Original Article

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Ivermectin Induces Oxidative Stress and DNA Damage in Breast Cancer Cells

İvermektin Meme Kanseri Hücrelerinde Oksidatif Stres ve DNA Hasarı Oluşturuyor

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ABSTRACT

Objective: Breast cancer (BC) remains to be one of the most diagnosed cancer types among women around the world. Drug repurposing is suggested to be a convenient alternative for drug development in cancer treatment. Ivermectin, the antiparasitic agent produced by the bacterium *Streptomyces avermitilis*, is currently being examined thoroughly in oncology and has begun to be seen as a potential drug candidate for BC therapy. However, studies are limited, and the exact anti-tumorigenic mechanism is not yet clarified in breast cancer.

Methods: For elucidating the molecular mechanisms of Ivermectin's potential anticancer effects, we have examined its *in vitro* effects on BC cells in terms of cell viability, intracellular ROS levels, glutathione levels, mitochondrial membrane potential, apoptosis, and DNA damage.

Results: Ivermectin induces apoptosis via oxidative stress and DNA damage in BC cells.

Conclusion: The *in vitro* mechanistic studies of promising anticancer agents for repurposing are essential guides for drug developers. For this purpose, ivermectin should be further studied as a drug candidate for its potential in the treatment of breast cancer.

Keywords: Ivermectin, breast cancer, drug repurposing, DNA damage, oxidative stress

ÖZ

Amaç: Meme kanseri (MK), tüm dünyada kadınlar arasında en sık teşhis edilen kanser türlerinden biri olmaya devam etmektedir. İlaçların yeniden hedeflendirilmesinin (repurposing) kanser tedavisinde ilaç geliştirme için uygun bir alternatif olduğu ileri sürülmektedir. *Streptomyces avermitilis* bakterisi tarafından üretilen anti-paraziter ajan olan İvermektin, şu anda onkolojide kapsamlı bir şekilde incelenmektedir ve MK tedavisi için potansiyel bir ilaç adayı olarak görülmeye başlanmıştır. Bununla birlikte, çalışmalar sınırlıdır ve MK'de kesin anti-tümörijenik mekanizma henüz açıklığa kavuşturulmamıştır.

Yöntemler: İvermektin'in potansiyel antikanser etkilerinin moleküler mekanizmalarını aydınlatmak için MK hücreleri üzerindeki *in vitro* etkilerini hücre canlılığı, hücre içi ROS seviyeleri, glutatyon seviyeleri, mitokondriyal membran potansiyeli, apoptoz ve DNA hasarı açısından inceledik.

Bulgular: İvermektin, MK hücrelerinde oksidatif stres ve DNA hasarı yoluyla apoptozu indükler.

Sonuç: Yeniden hedeflendirmek için umut verici anti-kanser ajanlarının *in vitro* mekanik çalışmaları, ilaç geliştiriciler için temel kılavuzlardır. İvermektin, bu amaçla MK tedavisindeki potansiyeli açısından bir ilaç adayı olarak incelenmeye devam edilmelidir.

Anahtar Sözcükler: İvermektin, meme kanseri, ilaç yeniden hedeflendirilmesi, DNA hasarı, oksidatif stres

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Introduction

Breast cancer (BC) remains to be one of the most diagnosed cancer types among women around the world (1). Prognosis is poor and survival is low in BC, although various treatment recommendations such as local and systemic therapy are offered along with new generation targeted therapies depending on the subtype of the cancer (2-4). BC can be categorized into four subtypes: Luminal A, luminal B, HER2-enriched, and basal-like (4,5). Luminal-like subtypes, which constitute 70% of patients, are hormone receptor-positive (estrogen and/or progesterone) but lack human epidermal growth factor 2 receptor (ERBB2; HER2). The HER2-enriched subtype constitutes 15-20% of patients with BC. And lastly, the basal-like subtype (15%), also called "triple-negative", lacks the receptors of estrogen, progesterone, and HER2 (4).

