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Original Article

Saponarin and its Role in Nursing Care for the Adjuvant Treatment of MDA-MB-231 Triple-Negative Breast Cancer Cells: An *in vitro* and *in silico* Approach

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ABSTRACT

Background: Breast cancer caused 670,000 deaths worldwide in 2024. Saponarin (SAP) is mostly present in vegetables and citrus fruits and has demonstrated encouraging anti-proliferative properties in various studies. This investigation examines the possible synergic effects of SAP in breast cancer MDA-MB-231 cells. **Materials and Methods:** Breast cancer cells MDA-MB-231 cells were undergone to various doses of SAP treatment to assess cell viability, Lactate Dehydrogenase (LDH) assay and measure the Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione (GSH) oxidative stress level. In addition, an *in silico* approach was used to perform Lactate Dehydrogenase B (LDHB) expression analysis and molecular docking. **Results:** The impact of SAP on the cell viability revealed that its cytotoxic potential increased in a dose-dependent pattern and IC_{50} concentrations were chosen for further experiments. The release of LDH enzyme after treatment of cells with a SAP was measured as a biological measure of cell membrane cytotoxicity. This is achieved by remarkably augmenting oxidative stress. Hence, SAP shows promise for pharmacological use in breast cancer chemotherapy by triggering oxidative stress and death in MDA-MB-231 cells. The molecular docking analysis revealed that the SAP interacted well with the LDHB with the residues GLU105, ASP196(2), HIS194, and ARG107 via hydrogen bonds and with residues ARG100, ILE243, and TYR240 by extending pi-alkyl and pi-pi-T-shaped bond. Additionally, the hydrophobic residues that include VAL32, GLN101, SER106, ASN139, PRO140, GLU193, HIS194, SER197, GLY195, THR249, and ILE253 surrounded the docked complex via van der Walls interactions with the binding affinity of -8.4 kcal/mol and the estimated RMSD of 2.707Å which might trigger that activity of LDHB. Nursing care is vital in integrating research findings into clinical practice, educating patients about treatments, managing side effects, and collaborating with other healthcare professionals to improve patient outcomes. **Conclusion:** This study's findings endorse the heightened SAP as an approach to reducing cell growth in breast cancer. All these results suggest that Sap can be used as a potent anti-cancer drug for breast cancer cells.

Keywords: Antioxidants, Breast Cancer, Saponarin, "*in silico* studies", "Molecular Docking".

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INTRODUCTION

Breast cancer has rapidly emerged as 1 of the most important health problems facing women in the last few decades.[1] Although breast cancer diagnosis and treatment technology has made significant progress, it still poses a burden on the health system.[2] This is mainly because cancer incidence is higher in individuals with poor health and risk factors.[3] Breast cancer often occurs in the breast ducts or lobules and can be invasive. It can metastasize to other body parts and spread to the lymph nodes. Treatment of this disease requires a comprehensive approach that includes surgery, endocrine therapy, chemotherapy, radiation, and targeted therapy directed against Human Epidermal growth factor Receptor 2 (HER2), based on the stage and molecular subtype of the disease.[4-6] Despite advances in breast cancer treatment, the narrow range of effective drugs and radiation exposure may cause side effects that reduce patient compliance.[7] Therefore, new drugs specific to breast cancer cells that are less damaging to healthy cells are needed.[8]

Flavonoids have demonstrated their anti-inflammatory properties through various mechanisms, such as improving oxidative stress and apoptosis in many cancers.[9-12] These effects reduce cell proliferation, migration, and invasion. SAP is a well-known

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flavonoid found in many plants, including berries, broccoli, citrus fruits, and onions. SAP is the nonreactive form of flavanone glycosides called saponins. The pharmacological properties of SAP have been evaluated *in vitro* using various tumor cell and *in vivo* xenograft models with encouraging results.[13] However, flavonoids usually need to be administered in large amounts due to their effectiveness in causing cancer death.[14] When flavonoids are taken together, they work better together at lower doses, increasing their capacity to target and kill tumor cells. We believe that the bioactive substances found in plants are more potent in their original components than in their isolated or isolated form. Specific compounds found in certain plants can affect biological processes.[15]

