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A Comparative Analysis of D-Mannoheptulose and Trastuzumab in Breast Cancer: Targeting the Warburg Effect versus HER2 Signaling

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Abstract

Background: D-Mannoheptulose (MH) is a hexokinase inhibitor that hampers glycolysis whereas, Trastuzumab (TZB) is an immunotherapeutic drug that causes apoptosis in cancer cells. Interestingly, Cancer cell metabolism modulation serves as one of the excellent targets for treatment translation for the reason that it is capable of preventing cell proliferation. **Objective**: Aiming to determine the therapeutic prospects of D-Mannoheptulose and Trastuzumab, this study is designed to examine their cytotoxic characteristics against normal and breast cancer cells. **Method**: MCF-7 breast cancer cell line and Human mammary epithelial cells (HMECS) were cultured by RPMI-1640 and Minimum Essential Medium (MEM). Subsequently, the synthesis of MH and TZB was conducted. Afterwards both cell lines were treated to different doses of the agents prepared. Cellular toxicity was completed via MTT assays. **Results**: Results showed that both MH and TZB induced significant antimetastatic and antiproliferative activities against breast cancer MCF-7 cells with the IC50 values proving efficacy. In addition, MH and TZB had no observable cytotoxic effects on HMEC which were the healthy and tumor nontarget cells. **Conclusion**: Through increased cytotoxicity and apoptosis induction, D-Mannoheptulose and Trastuzumab can help deal with the metabolic aspect of tumor cells in Her2 positive breast cancer patients.

Key words: Apoptosis, Glycolysis inhibition, Cytotoxicity, MCF-7 cell line and Herceptin

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تحليل مقارن لـD مانوهيبتولوز وتراستوزوماب في سرطان الثدي: استهداف تأثير واربورغ مقابل إشارات HER2

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الخلاصة

المقدمة: يُعدّ د-مانوهيبتولوز (MH) مثبطًا للهيكسوكيناز، مما يعيق عملية تحلل الجلوكوز، بينما يُعدّ تراستوزوماب (TZB) دواءً مناعيًا يُحفّز موت الخلايا المبرمج في الخلايا السرطانية. ومن المثير للاهتمام أن تعديل استقلاب الخلايا السرطانية يُعدّ أحد الأهداف الممتازة لتطوير العلاجات، نظرًا لقدرته على منع تكاثر الخلايا. **الهدف:** تهدف هذه الدراسة إلى تحديد الإمكانيات العلاجية لكل من د-مانوهيبتولوز وتراستوزوماب، وذلك من خلال فحص خصائصهما السامة للخلايا ضد الخلايا الطبيعية وخلايا سرطان الثدي. **المنهجية:** زُرعت خلايا سرطان الثدي MCF-7 وخلايا ظهارة الثدي البشرية (HMECS) في وسط RPMI-1640 ووسط .MEM بعد ذلك، تم تحضير MH و TZB ثم عُولجت كلتا السلالتين الخلويتين بجرعات مختلفة من المواد المحضّرة. تم تقييم السمية الخلوية باستخدام اختبار .MTT **النتائج:** أظهرت النتائج أن كلاً من مانوهيبتولوز (MH) وتراستوزوماب (TZB) أحدثا نشاطًا مضادًا للانتشار والتكاثر بشكل ملحوظ ضد خلايا سرطان الثديMCF-7 ، حيث أثبتت قيم IC50 فعاليتهما. بالإضافة إلى ذلك، لم يُلاحظ أي تأثير سام للخلايا على خلايا HMEC السليمة وغير المستهدفة بالورم. **الاستنتاج:** من خلال زيادة السمية الخلوية وتحفيز الاستماتة الخلوية، يمكن أن يُسهم كل من مانوهيبتولوز وتراستوزوماب في معالجة الجانب الأيضي للخلايا السرطانية لدى مريضات سرطان الثدي الإيجابي لمستقبلات.HER2