Drug repurposing is suggested to be a convenient alternative for drug development in cancer treatment (6). In this regard, ivermectin, the antiparasitic agent produced by the bacterium *Streptomyces avermitilis,* which has been used since 1987 (7), is currently being examined thoroughly in oncology and has been found to have *in vitro* antiproliferative/cytotoxic properties against cervical cancer, gastric cancer, ovarian cancer, colon cancer, glioblastoma, leukemia and melanoma (8-15).

Ivermectin has also been tested against different types of BC cell lines and thus began to be seen as a potential drug candidate for BC therapy (16-19). Although ivermectin was reported to block the PAK1/Akt Axis in BC cells and induce autophagy both *in vitro* and *in vivo* (16,17) the exact molecular mechanism of ivermectin's inhibitory effects is not yet clarified. For this reason, ivermectin was selected to be studied for elucidating the molecular mechanisms of its potential anticancer effects. We examined the drug's *in vitro* effects on BC cells in terms of cell viability, intracellular ROS levels, glutathione levels, mitochondrial membrane potential, apoptosis, and DNA damage. The promising effects of ivermectin against cancer cell lines make it suitable for further evaluation, suggesting its potential for taking place as an alternative in the treatment of breast cancer.

Methods

Chemicals

Ivermectin, fetal bovine serum (FBS), Leibovitz's L15 Medium, Eagle's Minimum Essential Medium (EMEM), dimethylsulfoxide (DMSO), 2'7'-dichlorodihydroflorescein-diacetate (H_2DCF-DA), penicillin/streptomycin (P/S), acridine orange (AO), ethidium bromide (EB), and 3,3'-dihexylocarbocyanine iodide [DiOC6 (3)] were purchased from Sigma-Aldrich (Seelze, Germany). The luminometric ATP kit was supplied by Promega (CellTiter-Glo® Luminescent Cell Viability Assay, Madison, USA). Other chemicals and solutions used in the experiments were all used in analytical quality.

Cell Culture

Human BC cell lines MCF7 American Type Culture Collection (ATCC® HTB-22™), MDA MB231 (ATCC® CRM HTB-26™), and healthy human breast epithelial cell line 184A1 (ATCC® CRL8798™), which were used in the experiments, were commercially obtained from the ATCC. MCF7 was cultured and cultivated in complete media containing 10% FBS, 1% P/S, and 0.01 mg/mL in EMEM at 37 °C in incubators containing 5% CO_2. MDA MB231 was cultured and cultivated in complete media containing 10% FBS and 1% P/S, Leibovitz's L15 Medium at 37 °C in incubators containing 5% CO_2. 184A1 was cultured and cultivated in base medium (MEBM), and the MEGM kit included 0.005 mg/mL transferrin and 1 ng/mL cholera toxin at 37 °C in incubators containing 5% CO_2. Cells were seeded $5x10^3$/well to 96 opaque well plates (black or white) for cytotoxicity, intracellular ROS, glutathione, and mitochondrial membrane potential levels. For genotoxicity and apoptosis, $50x10^3$ cells per well were seeded in 6-well plates. All experiments in the study were left for 24 h incubation for the adhesion of the cells, and another 24 h at 37 °C to examine the cytotoxic, genotoxic, and apoptotic, and other effects of the administered substances. Before all experiments, the viability of the cells was checked with trypan blue, and the number of cells was counted in the Thoma slide.

Cytotoxicity of Ivermectin

In the study, a luminometric ATP assay was used to analyze cytotoxicity. The commercially purchased CellTiter-Glo® luminescence cell viability kit is a similar method based on the amount of ATP that indicates the presence of living cells. The principle of the method is that the amount of ATP is proportional to the number of cells (20), and in the presence of ATP, luciferin in the cells in the medium emits luminescence by converting to the recombinant luciferase enzyme (21). Ivermectin (2.5-250 µM) prepared at different concentrations was added to $5x10^3$ cells after 24 h in opaque white 96-well plates and incubated for 24 h. After incubation, the ATP solution was added, and luminescence measurement was taken in Thermo Varioskan multiplate reader (Varioskan Flash Multimode Reader, Thermo, Waltham, USA) within 5 minutes. Luminescence spreading in the presence of ATP was reported as relative luminescence units. Cell viability was expressed relative to the control group considered 100% (Figure 1). Half maximum growth inhibitor concentration (IC_{50}) values were calculated from the non-linear regression analysis dose-response curves. All doses were repeated four times.