They contribute to the accumulation of free radicals such as (OH) or Hydrogen Peroxide (H_2O_2) and singlet Oxygen ($^1O_2$). Enzymatic or non-enzymatic antioxidants can attack and degrade those.[16] Oxidative damage and stress in cellular components such as the nucleus, mitochondrial DNA, and lipid membranes occur when there is a disproportion between the ROS accumulation and the action of antioxidant mechanisms.[17-19] Flavonoids have been shown to demonstrate antioxidant properties in different cancers through different mechanisms, such as the production of free radicals through autoxidation, the reduction of antioxidants, and the inhibition of enzymatic antioxidant activity.[20]

In this study, nursing is important in terms of translating scientific research into clinical practice, educating patients about new treatments, monitoring and managing side effects, providing support to patients participating in clinical trials, providing counselling, and collaborating with other physicians for this purpose. It is important to create a treatment plan. Physicians play an essential role in filling the gap between research and patient care, and in ensuring that cancer treatment is modified to improve individual pain. Therefore, this work focused to assess the antitumor properties of SAP on breast cancer MDA-MB-231 cells. Therefore, MDA-MB-231 cells were utilized to evaluate the properties of SAP on oxidative stress and cell growth.

MATERIALS AND METHODS

Cell culture and treatment

MDA-MB-231 (breast cancer) cells were maintained in Dulbecco's Modified Eagle Culture Medium (DMEM) enriched with 10% Foetal calf serum, streptomycin (100 µg/mL), and penicillin (100 U/mL). They were stored at 37°C in humidified 5% CO_2.

MDA-MB-231 cells were cultivated in a 96-well plate (3×10^3 cells per well) for 24 hr to form a semi-confluent culture. The next day, the cells were coated with diverse dosages of SAP (control, 5, 10, 20, 40, 80 and 100 µg/mL) for 24 hr. Finally, 5 mg/mL MTT reagent in PBS was mixed into each well and the formation of formazan crystals was observed. Then, combine DMSO (100 µL/well) to absorb the blood formazan crystals at 475 nm. Cell growth was determined and depicted as the percentage of aeration of the sample compared to the control. The IC_{50} value (standard concentration causing a 50% loss in cell growth) was measured in a dose-response using a horizontal bar.[21]

Lactate Dehydrogenase (LDH) assay

MDA-MB-231 cells were grown (1×10^5 cells/mL) on 96-well plates in to estimate the activity of the LDH enzyme liberated from the cytosol when cells were disrupted under stress. A commercial kit was employed to measure the LDH activity. The number of live cells was then correlated with the absorbance readings to determine the cytotoxic action.

Investigation of antioxidant enzymes

After introducing SAP to the cells in accordance with the instructor's guidelines, the commercial SOD assay kit was utilized to gauge SOD activity. A plate reader was used to calculate the samples' absorbance at 450 nm. The CAT assay kit was used in accordance with the instructions to gauge the CAT enzyme's activity. The kit measures the enzyme based on how hydrogen peroxide breaks down. Moreover, a kit was used to assess the levels of GSH activity. By continually reducing 5,5′- dithiobis(2-nitrobenzoic acid) in the presence of catalytic amounts (nmoles) of GSH, a kinetic assay is used to calculate the quantity of GSH.

LDHB Performance Analysis

LDHB estimation is using TCGA normal and BC criteria (including different stages, age groups, and race of BC patients) using the University of Birmingham Alabama Cancer (UALCAN) data analysis portal (UALCAN) performance. http://ualcan.path.uab.edu/index.html accessed: July 10, 2024).[22]

The dataset retrieval and its preparation for molecular docking analysis

Retrieve the target LDHB (PDB ID: 7DBK Chain ID: A)[23] from Protein Data Bank (PDB) for molecular docking analysis. LDHB preprocessing, including removing water bonds and ligands, was performed using Discovery Studio Visualizer (DSV) v19.1.0.18287 (www.accelerys.com). Saponin 3D atomic coordinates were obtained from the PubChem compound database in SDF format[24] and then converted to PDB format using DSV.