Introduction

Cancer is categorized as a collection of diseases characterized by the uncontrolled growth and spread of malignant cells that can lead to mortality (Tarver T., 2012). While inside the normal process, ordinary cells divide then mature to die, the contrary happens to abnormal cells as they don't progress and therefore lead to cancer (Kim JH. et al, 2025). Global estimates show that there is an upward trend in cancer cases which has become a leading cause of death globally (Bray F., et al., 2018). Cancer cells exhibit a quick rise in their glycolytic flux to produce ATP and utilize this biosynthetic pathway to promote and aid in the cell division. There is a possibility that targeting glycolysis could be useful in the treatment of tumors (Al-Ziaydi AG. et al.,2020). As postulated by the Warburg effect, tumor cells have a better tendency to make use of glycolysis to meet their ATP requirement (Vander Heiden MG., et al.,2009). In general, Normal tissues require less glucose than cancer cells, which is the basis for cancer therapy (Aft RL., et al., 2002), (Jasib Almzaiel A., et al., 2021). Apoptosis results from a multistep process triggered by mitochondrial and endoplasmic reticulum stress responses, as well as reactive oxygen species. The strategies for cancer management emphasize the induction of apoptosis as the proposed target mechanism to regulate cancer cells. (HMECs) are a distinct cell type that forms epithelial tissue of breast glands. They are crucial for the normal growth of the breast and breastfeeding, yet they also serve as precursors to breast cancer. HMECs, derived from the breast tissue of adult women, are specifically prepared for the investigation of breast cancer development and are extensively utilized in breast cancer research, whereas normal human mammary epithelial cells were previously employed to characterize epithelial cell properties. These normal human mammary epithelial cells (HMEC) were procured from a 50-year-old Caucasian female and grown under primary conditions (Bertram C, Hass R., 2008). Cell culture refers to the growth of cells which can be prokaryotic, eukaryotic or plant cells in artificial controlled environment. In this technique, cells are first taken out from a certain type of tissue, then broken down into smaller aggregates by chemical agents or physical methods before being cultured. Primary culture refers to the initial cell culture established by isolating cells from tissue, which can then be classified as a cell line or sub-

clone following its first subculture (Freshney RI., 2025). Cell culture techniques have an extensive application in both of biology fields at the cellular level and molecular level enabling remarkable model systems to help in determining normal cell physiology, the biochemistry of cells, mutagenesis and carcinogenesis (Vunjak-Novakovic G, Freshney, 2024). According to the scientists who believe in the significance of D-Mannoheptulose (MH) in cancer therapy and their findings suggest methanolic extracts of avocado fruit and leaves are efficient in cell ant mutagenesis targeting and curing, (Paul R., et al., 2011), Also planting this plant is. said to be highly beneficial (Nordal A, Benson AA., 1954). According to recent M. Bullatti Chloroform fractions of avocado extracts have been found to selectively induce apoptosis in cancer cell line human oral epithelial patients in vitro (Ding H., et al., 2009). The phosphorylation of glucose is the first and the key process involved in glycolysis the initial step. This is catalyzed by the enzyme hexokinase HK to form glucose-6-phosphate Glu-6-P. The Glu6p then gives rise to 2ATP molecules (Waugh A, Grant A., 2009).

Breast cancer is a complex illness comprising multiple genetic subtypes that display unique biological properties and clinical behaviors. HER2-positive breast cancer is defined by the overexpression or amplification of the HER2 gene. HER2-positive breast cancer is often more aggressive and has a lower prognosis than other subtypes (Slamon DJ. et al 2001). Indeed, the management of HER2-positive BC has experienced a revolution with the use of trastuzumab, referred to as Herceptin in the literature. Trastuzumab selectively bind to the HER2 receptor since the receptor is overexpressed on the surface of HER2-positive breast cancer cells (Hudis CA., 2007). It has been illustrated that Trastuzumab has anticancer effects due to interaction with the HER2 receptor and interference of many activities successively; these include HER2-mediated signaling, cytotoxic effects of ADCC (Valabrega G., 2007). Trastuzumab has been proved to be therapeutically effective in the treatment of HER2-positive breast cancer in many clinical trials, as it has been studied extensively. The integration of trastuzumab into their conventional monoclonal antibody boosted the general durability of life and the durability of survival without the progression of the illness in patients with HER2-positive metastatic breast cancer as investigated

by (Slamon DJ., et al., 2001). It has also been summarized that there is evidence in further research that trastuzumab has advantages in both the metastatic and early treatment settings (Piccart-Gebhart MJ., et al., 2005).

Settings and Design:

The research was carried out in a laboratory environment. It was hypothesized that the addition of D-Mannoheptulose, an inhibitor of glucose metabolism, into human mammary epithelial cells would reduce the action of the anti-HER-2 monoclonal antibody Trastuzumab when compared to normal (untransfected) cells. This study employed a comparative study design and the cross-over study design. D-Mannoheptulose was compared with Trastuzumab in terms of cell cytotoxicity against normal human mammary epithelial cells and breast cancer cell lines (MCF-7).

