Negative Effect on the Anti-Oxidant Potential of Cilnidipine in Breast Cancer Cell Lines – An in Vitro Study

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Abstract

Background: One of the malignancies that is most frequently diagnosed worldwide is breast cancer. According to recent data, approximately 2.3 million women worldwide receive a breast cancer diagnosis, and 6,85,000 of them pass away. One of the treatment modalities for breast cancer is chemotherapy. Chemotherapy can induce cytotoxicity in normal cells as well. Studies have shown cytotoxicity effects of calcium channel blockers. Cilnidipine is one of the dihydropyridine calcium channel blockers. The antioxidant effects of Cilnidipine have been observed through its benefits in cardioprotection, reno protection and neuroprotection. This study aims to find the anti-oxidant potential of Cilnidipine in MDA-MB 231 breast cancer cell lines.

Methodology: The study was conducted in a tertiary care hospital , Chennai, Tamilnadu from December 2021-July 2022. The anti-oxidant activity is evaluated by Lipid peroxidase assay. In this assay, $4x10^5$ MDA MB 231 cells were incubated for twenty-four hours. After incubation, the cells were either left untreated (control group) or given cilnidipine treatments at various concentrations. After this, the cells were exposed to 1 mM hydrogen peroxide for six hours to promote oxidative damage. Cells were taken after treatment and sonicated for 10 seconds to lyse them. The cell lysates were then centrifuged for 10 minutes at 4°C using 10,000 rotations per minute. The supernatants were then combined with a solution containing an equivalent volume of 0.375 TBA (thiobarbituric acid), 15% trichloroacetic acid, and 0.25 N HCl solution and were heated for 15 minutes in a boiling water bath before being centrifuged at 10,000 rotations per minute for 5 minutes. Finally, the supernatant's absorbance at 535 nm was determined.

Result: Cilnidipine shows oxidative effect and produces cytotoxicity in the cancer cells.

Conclusion: Cilnidipine causes cytotoxicity like conventional anti-cancer drugs by causing oxidative damage to the cancer cells.

Keywords: Anti-oxidant, Cilnidipine, MDA MB 231 breast cancer cell lines, anti-cancer; cytotoxicity.

INTRODUCTION

Cancers are spreading throughout the world and its incidences are increasing worldwide over the years. It can develop in almost any mammalian organ giving rise to a wide array of clinical outcomes[1]. Breast cancer is one of the most often diagnosed cancers and the second most common cause of cancer-related death in women worldwide. [2]According to recent data, approximately 2.3 million women worldwide receive a breast cancer diagnosis, and 6,85,000 of them pass away. [3]In India, 1 in every 28 women in urban areas and 1 in every 60 women in rural areas are affected by breast cancer. [4]The etiology of breast cancer can be familial or genetic factors, endocrine factors and environmental factors. [5]Treatment of breast cancer can be hormonal

therapy, chemotherapy, radiation therapy, and surgical. Most of the anticancer drug regimens contain cytotoxic drugs intended to kill cancer cells. It is observed that while destroying the cancer cells, these drugs produce damage to the normal cells in the body and result in side effects. Some of the common side effects associated with anticancer drugs are hair loss, diarrhoea, and bone marrow suppression.[6]

Calcium is one of the most important molecules in human body. Calcium is essential for various physiological processes in human body. Studies show that in cancer an increase in intracellular calcium facilitates proliferation, metastasis, and resistance to apoptosis probably by calcium dependent activation of concerned enzymes. [7]G R Crabtree et al observed that the expression of genes related to cell growth and death is controlled by nuclear transcriptional factors. NFAT (Nuclear Factor of Activated T cells) proteins are transcription factors that depend on calcium. Calcium/calmodulin-dependent protein phosphatase and calcineurin control its nuclear translocation and transcriptional activity[8]

Calcium channel blockers are the one of the safest drugs used in conditions like hypertension, arrhythmias, angina pectoris and migraine. Calcium channel blockers can be dihydropyridines like Amlodipine, Cilnidipine, Nicardipine and non-dihydropyridines like Verapamil and Diltiazem. Cilnidipine is an L and N-type calcium channel blocker and by blocking N-type calcium channels, Cilnidipine prevents reflux tachycardia by inhibiting sympathetic system activation.[9]