Molecular docking and visualization

Molecular docking studies were executed using Autodock Vina, an advanced tool with a matching algorithm in PyRx software.[25] This approach helps to predict the optimal combination of LDHB and saponins. Autodock Vina uses a new measuring mechanism.

$$C=\sum_{i<j} f_{titj}(r_{ij}),$$

C-Sum of intermolecular and intramolecular distance; Σ-Over all of the pairs of atoms; f_{titj} - Symmetric set of interaction functions; r_{ij}-Interatomic distance.

The LDHB and Saponarin were initially converted to PDBQT format. Subsequently, the PyRx virtual screening tool was utilized to identify interactions with targeted LDHB biomarkers. Additionally, the properties of the grid box were set to size_x=72.97 Å; size_y=97.96 Å; size_z=65.11 Å. Following the execution of molecular docking, we conducted a comprehensive analysis of LDHB's interactions with Saponarin in 3D and 2D using Discovery Studio Visualizer v19.1.0.1828, a product of Dassault Systèmes BIOVIA. This software can be obtained from Rue Marcel Dassault, Vélizy-Villacoublay, France (URL: www.accelerys.com, Accessed on: 9 July 2024).

Statistical Analysis

The information is based on three distinct experiments and was presented as mean±SD. GraphPad Prism was used to statistically analyze the results. A noticeable difference between the groups was indicated by $p<0.05$. Software called SPSS was used to perform statistical analysis. Tukey's test is used after a one-way Analysis of Variance (ANOVA) to determine any significant differences among the groups.

RESULTS

MDA-MB-231 cell viability

The toxicity of SAP to MDA-MB-231 breast cancer cells was evaluated using the MTT test. Administering SAP at dosages ranging from (control, 5, 10, 20, 40, 80 and 100 µg/mL) reduced the toxicity to MDA-MB-231 cells than the treated cells. SAPs did not exhibit a response that varied with the dosage, as indicated by the displayed IC_{50} values of 45.52 µg/mL (Figure 1).

LDH activity

The amount of the LDH enzyme produced from the cytosol rises when cells are harmed under stress. The enzyme activity was analyzed to determine whether treating the cells with SAP caused an increase in LDH release, and the findings are depicted in Figure 2. It was revealed that with increasing SAP concentration, the LDH activity also elevated, displaying the highest LDH activity, indicating the damaging effect of SAP on MDA-MB-231 cells.

Investigation of antioxidant enzymes characteristics

The IC_{50} concentration of SAP may exhibit antioxidant characteristics. The SOD, CAT, and GSH levels were determined as indicated in Figure 3. The findings demonstrated a considerable elevation in the SOD, CAT, and GSH levels in MDA-MB-231 cells exposed to SAP at the IC_{50} concentration, as compared with control. Nevertheless, MDA-MB-231 cells exposed to a dosage of 45.52 µg/mL exhibited reduced levels of SOD, CAT, and GSH activity.

In silico approach

The LDHB expression rate in the normal and SAP was analyzed. The expression rate of LDHB in SAP is drastically reduced and measured as statistically significant $<1e^{-12}$ (Figure 4A). The study estimated the expression rate of LDHB based on the SAP categorized by stage, ranging from benign to metastasis. Significant statistical variations ($p\leq0.05$) was observed in the expression of LDHB from the normal (N)-vs-stage (S) 1 ($P=5.16e^{-14}$), N-vs-S2 ($P=1.11^{e-16}$), N-vs-S3 ($<1e^{-12}$), and N-vs-S4 ($1.36e^{-08}$) (Figure 4B). The findings revealed a marked reduction in LDHB expression from normal samples to stage 1, with a subsequent decline as the cancer progressed towards metastasis. As per the Centres for Disease Control and prevention (CDC), the chances of progressing breast cancer higher with age, with the majority of cases being diagnosed after the age of 50 (CDC, 2024). Hence, the LDHB expression rate was measured in the different age groups of patient samples affected by BC. The LDHB expression rate was primarily reduced when getting older. A statistically significant reduction in the expression rate of LDHB was seen when compared with the normal samples (N-vs-age (21-40 years=$2.49e^{-05}$; N-vs-age (41-60 years=$1.15e^{-13}$); N-vs-age (61-80 years=$<1e^{-12}$); N-vs-age (81-100 years)=$4.26e^{-06}$). Also, the statistically significant downregulation of LDHB was seen in the age group (21-40 years)-vs-age (61-80 years) ($p=5.20e^{-04}$) and age (41-60 years)-vs-Age(61-80Yrs) ($p=1.60e^{-05}$) (Figure 4C). Meanwhile, the LDHB expression rate was evaluated in Caucasians, African and American nationals, and Asians. This reduced the LDHB expression rate in Caucasians and Asians compared to the normal samples. The statistical significance was observed between all the races and normal samples (N-vs-Caucasian ($p=1.62e^{-12}$); N-vs-African-American nationals ($p=2.54e^{-13}$); N-vs-Asians ($6.65e^{-08}$)) (Figure 4D). However, no

