, and cytotoxic effects were determined using the MTT viability assay. According to the test results, in the MDA-MB-231 cell line, cell viability decreased in increasing application groups of Artemisinin, and the LD_{50} value was determined as the concentration at which 50% of the cells died and was found to be 25 μM. In the L929 cell line, the LD_{50} dose was found to be 50 μM; however, for comparison purposes, the doses used for the MDA-MB-231 cell line were applied to the L929 cell line for the other parameters studied.

The comet assay is a quantitative measure used to assess DNA damage. In an alkaline environment, it detects single- and double-strand breaks, unstable DNA fragments, and deficiencies in repair sites, while also revealing DNA-protein and DNA-DNA cross-links[39]. In our study, Artemisinin applied to MDA-MB-231 cells at increasing doses caused DNA damage in the cells, but damage was only effective at high doses in L929 cells.

Another parameter used to determine the effect of Artemisinin on cell lines is the antioxidant system. The antioxidant system neutralises reactive oxygen species formed in the body, rendering them harmless. Researchers have examined antioxidant expression in non-small cell lung cancer tissue. The study showed that total SOD activity increased, CAT activity decreased, and GSH and GPx levels were similar in tumours compared to tumour-free lung tissue[40]. SOD is an enzymatic antioxidant that converts superoxide

radicals into O_2 and H_2O_2[41]. In this thesis study, a decrease in SOD activity was determined between the group treated with Artemisinin and the control group in MDA-MB-231 cells. When Artemisinin was administered at increasing doses, a statistically significant decrease in CAT enzyme activity was detected in the 25 µM and 50 µM treatment groups compared to the control group. In the L929 cell line, no statistically significant difference was found at the 25 µM dose, but it was found to decrease enzymatic activity at the 50 µM dose. This decrease in CAT enzyme activity suggests the possibility of H_2O_2 accumulation at high concentrations within the cell.

H_2O_2 is utilised by the active glutathione (GSH), an antioxidant enzyme known as GPx, to prevent the formation of hydroxyl radicals within the cell. Additionally, GSH facilitates the removal of oxidised and reduced free radicals by converting into H_2O and GSSG forms[42]. In this thesis study, it was observed that GPx enzyme activity was lower in the groups treated with Artemisinin in the MDA-MB-231 cell line compared to the control group. In the L929 cell line, no statistically significant difference was found at a dose of 25 µM, but it was determined that it reduced enzymatic activity at a dose of 50 µM.

The molecular docking analyses conducted with artemisinin, a potential anti-cancer drug, has demonstrated its significant affinity to bind to JAK2 protein, a key mediator of acute proliferative signalling in MDA-MB-231 Breast Cancer cell line, with a calculated binding energy of -8.8 kcal/mol and corresponding inhibition constant, support for the potential for Artemisinin to interfere with options within JAK2-mediated oncogenic pathways. Moreover, in the context of the experimental results, which showed dose-dependent cytotoxicity of Artemisinin to MDA-MB-231 Cells, the increased DNA damage, and significant reductions in antioxidant enzyme activity are supported by these findings. These findings further suggest that artemisinin modulates cancer cell responses through multiple complementary mechanisms. This is further evidenced by JAK2/PD-L1 axis well-known immune evasion mechanism of triple-negative breast cancer; Therefore, artemisinin's inhibition of JAK2 is suggested to inhibit not only proliferation of cancer cells, but also its potential to inhibit immune escape from tumors. Therefore, the cumulative potential of Artemisinin indicates that its anti-cancer potential will be more than just through oxidative stress and/or genotoxicity, but also due to its specific molecular targeting of JAK2 as a candidate for future therapeutic targeted drug development for cancer.

The results showed that Artemisinin caused cell death in live cells at increasing doses in the MDA-MB-231 and L929 cell lines using the MTT method and caused DNA damage using the comet assay. Regarding its effect on enzyme activities, a decrease in SOD, CAT, and GPx enzyme activities was observed. Similar results were obtained in the L929 cell line at the same doses, but they were not statistically significant.

Conclusion

The findings of this study demonstrate that Artemisinin exerts significant cytotoxic and genotoxic effects on MDA-MB-231 breast cancer cells, while showing relatively lower toxicity on normal L929 fibroblast cells at low concentrations. The dose-dependent reduction in cell viability and the increase in DNA damage in cancer cells indicate the potential of Artemisinin as a selective anticancer agent. Furthermore, the observed decrease in antioxidant enzyme activities (SOD, CAT, GPx) in MDA-MB-231 cells suggests that oxidative stress may be a key mechanism underlying its cytotoxic action. Importantly, the limited impact on normal cells at concentrations up to 25 µM highlights its therapeutic potential with a favorable safety profile. In parallel with these experimental observations, molecular docking analysis revealed that Artemisinin exhibits a stable and energetically favorable interaction with the JAK2 protein, a critical regulator of proliferative and immune-evasive signaling pathways in triple-negative breast cancer. This interaction supports the notion that Artemisinin may not only induce cytotoxicity through oxidative and genotoxic mechanisms but also interfere with JAK2-mediated oncogenic signaling at the molecular level. Importantly, the limited impact on normal cells at concentrations up to 25 µM highlights its therapeutic potential with a favorable safety profile. Overall, these results suggest that Artemisinin exerts its anticancer effects through a multi-targeted mode of action, combining cellular toxicity with specific molecular interactions, and may serve as a promising natural compound for targeted anticancer therapy, although further in vivo studies and clinical investigations are required to validate its efficacy and safety.

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Data availability:

The corresponding author will provide the datasets used and/or analyzed during the current work upon reasonable request.

Conflict of interest:

The authors declare no conflicts of interest.

Ethical Approval:

Not applicable.

Consent to Participate:

Not applicable.

Consent to Publish:

All authors have read and agreed to the published version of the manuscript.

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