In the kidneys, it decreases proteinuria without increasing serum creatinine concentration. [10]Takeshi Soeki et al, showed the renoprotective characteristics of cilnidipine. They concluded that Cilnidipine, when compared to Amlodipine, decreased the urinary excretion of albumin and other proteins, including 8-hydroxy-2'-deoxyguanosine (OHdG) and liver-type fatty-acid-binding protein (L-FABP), in the hypertensive patients, probably because of its antioxidative properties.[11,12]

Cilnidipine also has better anti-ischemic action. Takatsu M et al, observed the cardioprotective effect of Cilnidipine. The researchers found that in Dahl salt-sensitive mice, Cilnidipine reduced left ventricular (LV) fibrosis, diastolic dysfunction, and LV concentricity more than Amlodipine did. It is likely that cilnidipine's higher anti-oxidant and anti-inflammatory effects are what contribute to its improved cardioprotective efficacy.[13,14]

Lee YJ et al detected the neuroprotective effect of Cilnidipine. According to their research, cilnidipine kills free radicals, lowers oxidative stress, and has a neuroprotective impact.[15]

The antiproliferative properties of cilnidipine in vascular endothelial cells have been explained by Wen-Yang et al. According to their research, Cilnidipine has an antiproliferative effect by preventing DNA synthesis brought on by factors that promote cell development and by suppressing the expression of TGF-1 mRNA in vascular smooth muscle cells from spontaneously hypertensive rats.[16]

Thus, Cilnidipine is a dihydropyridine with relatively safe and efficacious profiles when compared to other dihydropyridines.

According to studies, calcium channel blockers can be used to slow the spread of cancer by stopping calcium from entering cells. [17]Godfraind T. et al. showed that calcium channel blockers have antioxidant properties and can be used to treat hypertension. [18]There are no conclusive evidence for anti-oxidant effect of calcium channel blockers in cancer cells. This study aims to find whether Cilnidipine has anti-oxidant activity in breast cancer cell lines which can protect the normal cells when it is used in anticancer therapy.

METHODOLOGY

The study was conducted in a tertiary care hospital, Chennai, Tamilnadu from December 2021-July 2022 in collaboration with Whizbang Bioresearch Pvt Ltd, Chennai, after obtaining Institutional ethics committee approval. The Department of Biotechnology at the National Center for Cell Sciences (NCCS), located in Pune, India, provided the MDA-MB-231 human breast cancer cell lines.

Antioxidant activity assessment by measurement of lipid peroxidase:

Oxidative stress causes the lipids in the cell membrane to breakdown in lipid peroxidation. During this procedure, free radicals are extracted from the lipids. To evaluate oxidative stress, lipid peroxidation must be quantified. As byproducts, reactive aldehydes such malondialdehyde (MDA) and 4-hydroxynonenal (4- HNE) are created. To detect oxidative stress and evaluate lipid peroxidation, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are widely utilised.[19]

Malondialdehyde (MDA), a product of cellular lipid peroxidation, is measured using the thiobarbituric acid-reactive substances (TBARS) method to quantify lipid peroxidation. In a nutshell, $4x10^5$ MDA MB 231 cells were incubated for twenty four hours. After incubation, the cells were either left untreated (control group) or given cilnidipine treatments at various concentrations. After this, the cells were exposed to 1 mM hydrogen peroxide for six hours to promote oxidative damage. Cells were taken after treatment and sonicated for 10 seconds to lyse them. The cell lysates were then centrifuged for 10 minutes at 4°C using 10,000 rotations per minute. The supernatants were then combined with a solution containing an equivalent volume of 0.375 TBA (thiobarbituric acid), 15% trichloroacetic acid, and 0.25 N HCl solution and were heated for 15 minutes in a boiling water bath before being centrifuged at 10,000 rotations per minute for 5 minutes. Finally, the supernatant's absorbance at 535 nm was determined.[20]

RESULT

The study result shows that Cilnidipine produces oxidative damage to the cancer cells while the control showed no oxidative damage to the cells. The Lipid Peroxidation IC50 value of Cilnidipine in MDA -MB -231 cell line against H2O2 induced oxidative injury was found to be 128.418 uM. The results are shown in figures