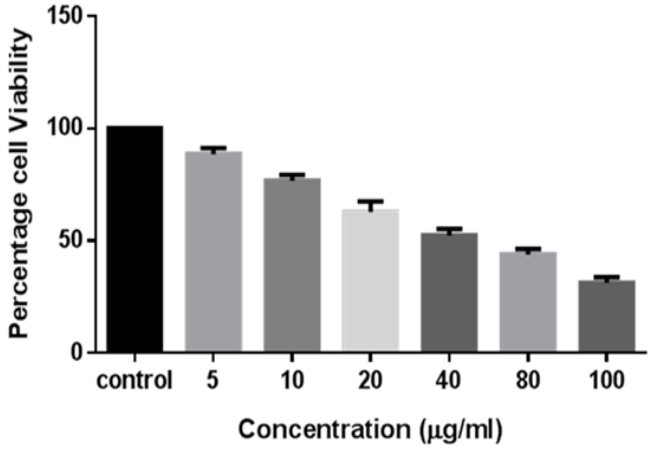

Figure 1: Effect of SAP at various doses on MDA-MB-231 cells for 24 hr as assessed by MTT assay and cell viability was determined. Note that at dosages of SAP above IC_{50} value 45.52 µg/mL, a rapid decrease in the growth was noted that increases with concentration. Each point indicates the mean of three replicate experiments of incubations of cells.

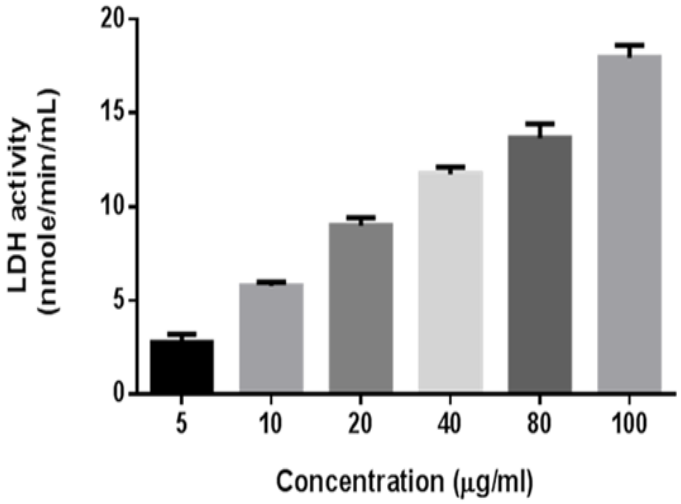

Figure 2: Measurement of lactate dehydrogenase activity in SAP-treated MDA-MB-231 cells. Data were revealed as the mean of three replicate assays.