Experimental Design:

A series of in vitro experiments were designed and coordinated to determine the cytotoxicity along with the antiproliferative effects of MH and TZB as follow:

1: Stock Solutions Preparation: This was necessary for D – Mannoheptulose (2.5 mg/ml) and Trastuzumab (7500 µg/ml) in order to make subsequent dilutions easier.

2: Treatment: All groups of both cell lines were treated with different doses of both agents. The doses were in the range of 39 – 1250 µg/ml and 9.375 – 600 µg/ml for MH and TZB respectively.

3: Incubation: Different sets of cells in a microplate containing 96 wells were incubated for 72 hours with the agents followed by a control setup where serum free medium (SFM) was added.

4: Cytotoxicity Assessment: Post the different doses of the agents the cell viability was calculated using the MTT assay where by measuring the optical density (OD) at 492 nm, we were able to determine the level of cytotoxicity through the set formulas.

5. Statistical Analysis: The results were analyzed concerning significance employing appropriate statistical techniques with the values presented as mean ± standard deviation.
This study design aimed to elucidate the distinct effects of MH and TZB on

breast cancer cells compared to normal mammary epithelial cells, hence highlighting their potential therapeutic applications.

Methodology

All materials were created to the manufacturer's specifications and are explained below as follows:

Preparation of RPMI-1640 Medium

Applying the RPMI-1640 grade components within the Distilled water, combine 10.4 grams of the RPMI grade components with around 600 milliliters of the water (TDW). Post that, integrate the following components to the solution:

2.2 grams of sodium bicarbonate, 1 milliliter of ampicillin solution (100 µg/ml), 0.5 milliliters of streptomycin solution (50 µg/ml), and 100 milliliters of Fetal Bovine Serum (FBS) (10%). Post mixing the components, add enough distilled water (TDW) up to 1 liter to the solution. 25 millimolar's of the HEPES buffer and 2 millimolar's of L-Glutamine is also added to ensure stability of the pH. or the solution and further ensure a smooth cell culture application in the future. Drops of KOH (1N) for a neutral solution. Make sure to sterilize the water after placing it inside Nalgene filter with a 0.22 µm filter device, following the rules the device mentions.

Preparation of (MEM) Minimum Essential Medium

Dissolve 11 grams of MEM powder with L-glutamine. (HiMedia, India) in around 600 milliliters of distilled water in order to make Minimum Essential Medium (MEM). Then, include 10 milliliters of a 1M HEPES buffer, 2.2 grams of sodium bicarbonate, 100 grams of Fetal Bovine Serum (FBS), 100 micrograms of ampicillin and 50 micrograms of streptomycin. Once those ingredients have been prepared, dilute the solution to one liter using dried TDW. Drops of KOH (1N) for a neutral solution. Ensure that the unit is adequately treated with a Nalgene filter 0.22 µm in order to make the medium useful in cell culture applications (Freshney RI., 2015).

