Anticancer Potential Of Manzamine A In Mcf-7 And Mda-Mb-231 Breast Cancer Cells: Evaluation Of Cytotoxicity, Oxidative Stress, And Apoptotic Morphology

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Abstract

Background: Breast cancer remains one of the most prevalent malignancies among women worldwide, necessitating the discovery of novel therapeutic agents with improved efficacy and reduced toxicity. Marine-derived bioactive compounds have emerged as promising candidates in anticancer research.

Objective: This study aimed to evaluate the anticancer potential of the marine alkaloid manzamine a in two human breast cancer cell lines: mcf-7 (hormone receptor–positive) and mda-mb-231 (triple-negative). Cytotoxic effects of manzamine a were assessed using mtt and ldh assays following treatment with varying concentrations (10–100 µm) for 24 and 48 hours. The ic_{50} values were determined and used for further mechanistic investigations. Morphological alterations, intracellular reactive oxygen species (ros) generation, and apoptotic changes were evaluated using dcfh-da staining, propidium iodide (pi) staining, and acridine orange/ethidium bromide (ao/etbr) dual staining through fluorescence microscopy.

Results: The results demonstrated a dose- and time-dependent reduction in cell viability in both breast cancer cell lines, with a more pronounced cytotoxic effect observed after 48 hours of treatment. Increased ldh release indicated membrane damage, while fluorescence imaging revealed elevated ros production and characteristic apoptotic features, including chromatin condensation, nuclear fragmentation, and membrane blebbing.

Conclusion: These findings suggest that manzamine a exerts significant anticancer activity by inducing oxidative stress–mediated apoptosis in both hormone-positive and triple-negative breast cancer models. Overall, the study highlights manzamine a as a promising marine-derived lead compound for breast cancer therapy, warranting further molecular and in vivo investigations to validate its therapeutic potential.

Keywords: Breast Cancer, Marine Derived Compound, Manzamine, Cell Viability, Morphological Alterations.

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INTRODUCTION

With 2.3 million new cases reported in 2020, breast cancer (BC) is most common cancer among women globally [1]. According to recent national cancer estimates, BC is most common cancer in India accounting for 192,020 new cases in 2022, and approximately 13.6% of all cancers reported across both sexes [2]. The current treatment options ranging from surgery and chemotherapy to hormonal and targeted therapies though effective continue to show limitations [3]. Their effectiveness in managing more severe subtypes like TNBC seems to be limited increasing the substantial interest in finding new anti-cancer agents from natural sources [4]. Several plant-derived compounds like curcumin, resveratrol and withaferin A have been shown to induce apoptosis, inhibit metastasis as well as modulate oncogenic pathways in BC models [5]. Marine-derived compounds like fucoidan, sarcophine, pseudopterosins and halichondrin B have demonstrated potent cytotoxic and anti-proliferative properties [6]. Despite this progress, the therapeutic potential of many marine alkaloids remains underexplored, particularly in the context of diverse breast cancer phenotypes.

Among these marine alkaloids, Manzamine A has garnered attention as promising anti-cancer compound because of its ability to regulate apoptosis, autophagy, metastatic signalling and inflammatory pathways [7]. However, its activity in breast cancer remains limited. MCF-7 represents hormone receptor positive BC, while MDA-MB-231 models highly invasive TNBC with poor therapeutic response [8]. Examining Manzamine A in these contrasting phenotypes gives an opportunity to elucidate its mechanism of action and identify subtype specific sensitivity. Therefore, aim of this investigation as well as compare anti-cancer effects of manzamine A in MCF-7 and MDA-MB-231 BC cells to explore its potential as a novel marine derived therapeutic agent.

Cancer starts when a single cell in the body undergoes a genetic change that makes it grow more than it should. This cell and its copies continue to look normal but multiply too much, a condition known as hyperplasia. Over time, one of these cells may develop another mutation, which can make it grow even more uncontrollably. The cells from this mutation may begin to look and behave differently from normal cells, a condition known as dysplasia. Eventually, a rare mutation may occur that changes how the cells behave even more. These cells may become more abnormal in shape and growth. At this stage, if the tumor has not spread beyond its original area, it is known as in situ cancer. This type of cancer may stay contained for a long time, but some cells may acquire more mutations. If these changes allow tumor to invade surrounding tissues or shed cells in bloodstream or lymph nodes, tumor becomes malignant. These cancer cells can then form new tumors in other parts of body, known as metastases. These new tumors can be life-threatening if they damage important organs [9].