Generation of Reactive Oxygen Species

Intracellular ROS production was evaluated using the fluorescent signal indicator 2,7-dichlorodihydroflorescein-diacetate (H_2DCF-DA). The colorless H_2DCF-DA is oxidized by intracellular ROS in the medium and converts to a green fluorescent DCF. There is a fluorescence correlation between the increasing amount of ROS and diffusing fluorescence (22). Ivermectin prepared at different concentrations (2.5-250 µM) was added to the cells, which were seeded in 96 opaque black

plates $15x10^3$ cell/well for 24 h treatment. After 24 h treatment, the media was removed and washed three times with 1xdPBS. 100 µL 10 µM H_2DCF-DA prepared in DMSO was added and incubated at 37˚C for 30 minutes in a CO_2 incubator. The fluorescence intensity of DCF formed after incubation was measured using the Ex: 488nm / Em: 525nm fluorescence plate reader (Varioskan Flash Multimode Reader, Thermo, Waltham, USA). Results were calculated relative to the control group, with 0.1% DMSO compared to ATP (iROS/ATP) (21). All doses were repeated four times.

Intracellular Glutathione Levels

In this study, a luminometric glutathione kit was used to measure intracellular glutathione levels. The commercially purchased GSH-Glo™ Glutathione Assay luminescence glutathione kit reduces glutathione with the glutathione-s transferase enzyme while converting the luciferin-NT substrate in the kit to luciferin. ATP emerges in the environment during this conversion. The luciferase that occurs in the environment transforms into recombinant luciferase enzyme and converts to oxyluciferin, and emits luminescence (23). Ivermectin prepared at different concentrations (2.5-250 µM) was added to $15x10^3$ cells after 24 h in opaque white 96-well plates and incubated for 24 h. After incubation, the glutathione solution was added, and luminescence measurement was done in Thermo Varioskan multiplate reader within 5 minutes. Results were calculated relative to the control group added with 0.1% DMSO. All doses were repeated four times.

Mitochondrial Membrane Potential

Mitochondrial membrane potential (MMP-$\Delta\psi$m) is an essential parameter of mitochondrial function and an indicator of cellular health and apoptosis. The fall of MMP indicates the loss of mitochondrial membrane integrity, which reflects the initiation of the proapoptotic signal. In this protocol, 3,3'-hexyloxycarbocyanine iodide [DiOC6(3)], which is a cell-permeable, green fluorescence, lipophilic dye, accumulates in mitochondria. After 24 h incubation of ivermectin (2.5-250 µM), the media was removed and washed. Fourty nM DiOC6(3) was added and incubated at 37 °C for 15 minutes, after washing three times with 1xdPBS. Fluorescence intensity was measured using Ex: 484 nm/Em: 501 nm fluorescence plate reader (Varioskan Flash Multimode Reader, Thermo, Waltham, USA). The results were calculated relative to the control group compared to ATP (MMP/ATP) (21,24). All doses were repeated four times.

Apoptosis

AO/EB dye is a double staining method used to evaluate morphological changes in cells (25). The AO is a vital dye which stains both living and dead cells. EB dye only stains cells that have lost membrane integrity. Healthy cells appear to be homogeneous green, and early apoptotic cells contain bright green spots, especially in their nuclei due to chromatin condensation and nuclear breakdown. Late apoptotic cells turn orange because they contain both AO and EB. However, necrotic cells appear to be distorted red since they have a different nuclear morphology and dye density as they contain condensed chromatin as opposed to healthy cells (26). Ivermectin's doses below IC_{50} (2.5-30 µM) were added in 6-well plates. After 24 h incubation, cells were removed with Trypsin-EDTA and washed three times with dPBS. Cells were centrifuged at 500x g for 5 minutes at +4 ˚C, and then the supernatant was discarded. Ten µL of cell pellet and 10 µL of AO/EB solution (100 µg/mL AO +100 µg/mL EB) were added to the empty slide, and the coverslip was closed. Images were evaluated and recorded under a fluorescence microscope (Leica DM 1000). A minimum of 100 cells were counted at each concentration in randomly picked areas. The percentage of apoptotic cells was proportional to the total cells (27).