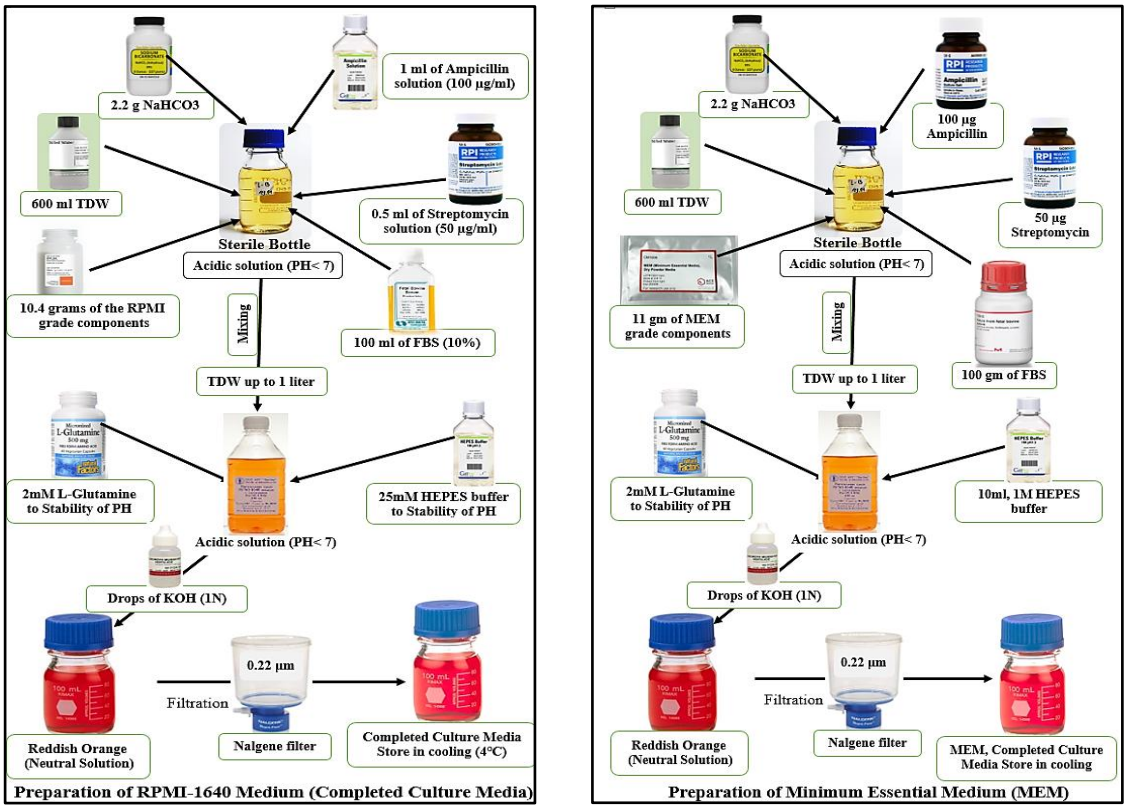


Figure 1: Preparation of RPMI-1640 Medium and Minimum Essential Medium

Cell culture and Cell line

This investigation employed the MCF-7 breast cancer cell line which was obtained from the breast of a 69-year-old woman of Caucasian origin suffering from metastatic breast cancer. The cells were subculture in a trypsin-EDTA solution to be preserved and further propagated. A cell suspension was obtained by diluting the cells in a new medium and the appropriate cell density was inserted in fresh vessels based on the requirements of the experiment. The RPMI 1640 medium was used MCF 7, HMECs on the other hand were cultivated in a MEM with six times 10−6 M Hydrocortisone, Fetal Bovine Serum (10 percent) and 1 percent penicillin-streptomycin. The humid condition was maintained at a temperature of 37 C

and 5 percent of CO2 was allowed to remain in the atmosphere. Monitoring of the cell growth as well as the viability was carried out routinely and the culturing media was changed after every 2 to 3 days. Since MCF 7 cells tend to formed in an adherent pattern, they could be passed on multiple times leading to more cells being produced (Todhunter ME., 2023).

Exposure of MH and TZB to MCF-7 and normal HMEC cell lines
MH and TZB Preparation

A stock solution of D-Mannoheptulose (MH), in the concentration of 2.5 mg/ml, was prepared by taking a 20 ml volume of PBS along with 50 mg of MH and using it for a variety of purposes. Making a 1250, 625, 312.5, 156.3, 78, and 39 µg/ml solution was one of it. In a similar manner, tubes were lined up with sterile Eppendorf richly marked, so they were not confused during the use of them, a solution of SFM and the stock solution of 2.5 mg/ml were mixed with. A 25 ml aqueous buffer solution was produced, consisting of 10 mM histidine buffer (pH 5.5-6.5), 240 mM trehalose, 0.02% polysorbate 20, and filtered water. Trastuzumab (TZB) stock solution [(7500 µg/ml), (7.5 mg/ml)] was formulated through dissolving 150mg of Trastuzumab in 20ml of an aqueous buffer solution. A diluted TZB solution (600 µg/ml) was prepared by combining 400 µl of a Trastuzumab stock solution (7500 µg/ml) with 5 ml of an aqueous buffer. This solution was subsequently utilized to prepare additional diluted Trastuzumab solutions (300, 150, 75, 37.5, 18.75, and 9.375 µg/ml) by including 600µl of cold SFM and 600µl of the diluted stock solution of Trastuzumab (600µg/ml), applying a six-fold serials dilution in sterile tubes as specified.