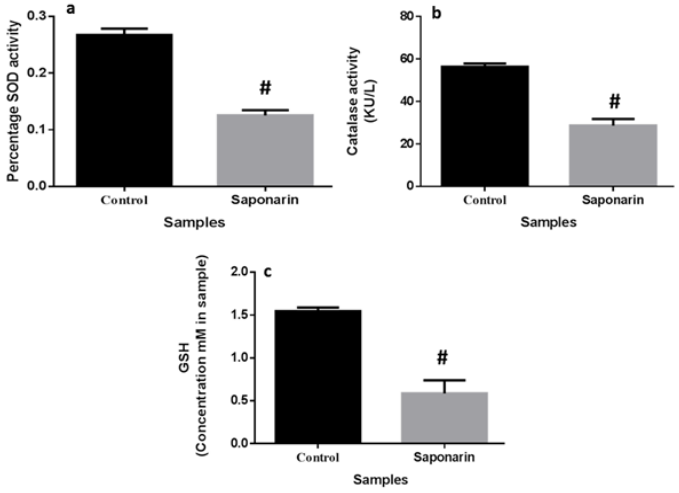

Figure 3: Protective effect of SAP on the antioxidant defences for MDA-MB-231 cells: SOD content (a) CAT (b) and GSH (c). Data were revealed as the mean of three replicate assays. Data were depicted statistically significant at #*p*< 0.05 as compared with control.

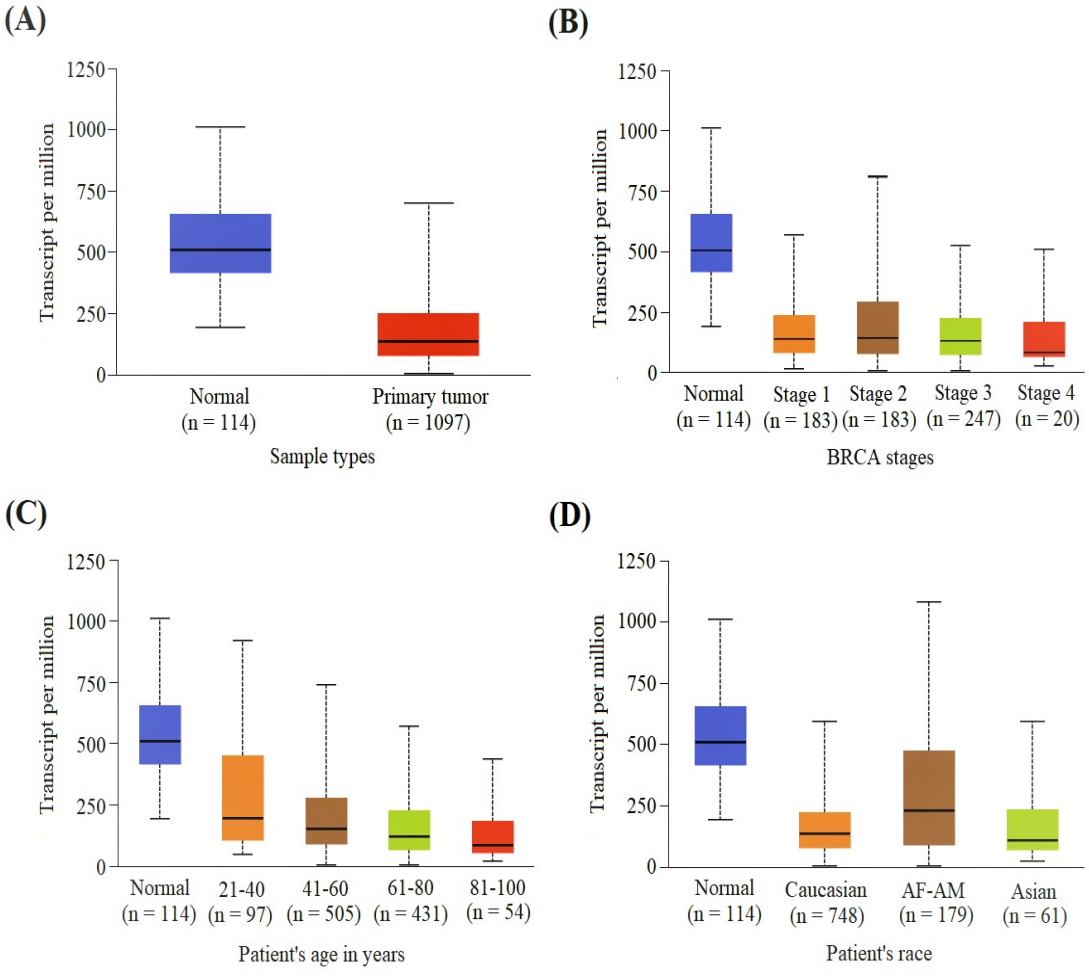

Figure 4: The LDHB expression rate was evaluated in the normal and BC samples based on the BC stages, patient age, and race. (A) Normal and BC (B) Normal vs BC stages (C) Normal vs BC Patient's age (D) Normal vs BC Patient's race.

statistical significance was noted for the expression of LDHB between the various groups of races affected by BC.