BC develops when cells within breast grow and divide uncontrollably. In many cases, it is first detected through a mammogram, a specialized X-ray that allows detailed imaging of breast tissue. Sometimes, breast cancer is identified when a woman or her healthcare provider feels a lump or mass in the breast. Breast cancers are categorized relied on availability or lack of certain receptor proteins on surface of tumor cells. These proteins influence both disease behaviour and treatment strategy [10]. Management and prognosis depend on both the stage at diagnosis and the biological type of cancer. For Stages 0–III, treatment aims for complete cure and usually includes combination of surgery, drug therapy, as well as sometimes radiation therapy. In contrast, Stage IV (metastatic) breast cancer is typically managed with systemic medications to control disease progression and maintain quality of life rather than to achieve cure [11].

Among natural compounds, alkaloids are of particular therapeutic interest due to their broad biological and pharmacological actions. Alkaloids have been used in oncology, and it has been found that they induce cancer cell death, prevent metastasis and angiogenesis, improve immune responses, and overcome drug resistance. Although they are effective, low natural yields frequently result in over harvesting making it necessary to adopt sustainable means of production. All in all, alkaloids and other phytochemicals have provided a good starting point to create safer and more

efficient anticancer drugs with multiple molecular pathways in tumor progression [12].

Manzamine A is alkaloid of marine origin that has been initially extracted out of the sponge *Haliclona sp.* gathered near Manzamo, Okinawa, Japan. It is the most chemically active and well-known β-carboline-containing macrocyclic alkaloid of the manzamine family, with its peculiar blend of extraordinary structural variety and powerful biological behavior. The polycyclic Manzamine A structure has attracted interest because of its wide pharmacological effects, such as antitumor, antimalarial, antibacterial, antiviral, and anti-inflammatory action. The study on manzamines has also shown that they can be active owing to the capacity to interact with numerous molecular targets, including kinases, DNA, and mitochondrial processes, and thus make good drug discovery candidates. The discovery of a number of natural analogues and derivatives of Manzamine A by further investigations into marine sponges has highlighted the importance of marine biodiversity in the creation of new therapeutic agents [13] [14] [15].

The aim of this research is to determine anticancer activities of marine alkaloid Manzamine A on human BC cell lines (MCF-7 (hormone-receptor-positive) as well as MDA-MB-231 (triple-negative). In particular, paper explores the cytotoxicity of Manzamine A in the MTT and LDH assays, and its effect in causing morphologic changes, oxidative stress and apoptosis in the immunofluorescence imaging, ROS (DCFH-DA) assay, and PI and AO/EtBr staining. The proposed study will explain the potential of Manzamine A as a promising phytochemical-based anticancer agent to be used against biologically different subtypes of BC.

METHODOLOGY

Cell culture

The "MCF-7 and MDA-MB-231 human breast cancer cell lines were obtained from the National Centre of Cell Science (NCCS) in Pune, India. The cells were then cultured in Dulbecco's Modified Eagle Medium (DMEM; HiMedia, India), enriched with 10% fetal bovine serum (FBS) and a penicillin-streptomycin antibiotic solution (1:1). All cultures were maintained in a humidified CO_2 incubator at 37°C with 5% CO_2 and regularly passaged to ensure" logarithmic growth [16].

