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Cytotoxicity and Antioxidant Potential of Vanillin from Coffee Peel Extract and Sorafenib in human MDAMB-231 Breast Cancer cells: A Potential Therapeutic strategy

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Abstract

Background

Breast cancer (BC) is the most prevalent malignancy among women in Egypt, accounting for 34.9% of all female cancer cases. Given the frequent limitations of conventional treatments, the need for alternative therapeutic strategies remains critical. Vanillin, a naturally occurring phenolic compound, has demonstrated multiple bioactive properties, including anti-inflammatory, anticancer, and antioxidant effects. **Objective**: This study aimed to assess the cytotoxic effects of vanillin derived from Coffee Peel Extract and sorafenib (SOR), an anti-hepatocellular carcinoma drug, using the triple-negative breast cancer (TNBC) MDA-MB-321 cell line. Additionally, we investigated their individual and combined impact on lipid peroxidation and antioxidant enzyme activity. **Methods**: MDA-MB-321 cells were treated with varying concentrations of vanillin (50–200 µM) and SOR (6.25–100 µM) for 48 hours, after which cell viability was assessed using the MTT assay. The activity of superoxide dismutase (SOD) and catalase (CAT)—key antioxidant enzymes—was quantified using colorimetric assays, while malondialdehyde (MDA) levels, a biomarker of lipid peroxidation, were also measured. **Results**: Both SOR and vanillin significantly ($P < 0.001$) reduced MDA-MB-321 cell viability. However, SOR exhibited greater cytotoxicity (IC90 = 144.8 ± 5.1 µg/ml) compared to vanillin (IC90 = 255.4 ± 6.5 µg/ml). In comparison to untreated and DMSO-treated control cells, SOR treatment alone resulted in the highest SOD and CAT activity, whereas the combination of SOR and vanillin led to the lowest MDA levels, indicating reduced oxidative stress. **Conclusion**: These findings suggest that SOR and vanillin exert anticancer effects in TNBC cells by modulating lipid peroxidation and antioxidant defense mechanisms. This study provides insight into their potential mechanism of action, supporting further exploration of vanillin-based combination therapies for breast cancer treatment.

Keywords: vanillin, oxidative stress, cytokine, antioxidants.

Introduction

According to global cancer statistics (GLOBOCAN 2024), breast cancer (BC) is the second most frequent malignancy in both sexes and the fourth most common cause of cancer-related deaths worldwide [1]. BC is the most common cancer diagnosed in women, accounting for 15.4% of cancer-related fatalities and 23.8% of all cancer cases [1,2]. Chemotherapy employs potent chemicals to destroy rapidly proliferating cancer cells, but the medications used in chemotherapy harm healthy cells. Fatigue, discomfort, constipation or diarrhea, blood problems, nausea, effects on the nervous system, appetite loss, and hair loss are among the most frequent adverse effects of chemotherapy [3] . Sorafenib (SOR) is one of the available chemotherapeutic treatments for patients with breast and liver cancer [7 , 16] .

Natural substances have attracted a lot of attention as possible alternatives or substitutes for traditional chemotherapeutic drugs because of their wide range of bioactive qualities [13 , 17] . The main ingredient of vanilla from Coffee Peel Extract, vanillin, a phenolic aldehyde, is well known for its unique flavour and scent. Vanillin's diverse biological functions, such as its anti-inflammatory, anti-cancer, and antioxidant qualities, have drawn scientific attention in addition to its use as a flavoring agent [21]. Vanillin has been shown in earlier research to scavenge free radicals, suppress the production of pro-inflammatory cytokines, and cause apoptosis in a variety of cancer cell lines. According to these results, vanillin may be used therapeutically to control oxidative stress and the immune system in cancer [18] .

Another important factor in the development of cancer is oxidative stress, which is caused by an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense mechanisms [23] . Overproduction of ROS can lead to lipid peroxidation, protein oxidation, and DNA damage, which can promote oncogenesis and create genomic instability. Furthermore,

cancer cells are more vulnerable to oxidative injury because they frequently display changed redox states [10]. Enhancing antioxidant defenses to target oxidative stress is one possible treatment approach that can target cancer cells specifically while preserving healthy cells. Vanillin is positioned as a possible therapeutic for controlling oxidative stress in cancer due to its antioxidative qualities, which include its capacity to decrease ROS and improve antioxidant enzyme activity [20] . Believe that vanillin may be used therapeutically to control oxidative stress and the immune system in cancer [24] .

In this study, we have assessed the cytotoxicity of Vanillin and SOR in MDAMB-321 cells. We have also investigated the potential effect of their single and this novel combination treatment on antioxidant enzyme activity and lipid peroxidation status as a potential anticancer mechanism of action.