Thus, the study was conducted to elevate LDHB activity significantly by utilizing SAP. The interaction between the LDHB and SAP was identified by implementing molecular docking analysis to carry out further *in vitro* studies (Figure 5A, B). The SAP interacted well with the LDHB residues via hydrogen bonds that include GLU105, ASP196(2), HIS194, and ARG107. Likely, the pi-alkyl and pi-pi-T-shaped bond formed with the residues ARG100, ILE243, and TYR240 with a binding affinity of -8.4 kcal/mol and an RMSD of 2.707Å. Additionally, the hydrophobic residues such as VAL32, GLN101, SER106, ASN139, PRO140, GLU193, HIS194, SER197, GLY195, THR249, and ILE253 are surrounded the SAP-LDHB complex (Figure 5A, B). The molecular docking analysis revealed that the SAP interacted well with the LDHB, which may trigger the activity of LDHB, and this was further validated by the *in vitro* studies.

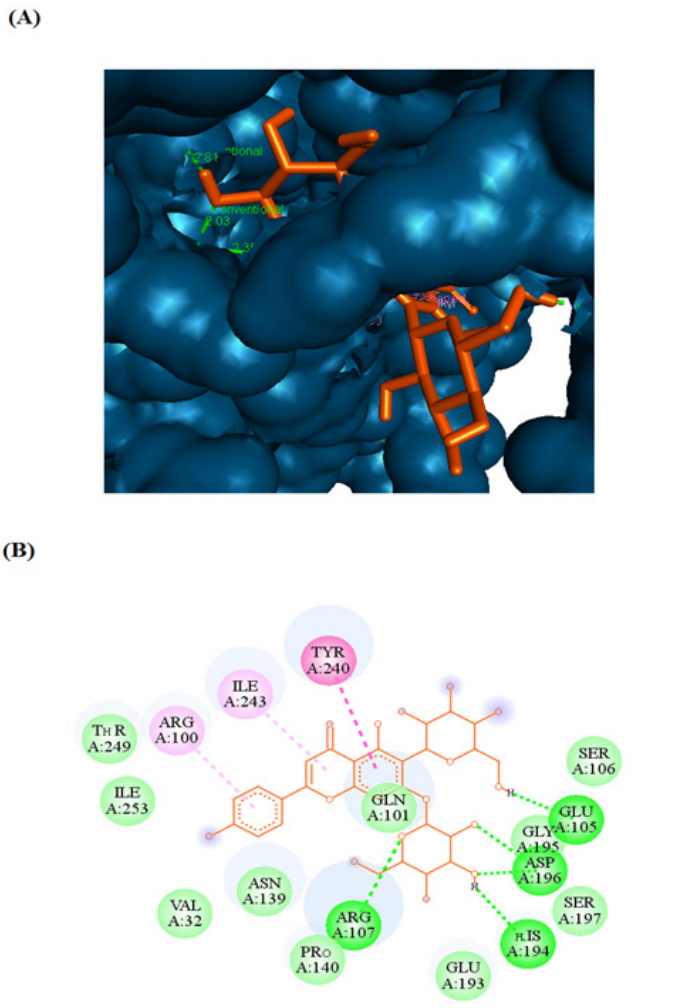

Figure 5: The 3D and 2D visualization of the docked SAP and LDHB complex. (A) The docked SAP and LDHB complex in 3D (B) The docked SAP and LDHB complex in 2D.