Experimental Design

Dose–response curves were generated by measuring cell viability (MTT) across the concentration series at 24 and 48 h. Percent viability was calculated. IC_{50} values are calculated for each cell line and time point. The IC_{50} concentrations were used for subsequent mechanistic assays when indicated. For each cell line the experimental groups given in Table 2 were used:

Table 2 – Experimental Design

	"Group I - Control	**Group II**	**Group III**
MCF-7	MCF – 7 Untreated cells	Manzamine A treated 24hrs	Manzamine A treated 48 hours
MDA-MB-231	MDA-MB-231 Untreated Cells	Manzamine A treated 24hrs	Manzamine A treated 48 hours"

Group I

This group consists of MCF-7 and MDA-MB-231 cells that have not been exhibit to Manzamine A. These untreated cells serve as the control, providing baseline data on cell morphology, viability, and physiological behaviour, against which the effects of the treatment can be compared.

Group II

In this group, both "MCF-7 and MDA-MB-231 cells have been treated with Manzamine A for 24" hours. It enables the evaluation of early cytotoxic and morphological effects caused by compound and its early effects on the cellular stress and apoptotic processes.

Group III

MCF-7 and MDA-MB-231 cells that had been incubated with Manzamine A after 48 hours are part of this group. The time-dependent effects, such as increased cytotoxicity, the build-up of oxidative stress, and the initiation of apoptosis, that are time-dependent can be evaluated under prolonged exposure of the compound, which can inform about the sustained anticancer effects of the compound.

The clinical treatment concentrations of Manzamine A were calculated on the IC_{50} values. The dose response curve gave the $IC_{50,}$ which was used as a measure of concentration of compound needed to inhibit half of the cell viability.

In vitro analysis

Cell Viability Assay

MTT Assay

Vitality of Manzamine A cells has been evaluate utilising MTT colorimetric test. "MDA-MB-231 and MCF-7 cells have been cultured in 96-well plates at density of 10^4 cells per well and incubated" overnight to facilitate adherence to substrate. Cells have been incubated with various doses of Manzamine A (10-100µM) that had been prepared in a mixture of dimethyl sulfoxide (DMSO) (1 percent) in DMEM, respectively, over 24 and 48 hours in a CO 2 incubator that was kept humid. After treatment, wells have been lightly washed with 1X phosphate-buffered saline (PBS) followed by addition of MTT solution into each well which was then incubated at 1 hour. Dissolution of the resulting formazan crystals was done in DMSO and absorbance has been measured at 495 nm [17] [18].

LDH Assay

LDH release assay has been utilised to determine cytotoxicity produced by Manzamine A. Given the differing concentrations of Manzamine A (10100 µM) used to treat each well, the culture supernatant in each well was finally pipette-transferred into a new plate to proceed to LDH analysis. The LDH activity in the supernatant was quantified using the manufacturer's protocol, and the extent of membrane damage has been expressed as percentage of LDH release relative to control groups [19].

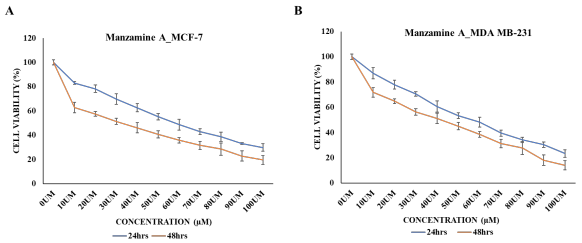
Immunofluorescence assays

The morphological alterations of MCF-7 and MDA-MB-231 cells following treatment with manzamine A (Control, IC50 concentrations for MCF-7 (55.83 µM for 24hrs and 29.74 µM for 48hrs and MDA MB-231 cells (60.87 µM for 24hrs and 39.785 µM for 48hrs) were examined using immunofluorescence and bright-field microscopy. We employed a 5-µM "non-fluorescent intracellular probe, 2',7'-dichlorofluorescein-diacetate (DCFH-DA), to evaluate the generation of reactive oxygen species (ROS). A total of $5x10^5$ MCF-7 and MDA-MB-231 cells have been treated with manzamine for 40mins. Thereafter, DCFH-DA was" introduced, and the cells were incubated "in darkness for an additional 20 mins at 37°C. The cells have been then resuspended in 1ml of ice-cold PBS. Propidium iodide (PI) and acridine orange/ethidium bromide (AO/EtBr) staining has been employed" to detect apoptotic cells. AO/EtBr solution has been added (10 µL) to treated cells that had undergone manzamine A treatment and the solution mixed by laying a coverslip over the sample. The condensed chromatin and fragmented nuclei of the apoptotic cells had specific reddish flakes of fluorescence that can be seen under a fluorescent microscope. The oxidative activity of ROS, along with the staining results from PI and AO/EtBr, was visualized utilising Olympus fluorescence microscope (#BX53, Tokyo, Japan), equipped with Zenoptik camera [20] [21].