DNA Damage

Genotoxicity was measured by the alkaline single-cell electrophoresis method (comet assay) developed by Singh et al. (28). The comet assay method is based on different migration characteristics of DNA in the field of electricity according to different electrical charges and weights. Cells are placed in agarose and lysed. If there is no damage in DNA, the appearance of DNA is compact; it does not form any tail (comet). However, if DNA is damaged and fragmented, since these fragments have different electrical charges and molecular weights, they act differently in the electrophoretic environment. Thus, when the DNAs are stained with the fluorescent dye (i.e., ethidium bromide) they form a tailed image (28,29). To determine the genotoxic potential of ivermectin, concentrations below IC_{50} were added in 6-well plates. After 24 h incubation, cells were removed with Trypsin-EDTA and washed three times with dPBS. Cells washed with 1x PBS were centrifuged at 500x g for 5 minutes at +4 ˚C, then the supernatant was discarded. After 10 µL of cell suspension was mixed with 85 µL of 0.65% low melting point agarose, and the mixture was added to pre-coated slides with 1% normal melting point agarose (NMA), the samples were incubated for 4 hours in lysis solution at +4 °C. After incubation, the samples were washed with cold 1xdPBS and incubated for 40 minutes to open DNA bonds in the electrophoresis buffer at +4 °C. The samples were then run under electrophoresis at +4 °C for 25 minutes at 300 mA. Slides were washed three times in neutralization buffer and fixed with ethanol. The dried samples were dropped with EB (2 µg/mL), and images were taken under a fluorescence microscope (Leica DM 1000, Solms, Germany). DNA tail percentages in the images were analyzed with the Comet Assay IV analysis program (30).

Statistics Analysis

Results were given as mean ± standard deviation. Data from all experiments were analyzed for statistical significance using analysis of variance (One-Way ANOVA). IC_{50} values of ivermectin on cell lines were calculated by nonlinear regression analysis. Relationships between all parameters were analyzed with the Pearson correlation coefficient. A p-value of <0.05 was considered statistically significant. All statistical analyzes were performed using the SPSS package program (Version 25 for Windows, Chicago, USA).

Results

Cytotoxicity of Ivermectin on Breast Cancer and Healthy Breast Epithelial Cells

The effect of ivermectin after 24 h treatment on cell viability was evaluated with the luminometric ATP viability assay on BC cells (MCF7 and MDA MB231) and healthy breast epithelial cells (184A1). Before the experiments, the viability of the cells were over 95%. Treatment of ivermectin reduced cell viability in a statistically significant manner ($p<0.001$). Results in increasing doses were calculated relative to the control group (0.1% DMSO). Cytotoxicity increased dose-dependently (Figure 1). IC_{50} levels of ivermectin in cancer and healthy cells were calculated from the dose-response curve (MCF= 24.04 µM, MDA MB231= 34.12 µM, 184A1=68.51 µM).

Ivermectin's Intracellular ROS Generation Effect

In BC cells and healthy cells, a fluorescence H_2DCF-DA probe was used to examine intracellular ROS levels. Twenty four h of ivermectin treatment significantly increased intracellular ROS levels in cancer and healthy cells in a dose-dependent manner ($p<0.001$). A relatively increased amount of intracellular ROS in cancer cells was observed to be higher compared to the healthy cells (Figure 2).

Intracellular Glutathione (GSH) Level

Ivermectin lowered GSH levels in human BC cells and healthy breast epithelial cells (Figure 3). After 24 h of ivermectin treatment, intracellular GSH levels were found to decrease significantly ($p<0.001$).

Changes in Mitochondrial Membrane Potential

The mitochondrial apoptotic pathway has been explored to show the mechanisms underlying apoptotic induction in both cancer and healthy cells. Decreased MMP leads to apoptosis. After 24 h incubation of ivermectin, a statistically significant decrease ($p<0.05$) was observed using the DiOC6(3) fluorescence probe (Figure 4).