DISCUSSION

Many studies have emphasized the importance of a plant-rich diet to prevent cancer, including breast cancer.[26] Vegetables contain many bioactive compounds, like phenolic compounds, carotenoids, and especially flavonoids, which may contribute to the health benefits of plants based on nutrition. Recently, the level of research on the full study of flavonoids has increased significantly due to their potential applications.[27-29]

Because they are harmful to healthy cells and have no anticancer effect.[30] Therefore, there is a need to develop new strategies, such as combination therapy, that target cancer cells while minimizing adverse effects. Earlier works have displayed that SAP supports the chemotherapy of breast cancer cells by decreasing cell growth and inducing apoptosis. However, large doses will cause minimal side effects. In addition, SAP has low water solubility, which may affect its ability to be absorbed by cells and thus reduce its effectiveness. Apoptosis-induced chemotherapy cytotoxicity is a cancer treatment method aimed at reducing cell proliferation.[31] Literatures have shown that different dosages of SAP (0-100 µg/mL) can affect the viability of breast cancer cells. This effect is mediated by increasing oxidative stress and apoptosis pathways.

The removal of the cytoplasmic enzyme LDH from MDA-MB-231 cells was evaluated as an indicator of the positive effect of SAP treatment. Our study showed that LDH release was increased in breast cancer treatment groups. The release of LDH enzyme after treatment of cells with a SAP was measured as a biological measure of cell membrane cytotoxicity. This confirmed the *in vitro* cytotoxic activity of SAP against breast cancer growth. According to our findings, LDH enzyme was released into the cells in large amounts, higher than the levels found in unfixed cells, due to the damage to the membrane of cells exposed to different antibodies.

SAP has been shown to have numerous therapeutic properties, such as wound healing, anti-inflammatory, anti-angiogenic, anti-inflammatory, anti-proliferative, and anti-cancer. SAP has also been reported to enhance the outcome of cancer treatment when combined with other medications. In addition, SAP has been shown to increase apoptosis in combination with other drugs. Different doses of SAP have shown cytotoxic effects of up to 50% in different cancer models. In addition, SAP has been documented to effectively support growth inhibition and cytotoxic effects in various cancer cells at doses of 4 and 20 µg/mL. Studies have also shown that flavonoids have anti-oxidative properties, especially in cancer cells. Thus, this process inhibits cell proliferation and causes apoptosis.[32] Saponins have been reported to have good antioxidant properties in tumor cells through ROS-dependent activation of ERK1/2 and blocking Glutathione peroxidase (GSH) activity. It can be observed from the present investigation (Figure 3) that, the cells treated with SAP at doses IC_{50} have notably low levels of SOD, CAT and GSH in contrast to the control which had a significantly higher

level. Therefore, further studies are recommended to confirm its anti-inflammatory potential.

The previous study stated that breast cancer has a disproportionately high impact on Black and Hispanic women, leading to higher mortality rates. Disparities persist in early diagnosis, timely treatment, and access to care, genetic testing, and reconstructive surgery. Further research is needed to address this gap.[33] Our results suggest that low expression of LDHB contributes to cancer progression of BC. Recently, it has been highlighted that LDHB can act as a potential target for the breast cancer microenvironment and thus enhance NK cell activity.[34]

CONCLUSION

The SAP demonstrates encouraging synergistic cell growth effects in MDA-MB-231 cells. This is achieved by remarkably augmenting oxidative stress and triggering apoptosis, resulting in cell viability and cytotoxicity. The findings presented here prove that a higher intake of vegetables and fruits containing these flavonoids is linked to a lower occurrence of various forms of cancer. We propose that these combinations of flavonoids should be further researched as chemotherapeutic drugs for treating breast cancerous cells. These flavonoids could be further studied and administered *in vivo* using suitable carriers in a nanoparticle delivery system such as biopharmaceuticals.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ABBREVIATIONS

SAP: Saponarin; **LDH:** Lactate dehydrogenase; **HER2:** Human epidermal growth factor receptor 2; **H_2O_2:** Hydrogen peroxide; **DMEM:** Dulbecco's modified Eagle culture medium; **DMSO:** Dimethyl sulfoxide; **SOD:** Superoxide dismutase; **CAT:** Catalase; **GSH:** Glutathione; **CDC:** Centres for disease control; **LDHB:** Lactate dehydrogenase B.

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