STATISTICAL ANALYSIS

Every experiment was performed "in triplicate (n = 3). The data was expressed as mean ± standard deviation (SD). Tukey's post hoc test" was utilized following one-way ANOVA for statistical comparisons between groups. A statistically significant result was established as
$p < 0.05$.

RESULTS



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IC50	24hrs (µM)	48hrs (µM)
MCF-7	55.83	29.74
MDA MB-231	60.87	39.785

Figure 1. Manzamine A inhibits MCF-7 and MDA MB-231 cell growth by MTT assay. MTT assay for "cell viability shows the increasing concentration of manzamine A (0-100µM) for 24-to-48-time intervals in MCF-7 (A) and MDA MB-231 (B) cells. GraphPad Prism 8 has been utilised to determine IC50 values. (C) IC_{50} values for manzamine A was mentioned for MCF-7 and MDA MB-231" cells.

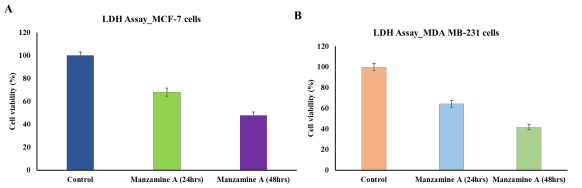

Figure 2. Manzamine A inhibits MCF-7 and MDA MB-231 cell growth by LDH assay. LDH assay for cell viability shows the increasing concentration of manzamine A (IC_{50} concentration) for 24-to-48-time

intervals in MCF-7 (A) as well as MDA MB-231 (B) cells.

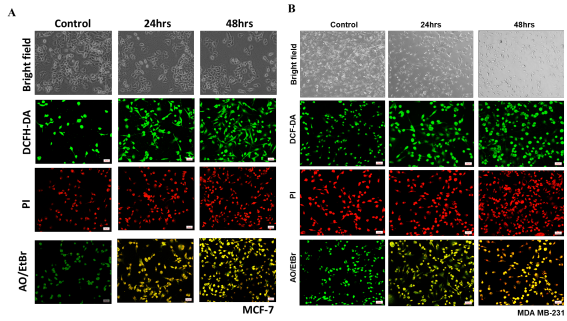

Figure 3. Cytomorphological changes were observed by treating compound (IC_{50} concentration) for 24-to-48-time intervals in MCF-7 (A) and MDA MB-231 (B) cells. Morphological investigation, DCFH-DA for intracellular reactive oxygen species activity, propidium iodide for nuclear staining, and "acridine orange/ethidium bromide staining for early and late apoptosis in KB cells at 20X magnification. Images have been examined with an Olympus microscope equipped with a Zenoptik camera and quantified using ImageJ" software.

DISCUSSION

Manzamine A inhibits cell growth in breast cancer cells

In "order to explore cytotoxic effect of manzamine A, we carried out a detailed analysis of the effects of this molecule on the MCF-7 and MDA-MB-231 cell lines. Through MTT" assay, we determined viability of cells subjected to different concentrations of manzamine A, 0-100 Muslims at three-time intervals: 24 and 48 hours. This method enabled us to determine the dose-time and time-dependent effects of manzamine A on these cancer cell lines. In Figure 1, we have observed a distinct "dose-dependent reduction of cell viability in both MCF-7 as well as MDA-MB-231 cells, with the cytotoxic effect being more" significant at 48-hour time interval than its effect at the 24-hour time interval. Manzamine A 24-hr treatment has less inhibition and percentile of cell death in 48 hours time interval is high in both cell lines. This implies that long-term effects of the exposure to manzamine A were more intense in inhibiting cell growth, with the best levels leading to significant decrease in viability of the two cell lines as well as the 48-hour treatment was preceded in the future experiments. Moreover, cytomorphological examination (Figure 2) showed that there were significant changes in cell structure after 48 hours of treatment. The key cellular signs of stress and cell death, such as cell shrinkage, membrane blebbing, and significantly decreased cell population, were found in treated cells. These morphological alterations provide the quantitative findings of the MTT assay, highlighting the fact that manzamine A can inhibit the growth of cancerous cells. Together, this preliminary study on cytotoxicity suggests that manzamine A has a significant dose- and time-dependent anti-cancer effect on both the MCF-7and MDA-MB-231 cells. More research is justified to understand mechanisms of its anti-cancer effect and to test its suitability in therapeutic utilisation of breast cancer.