Ivermectin's Effect on Apoptosis

Apoptotic defects/disorders are critical for tumor formation and treatment resistance. Different concentrations of ivermectin under IC_{50} concentrations were imaged under fluorescence microscopy using AO/EB double dye to clarify whether cancer and healthy cells caused apoptosis after 24 h of incubation. It was found that apoptosis increased in a dose-dependent manner, and the rate of apoptosis in cancer cells was observed to be higher compared to the healthy cells (Figure 5). As the concentration increased, the increase in percentage of apoptosis was found to be statistically significant ($p<0.001$). At least 100 cells were counted at each dose, and the number of semi-quantitative apoptotic cells was calculated (Figure 6).

DNA Damage

The comet assay method was studied after 24 h treatment to evaluate the different concentrations of ivermectin below IC_{50} levels for genotoxic damage in cancer and healthy cells. Damaged DNAs were bright and comet-like, and undamaged DNAs were round and large. At least 100 cells were displayed and recorded at each concentration. The degree of damage was given as tail density %. Increasing ivermectin concentrations significantly increased DNA damage (Figure 7) ($p<0.001$). Micrographs of DNA comet images formed with increased damage are presented in Figure 8.

Discussion

The concept that ivermectin being repurposed as an anticancer agent mostly relies on the preclinical studies of the drug (6,9).

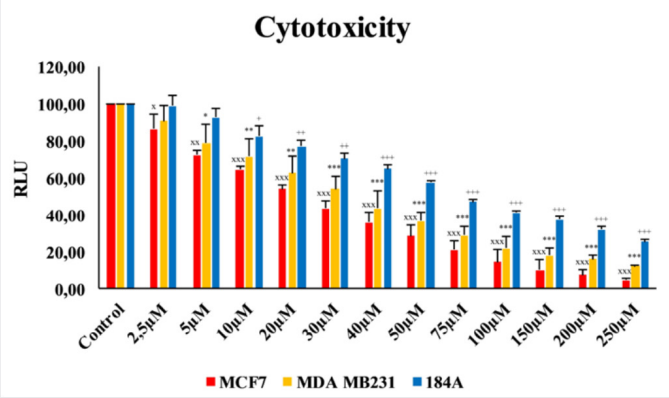

Figure 1. The effect of ivermectin on cytotoxicity. Different concentrations (2.5-250 µM) of ivermectin in MCF7, MDA MB231 and 184A1 cells were incubated for 24 hours. All results were calculated relative to the control (0.1% DMSO). Data represent four independent studies and are expressed as mean ± standard deviation. Differences in MCF7 cells, xp<0.05; xxp<0.01, xxxp<0.001; differences in MDA MB231 cells, *p<0.05; **p<0.01, ***p<0.001; differences in 184A cells, +p<0.05; ++p<0.01, +++p<0.001 values were considered statistically significant

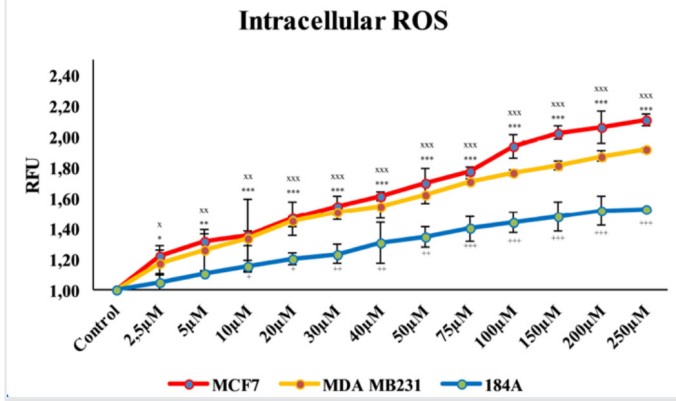

Figure 2. The effect of ivermectin on intracellular ROS level on MCF7, MDA MB231 and 184A1 cells. Ivermectin increased intracellular ROS levels in both healthy and cancer cells. Doses were calculated relative to the control and normalized with the viability level. Data represent four independent studies and are expressed as mean ± standard deviation. Differences in MCF7 cells, xp<0.05; xxp<0.01, xxxp<0.001; differences in MDA MB231 cells, *p<0.05; **p<0.01, ***p<0.001; differences in 184A1 cells, +p<0.05; ++p<0.01, +++p<0.001 values were considered statistically significant

Although it has been reported that ivermectin shows antitumoral activity through different mechanisms in various cancer cells, studies are limited, and the exact anti-tumorigenic mechanism is not yet clarified in BC. In the present study, we found that oxidative stress and DNA damage could be the underlying mechanism of ivermectin's anti-tumorigenic effects.