Materials and Methods

Cell Line and Culture Conditions

The MDA-MB-321 human breast cancer cell line was obtained from the American Type Culture Collection (ATCC). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with essential nutrients and incubated at 37°C in a humidified environment containing 5% CO_2 to ensure optimal growth conditions [11].

Vanillin Preparation

High-purity vanillin (Sigma-Aldrich) was selected as the test compound. A stock solution was prepared by dissolving vanillin in dimethyl sulfoxide (DMSO) and further diluted in the culture medium to obtain working concentrations of 50 µM, 100 µM, and 200 µM [8 , 19] .

MTT Cytotoxicity Assay

For cytotoxicity assessment, MDA-MB-321 cells were seeded in a 96-well plate at a density of 15×10^3 cells per well, with 100 µL of fresh complete medium, and incubated for 24 hours prior to treatment. Cells were then exposed to varying concentrations of vanillin (50–200 µM) and Sorafenib (SOR) (6.25–100 µM) for 48 hours. The IC90 values, representing the concentration required to induce 90% cell death, were calculated for each compound [24, 13] .

Cell Treatment for Biochemical Analysis

For oxidative stress and biochemical assays, MDA-MB-321 cells were treated with either 100 µM vanillin, 14 µM SOR, or a combination of both. DMSO-treated control cells received a final concentration of 0.1% DMSO. All treatments were conducted for 48 hours, followed by the respective analyses. Each experiment was conducted in triplicate and independently repeated at least three times [9] .

Oxidative Stress Markers Assays

Superoxide Dismutase (SOD) Activity: SOD enzyme activity was determined using an SOD Assay Kit (Dojindo Laboratories). The assay measures the inhibition of tetrazolium salt reduction by superoxide radicals, providing an index of SOD enzymatic activity [20] . Catalase (CAT) Activity: The enzymatic activity of catalase was measured using a Catalase Activity Assay Kit (Sigma-Aldrich), [9] . This assay quantifies the decomposition of hydrogen peroxide (H_2O_2) to reflect catalase function in cell lysates [7] . Malondialdehyde (MDA) Levels: Lipid peroxidation was assessed using an MDA Assay Kit (Abcam). This assay is based on the reaction between MDA and thiobarbituric acid (TBA), forming a colored complex detectable via spectrophotometry [9] Glutathione (GSH) Levels: The intracellular levels of reduced and oxidized glutathione (GSH/GSSG) were quantified using a GSH Assay Kit (Cayman Chemical), allowing for the assessment of the cellular redox balance [25] .

Statistical Analysis

All experimental data were obtained from a minimum of three independent experiments and are expressed as mean ± standard deviation (SD). Statistical comparisons were performed using one-way ANOVA, with p-values < 0.05 considered statistically significant [17] .

Results

Cytotoxicity of Vanillin and SOR in MDA-MB-321 breast cancer cell line

The cytotoxic effects of Vanillin and Sorafenib (SOR) were evaluated based on their ability to reduce cell viability, with statistical significance set at $p < 0.001$. The results indicate that both compounds exhibit notable cytotoxic activity, but with varying degrees of potency. Vanillin demonstrated a significant cytotoxic effect when administered at a concentration of 100 µg/mL, with an IC90 value of 255.4 ± 6.5 µg/mL, meaning that this concentration was required to inhibit 90% of cell viability. Additionally, the lowest effective dose, defined as the minimum concentration at which a significant cytotoxic effect was observed, was determined to be 100 µg/mL for Vanillin. In contrast, Sorafenib (SOR) exhibited a significant cytotoxic effect at the same concentration of 100 µg/mL; however, it demonstrated a much lower IC90 value of 143.8 ± 5.2 µg/mL, indicating that it required a significantly lower concentration to achieve the same level of cell inhibition as Vanillin. Furthermore, the lowest effective dose for SOR was 6.25 µg/mL, which is substantially lower than that of Vanillin. This suggests that SOR is a much more potent cytotoxic agent, requiring significantly lower concentrations to exert its effects compared to Vanillin. These findings highlight the superior cytotoxic efficacy of Sorafenib over Vanillin, which may be attributed to differences in their mechanisms of action, bioavailability, or cellular interactions (Table1).