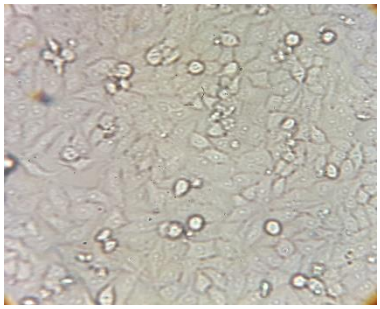

Fig 1a) Effect of cilnidipine at concentration of 12.5 µM

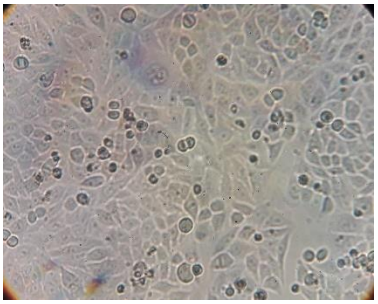

Fig 1b) Effect of cilnidipine at concentration of 25 µM

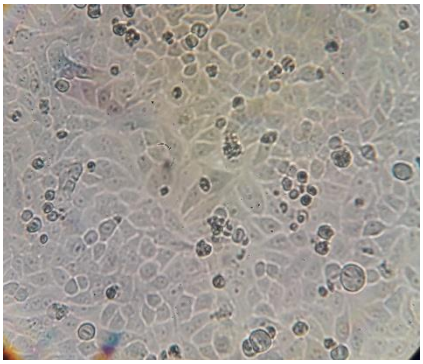

Fig 1c) Effect of cilnidipine at concentration of 50 µM

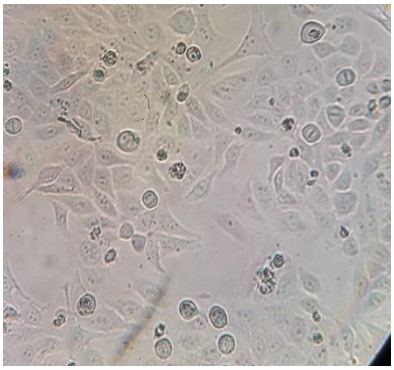

Fig 1d)Effect of cilnidipine at concentration of 100 µM

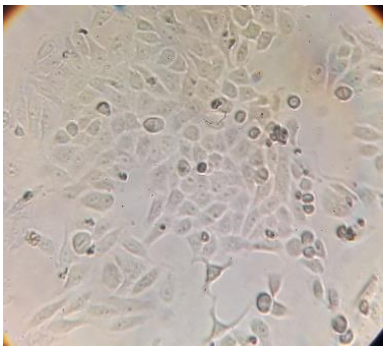

Fig 1e) Effect of cilnidipine at concentration of 200 µM

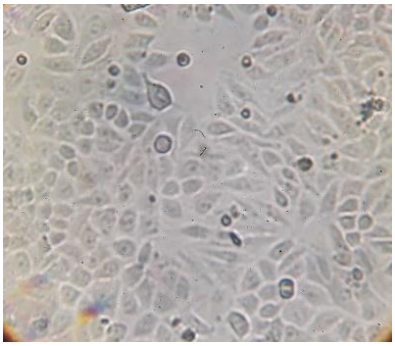

Fig 1f) Effect with Control

Table 1. Measurement of absorbance of the supernatant when the cells are not treated (control) as well as when the cells are treated with different concentrations of cilnidipine. Inhibition % calculated based on linear regression graph by employing the formula

(Absorbance of sample/Absorbance of control) X 100 = Cell Viability (%)

Concentrations of cilnidipine ((µM))	**Absorbance (I)**	**Absorbance (II)**	**Average**	**Inhibition %**
Control	**0.544**	**0.543**	**0.544**	**0.00**
12.5	**0.424**	**0.420**	**0.422**	**22.06**
25	**0.387**	**0.388**	**0.388**	**28.86**
50	**0.341**	**0.354**	**0.348**	**37.32**
100	**0.288**	**0.290**	**0.289**	**47.06**
200	**0.201**	**0.200**	**0.201**	**63.05**