In this study, the IC_{50} value of ivermectin was found much higher in the healthy breast epithelium cells (184A1) compared to the BC cell lines MCF7 and MDA MB231, indicating the potential of ivermectin to be used as an anticancer agent. MCF7 is a hormone receptor-positive cell line, while MDA MB231 is a triple-negative cell line of BC (31). We found that MCF7 was more sensitive to ivermectin, and this could be due to the primary ATP synthesis route, which was oxidative phosphorylation in MCF7 while it was glycolysis in MDA MB231(32).

Juarez et al. (8,9) stated that 5 µM of ivermectin showed antitumor effects and that this concentration was clinically relevant; hence, a significant inhibition of intracellular ATP level was recorded in our study in the BC cell lines while there was no significant difference in the healthy cells. Another study with MDA MB231 revealed ivermectin's preferential inhibition of cancer cell viability (18). Dou et al. (16) tested the drug's cytotoxicity with the MTT assay using BC cell lines and found similar results, furthermore, they also found that ivermectin inhibited 60% of tumor growth

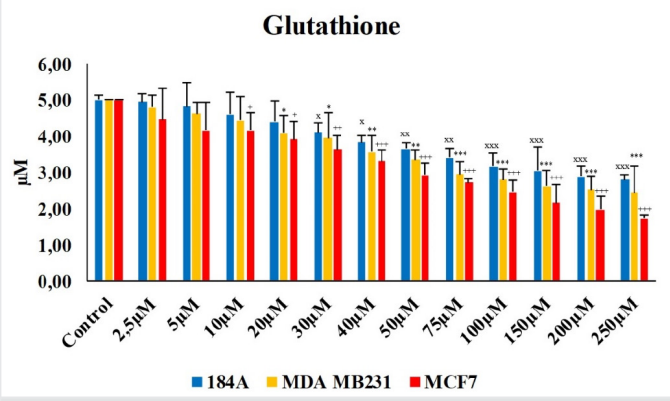

Figure 3. The effect of ivermectin on glutathione levels on MCF7, MDA MB231 and 184A1 cells. Ivermectin reduced glutathione levels in both healthy and cancer cells. Data represented four independent studies and were expressed as mean ± standard deviation. Differences in MCF7 cells, xp<0.05; xxp<0.01, xxxp<0.001; differences in MDA MB231 cells, *p<0.05; **p<0.01, ***p<0.001; differences in 184A1 cells, +p<0.05; ++p<0.01, +++p<0.001 values were considered statistically significant

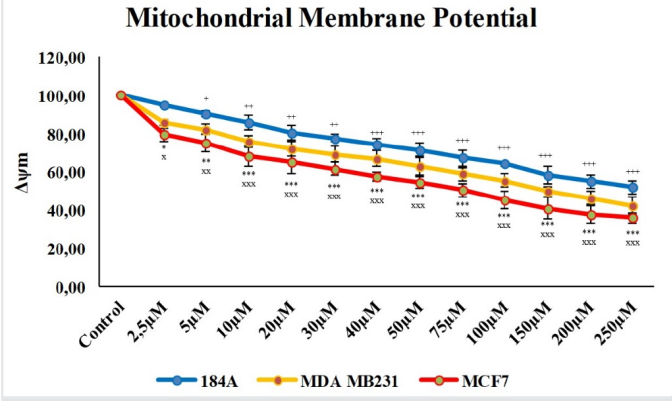

Figure 4. The effect of ivermectin on mitochondrial membrane potential on MCF7, MDA MB231 and 184A1 cells. Ivermectin reduced mitochondrial membrane potentials in both healthy and cancer cells. All doses were calculated relative to the control and normalized with the viability level. Differences in MCF7 cells, xp<0.05; xxp<0.01, xxxp<0.001; differences in MDA MB231 cells, *p<0.05; **p<0.01, ***p<0.001; ivermectin in 184A1 cells, +p<0.05; ++p<0.01, +++p<0.001 values were considered statistically significant