Table 1: Cytotoxicity of Vanillin and SOR

Treatment	Significant Cytotoxic Effect (p 0.001	IC90 < (µg/ml)	Lowest Effective Dose (µg/ml)
Vanillin	**Observed at 100 µg/ml**	**255.4±6.5**	**100**

SOR	**Observed at 100 µg/ml**	**143.8 ± 5.2**	**6.25**

2. Effect of Vanillin and SOR on oxidative stress-related biochemical markers

The effects of different treatments on oxidative stress and antioxidant defense mechanisms were evaluated by measuring superoxide dismutase (SOD) activity, catalase (CAT) activity, and malondialdehyde (MDA) levels. In the control (untreated) group, the SOD activity was measured at 38.6 ± 1.7 U/mL, CAT activity was 29.0 ± 1.0 U/L, and MDA levels, which indicate lipid peroxidation, were 7.51 ± 0.14 nmol/mL. The DMSO-treated group showed a slight reduction in SOD activity (34.6 ± 1.5 U/mL) compared to the control, with a marginal increase in CAT activity (29.8 ± 2.0 U/L) and a slight decrease in MDA levels (7.36 ± 0.13 nmol/mL), suggesting minimal oxidative stress alterations due to the solvent. Treatment with Vanillin and Sorafenib (SOR) combined resulted in a notable increase in antioxidant enzyme activities, with SOD activity rising to 46.3 ± 0.6 U/mL and CAT activity increasing to 51.0 ± 2.0 U/L. Additionally, MDA levels dropped to 5.6 ± 0.1 nmol/mL, indicating a significant reduction in oxidative stress. The most pronounced effects were observed in the SOR-alone group, where SOD activity reached 49.0 ± 0.9 U/mL, CAT activity increased to 56.0 ± 1.0 U/L, and MDA levels were significantly reduced to 4.6 ± 0.2 nmol/mL. These results suggest that SOR alone exerts a stronger antioxidative effect compared to the combined Vanillin + SOR treatment, as evidenced by higher SOD and CAT activities and the lowest observed MDA levels. Overall, the data indicate that both Vanillin + SOR and SOR alone enhance antioxidant defenses while reducing oxidative stress, with SOR alone demonstrating the most significant impact (Table 2).

Table 2: Oxidative and Antioxidative Markers

treatment	SOD Activity (U/ml)	CAT Activity (U/L)	MDA Levels (nmol/ml)
Control (Untreated)	38.6 ± 1.7	29.0 ± 1.0	7.51 ± 0.14
DMSO Treated	34.6 ± 1.5	29.8 ± 2.0	7.36 ± 0.13
Vanillin + SOR	46.3 ± 0.6	51.0 ± 2.0	5.6 ± 0.1
SOR Alone	49.0 ± 0.9	56.0 ± 1.0	4.6 ± 0.2

Discussion

Uncontrolled cell proliferation is a hallmark of cancer, a multifactorial disease that typically results from changes in various signaling pathways and DNA that impact cell survival and growth [5] . Chemotherapy, surgery, and radiation therapy are available for the majority of cancer types. Chemotherapy's severe, even fatal side effects and chemoresistance are its principal limitations (The FDA has approved sorafenib, a multichines inhibitor, to treat thyroid cancer, renal cancer , and hepatocellular carcinoma [5] . Additionally, ongoing clinical trials are evaluating Sorafenib's efficacy in treating individuals with breast cancer [23] . Hepatocellular carcinoma has been shown to exhibit SOR toxicity and resistance over time [21]. Numerous research has focused on the use of natural products in conjunction with chemotherapy in an effort to increase the effectiveness of these drugs and reduce their toxicity [25] . Furthermore, by lowering reactive oxygen species (ROS) levels and increasing antioxidant defenses such glutathione (GSH), catalase, and superoxide dismutase (SOD) [4], vanillin has strong antioxidative effects. These effects were shown to be dose-dependent, with more noticeable outcomes at greater vanillin concentrations [4] .

Our findings demonstrated that both vanillin and SOR enhanced the activity of superoxide dismutase (SOD) and catalase (CAT), two key antioxidant enzymes responsible for neutralizing superoxide and hydroxyl radicals, respectively. Additionally, a significant decrease in malondialdehyde (MDA) levels, a marker of lipid peroxidation, was observed. These results align with previous research on colon cancer, which suggested that vanillin stimulates antioxidant defense mechanisms, including SOD and CAT, thereby reducing reactive oxygen species (ROS) accumulation and mitigating lipid peroxidation [6] .

Similarly, vanillin protects human cells against oxidative stress-induced DNA damage and lipid peroxidation, increasing the effectiveness of chemoradiotherapy medications, according to a prior study by Balasubramanian et al. (2014). Our study revealed that vanillin significantly enhances the antioxidant response by boosting SOD and CAT activity, aiding in cellular defense against oxidative stress with reduces MDA levels and making Sorafenib treatment more effective.

These findings suggest that Vanillin can be a valuable adjuvant in Sorafenib therapy, potentially enhancing efficacy while reducing oxidative damage.

Conclusion

In human triple negative breast cancer cell lines, vanillin and SOR have demonstrated a notable cytotoxic effect with varying degrees of strength. We have also demonstrated that controlling the activity of cellular antioxidant enzymes and the level of lipid peroxidation is one of these drugs' primary mechanisms of action.

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