Immunofluorescence staining by treating manzamine A in MCF-7 and MDA MB-231 cells

We examined cytotoxic properties of manzamine A against MCF-7 and MDA-MB231 using nuclei staining using propidium iodide (PI) staining, indicating that there was significant dead cell count among the treated cells. Manzamine A at specific concentrations induced a high level of cytotoxicity as data from both MCF-7 and MDA-MB-231 cells has been characterized by significant increase in cell mortality in both cell lines relative to untreated strain group. These investigations propose that manzamine A is a good activator of cell death pathways in these cancer cells. Also, activity of ROS in cells after the treatment with manzamine A was high, which means that this level of activity was increased, indicating that the oxidative stress response was higher. This rise in the level of ROS supplements our PI staining results as oxidative stress is known trigger of cancer cell death. The increased ROS activity due to the presence of manzamine A highlights its ability to cause cell damage, which is among the effects on cancer in a dose-dependent fashion. To further demonstrate the cell death mode, nuclear staining using "acridine orange/ethidium bromide (AO/EB) has been done to determine presence of an apoptotic cell. Cell populations of MCF-7 and MDA-MB-231" were found to exhibit a significant increase in early as well as late apoptotic approaches in cells after exposure to manzamine A after 24 and 48 hours as indicated in Figure 3. The AO/EB staining exhibited characteristic apoptotic characteristics, including chromatin condensation, nuclear fragmentation, and altered membrane permeability, indicative of programmed cell death. These investigations suggest that manzamine A may exert cytotoxic effects by inducing apoptosis and enhancing oxidative stress, potentially serving as an effective anti-cancer agent. The fact that apploptotic markers and oxidative stress were also observed means that manzamine A has the potential to affect biomolecular processes of early and late apoptosis. Mazamane A therefore might be viewed as a promising

therapeutic candidate that may be used to forestall growth as well as progression of BC.

Conclusion and Future Aspect

Overall, this research paper shows that manzamine A has a high level of "cytotoxic activity against MCF-7 along with MDA-MB-231 breast cancer" cells. Compound elicited visible dose- as well as time-dependent cell-viability drop with a 48-hour exposure being the most effective inhibitory effect. Further confirmation of the morphological alterations and fluorescence-based techniques (PI and AO/EB stains) showed that manzamine A causes extensive cell death by apoptosis, and an increase in levels of ROS is observed.

All these findings are pointing towards the fact that manzamine A inhibits growth of breast cancer cells mostly via apoptotic processes mediated by ROS. Since it has a uniform cytotoxic effect on hormone-positive and triple-negative BC models, manzamine A is a promising lead molecule that can be subjected to additional preclinical studies.

All these findings are pointing towards the fact that manzamine A inhibits growth cells of BC mostly via apoptotic processes mediated by ROS. Since it has a uniform cytotoxic effect on hormone-positive as well as triple-negative BC models, manzamine A is a promising lead molecule that can be subjected to additional preclinical studies.

In order to increase clinical potential, it might also be studied how to optimize the structure and formulation approach, including analogy development, nano preparation, or combination with the available chemotherapeutic therapy, which could increase the potency, stability, and targeted delivery.

All in all, further exploration on the level of molecular, cellular and in vivo will be necessary to prove manzamine A as the potential lead compound in treating BC and move it to the next research stage of preclinical and later clinical study.

CONFLICT OF INTEREST

The authors state that there are no conflicts of interest.

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