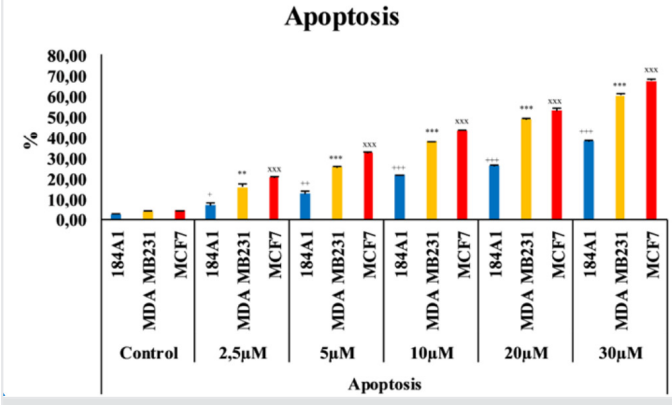

Figure 5. The effect of ivermectin on apoptosis on MCF7, MDA MB231 and 184A1 cells. Ivermectin induced apoptosis in both healthy and cancer cells. Cells counted semi-quantitative were expressed as mean ± standard deviation. Differences in MCF7 cells, xp<0.05; xx p<0.01, xxxp<0.001; differences in MDA MB231 cells, *p<0.05; **p<0.01, ***p<0.001; differences in 184A1 cells, +p<0.05; ++p<0.01, +++p<0.001 values were considered statistically significant

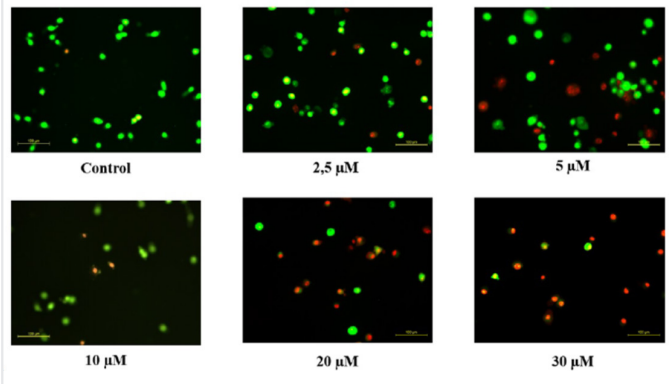

Figure 6. Effect of different concentrations of ivermectin on MCF7 cell on apoptosis. Healthy living cells in cells stained with AO/EB double dye after 24 hours were adequately structured and appeared in green; apoptotic cells appeared in yellow-orange with condensed chromatin and fragmented nucleus; necrotic cells appeared in red

AO/EB: Acridine orange/ethidium bromide

in mice. Dou et al. (16) found that 24 h ivermectin treatment to the cells did not induce apoptosis; instead, autophagy was induced. Conversely, we found that apoptosis increased in a dose-dependent manner, and the rate of apoptosis in cancer cells was observed to be higher compared to the healthy cells. In human and mouse BC cell lines, Draganov et al. (33) found that ivermectin induced apoptosis and necrosis.

Along with ATP inhibition and increased apoptosis, we found decreased MMP in a dose-dependent manner. Since oxidative phosphorylation in mitochondria is the primary source of ATP, and the MMP is a prerequisite of proper ATP synthesis, decreased MMP presumably leads to apoptosis (34). In this regard, it seemed logical that we found the MCF7 cells more sensitive to ivermectin-induced mitochondrial and oxidative stress since the cells relied on oxidative phosphorylation as the route for ATP synthesis. A study using renal cell carcinoma cell lines shows that ivermectin decreases MMP and ATP production explaining it as a consequence of mitochondrial dysfunction leading to oxidative stress (35). Liu et al. (14) also detected decreased mitochondrial membrane potential, ATP levels, and increased mitochondrial superoxide in ivermectin-treated glioblastoma cells. These inhibitory effects of ivermectin were significantly reversed in these studies when the cells were treated with antioxidants, which supported the presence of ivermectin-induced oxidative stress.