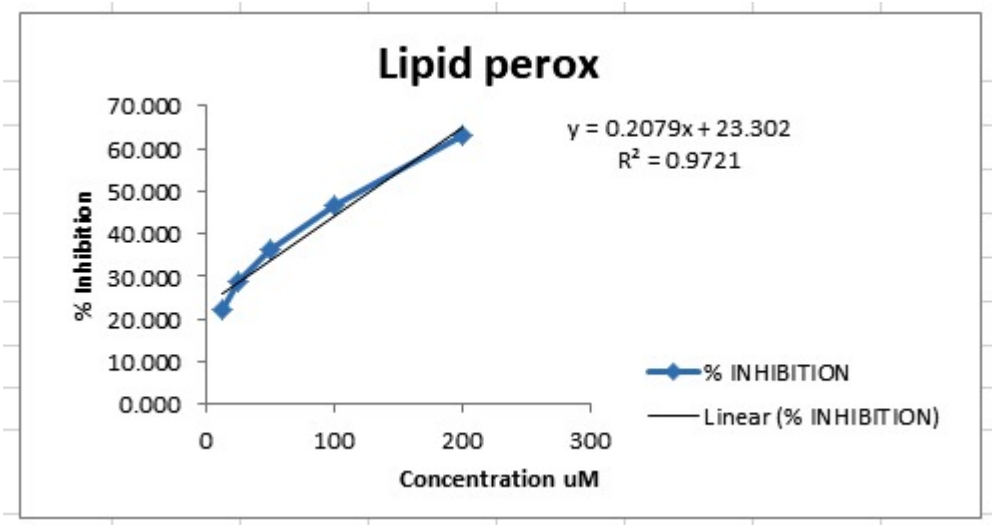

Fig 2. Linear regression graph showing the inhibition % at different concentrations of Cilnidipine.

DISCUSSION

The global incidence of cancers is increasing over the decades. This trend is very much related to lifestyles such as diet, obesity, physical inactivity, smoking, alcohol consumption, and hormonal imbalance. One of the treatment modalities for cancer is treatment with cytotoxic drugs. These medications cause cytotoxicity in both healthy and cancerous cells. Cytotoxicity to normal cells is associated with adverse effects like diarrhoea, hair loss and bone marrow suppression.

Researchers are now focused to generate anticancer drugs with very minimal side effects. Novel drug delivery of the existing anticancer drugs has also been carried out. Studies conducted by Chow et al, discuss about Nanocarrier-based anti-cancer drug delivery. They discovered that RES/mononuclear phagocyte system cells in the liver and spleen in particular, can quickly eliminate nanodrugs. This quick clearance may make them more harmful and less effective in these off-target organs[21,22,23]. The area of adverse effects associated with anticancer drugs hence remains unsolved still.

Calcium channel blockers are one the most efficacious and safest antihypertensive drugs. Among the calcium channel blockers, dihydropyridines are safer when compared to non-dihydropyridines. Cilnidipine, a dihydropyridine calcium channel blocker shows renoprotective, neuroprotective and cardioprotective properties probably by its anti-oxidant effect. Cytotoxicity of calcium channel blockers by blocking calcium entry has already been proved by various trials. Cilnidipine can thus be probably used as an anti-cancer agent.

The results of this study contradict these anti-oxidant benefits of Cilnidipine. In this study, Cilnidipine produces cytotoxicity by oxidative damage to cell walls of the breast cancer cell lines which is measured by the Lipid peroxidase assay.

Morakinyo AO et al showed that Calcium channel blockers produced reduced fertility in rats by inducing significant oxidative stress in the testes of male rats and resulting in decreased sperm count and motility.[24]

Berkowitz BA et al, showed that D-cis-Diltiazem can cause oxidative stress induced damage of rods in the retina of B6 mice models.[25]

The results of the study are consistent with some of the previous trials.

CONCLUSION

The results of the lipid peroxidase assay show that Cilnidipine causes cytotoxicity by oxidant activity which means that Cilnidipine can also induce cytotoxicity to normal cells like any other conventional anticancer drugs.

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Conflict of Interest

The authors declare that there was no conflict of interest.

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