The ROS produced in large quantities can activate cell death signaling pathways; furthermore, ROS accumulation and disruption of MMP in the presence of cellular stress can cause the inner membrane to break down leading to apoptosis (36). Besides increased ROS levels in our study, decreased levels of glutathione might pave the way to oxidative stress formation, which as a result, might cause mitochondrial damage and subsequently cell death. Zhu et al. (35) detected that ivermectin caused oxidative stress and DNA damage in ivermectin-treated renal carcinoma cells. It is known that DNA damage is an efficient factor that can induce cell death. Zhang et al. (13) suggested that ivermectin induced apoptosis via the mitochondrial pathway by recording ROS formation and MMP disruption along with DNA damage in ivermectin-treated HeLa cells. In our study, after ivermectin treatment DNA damage was more in the cancer cells compared to the healthy cells, and especially the cells with increased oxidative stress were seen to have more DNA damage.

Additionally, besides the potential anticancer effects, ivermectin can be considered to be used in combination with approved anticancer drugs of which resistance is a big problem to solve. Jiang et al. (37) and Kwon et al. (38) found that ivermectin could reverse drug resistance in cancer cells .

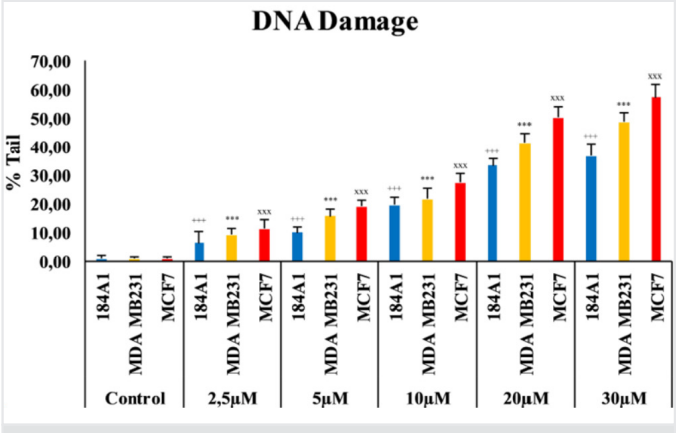

Figure 7. The effect of ivermectin on DNA damage on MCF7, MDA MB231, and 184A1 cells. Ivermectin increased DNA damage, in other words, genotoxicity, in both healthy and cancer cells. Results were given as % tail density and expressed as mean ± standard deviation

Conclusion

In conclusion, ivermectin induces apoptosis via oxidative stress and DNA damage in BC cells. The *in vitro* mechanistic studies of promising anticancer agents for repurposing are essential guides for drug developers.

Acknowledgments

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Ethics

Ethics Committee Approval: *In vitro* study.

Informed Consent: *In vitro* study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: E.M.G., Concept: E.M.G., Design: E.M.G., Data Collection or Processing: E.M.G., A.G.A., Analysis or Interpretation: E.M.G., A.G.A., Literature Search: E.M.G., A.G.A., Writing: E.M.G., A.G.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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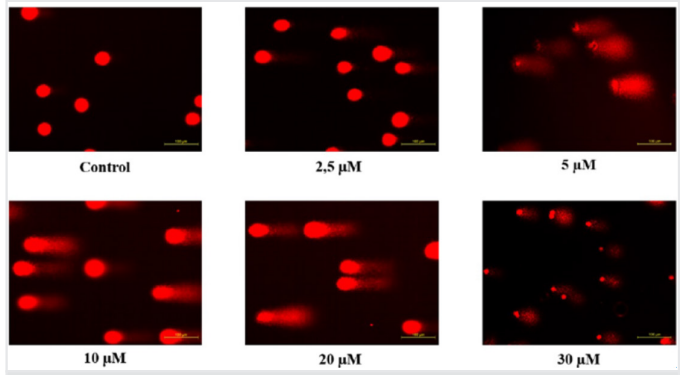

Figure 8. Effect of different concentrations of ivermectin on MCF7 cell on DNA damage. The comet image in the DNA formed after the damage increased in a dose-dependent manner

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