Exposure of MH, and Trastuzumab to MCF 7 cell line

The research involved the use of the MCF-7 breast cancer cell line upon a 96 well microplate. After cell culturing and its respective incubation period, the media was thoroughly removed and carefully setting up the number of pre prepared Eppendorf tubes, 100µL of either diluted D Mannoheptulose (MH) concentrations or diluted trastuzumab (TZB) drug was added to each well separately according to each column. Furthermore, 100µL of serum free medium (SFM) was added into remaining wells for control purposes. The

microplate was glue sealed with sterile adhesive, and the temperature was maintained at 37 degrees Celsius for 72 hours. The experiment was done in quadruplicate (n=4) for every concentration of changes being analyzed. Finally, the cytotoxicity ratio was calculated using MTT assay which was used to measure optical density (OD) (Al-Ziaydi AG., et al., 2020).

Exposure of MH, and TZB to normal HMEC s cell line

The normal human mammary epithelial cells (HMECs) maintained in a 96well microplate also formed the subject of the study. After that, incubation was continued and after its termination the medium was removed, then 100 µL of diluted trastuzumab (TZB) drug solutions or D-Mannoheptulose (MH) concentrations were added to each well without or independently with interval of twelve columns – depending on how many Eppendorf tubes had been prepared. (100µl) SFM was also added into the other wells as the control in each of the concentration levels under investigation, this experiment was repeated quadruplicates (n=4), the microplate was covered with sterile adhesive parafilm and incubate for 72 hours at 37 °C. Finally, the MTT test was applied in order to assert the optical density (OD) and the cytotoxicity ratio was determined (Panieri E, Saso L., 2021). was also incorporated to the other wells The experiment was conducted in duplicate for every concentration under investigation and the microplate was covered with a sterile sealing film before it was incubated at 37°C for 72 hours. In conclusion, MTT test was employed to assay the optical density (OD) and prepare the cytotoxicity ratio from it (Panieri E, Saso L., 2021).

MTT cytotoxicity assay

The MTT (Methyl Thiazolyl Tetrazolium) assay was employed to assess the cytotoxicity% of administering D- Mannoheptulose (MH) and trastuzumab (TZB) to the cell lines. The medium was removed from the 96-well microplate first. Then, 50 µL of serum-free medium (SFM) and 50 µL of MTT dye (2 mg/mL) were added to each research well. The plate was incubated at 37°C for three hours. After discarding the MTT dye solution, (100 µL) of 1% DMSO was added to solubilize a remaining MTT-Formazan crystals. The plate was then sealed with parafilm, gently rocked, and

incubated for an additional 15 minutes. The absorbance (A) of the treated and untreated cells was measured at a wavelength of 492 nm using an ELISA plate reader. The cytotoxicity% was calculated according to the provided Equation 1:

$$\text{Cytotoxicity \%} = (\text{A Control} - \text{A sample}) / \text{A Control} \times 100$$

A control refers to the average absorbance of the well without treatment, while a sample denotes the average absorbance of the well with treatment applied (22). To evaluate the influence of MH and TZB (the tumor cell viability assay), MTT was used.

Statistical Analysis

For the evaluation of cytotoxic effects of D-Mannoheptulose (MH) and trastuzumab (TZB) on MCF-7 cells and normal cells, SPSS and GraphPad Prism were used in conducting the cell assays. This, then, brings in the results to the MTT assay, which were computed as mean ± but with a standard deviation of SD and performed thrice separately. To arrive at the fitting of the response curves for dose with regards to the half-maximal inhibitory concentration (IC50), nonlinear regression analysis was good to go. In determining the extent of significance of variations experienced between the test groups, one-way analysis of variance (ANOVA) technique was performed, this is along with post hoc methods such as Tukey's and Dunnett's tests. A p-value of 0.05 was set as a threshold not to be crossed since results under this were set as significant values. To make use of MH and TZB together, retreating operating characteristic curves along with thickness increments under the curve was calculated in looking for sensitivity and specificity reasons.

Results and discussion

Cytotoxicity assay

The effect of cytotoxicity was assessed based on different concentrations (μg/ml) of Mannoheptulose and TZB using the MTT

cytotoxicity% assay. Increased amounts of inhibitor (MH) and the medicine (TZB) augmented cytotoxicity and inhibited cancer cell proliferation to a greater extent (Figure 2-3). The distinct effects of the MH and the medicine on the (MCF-7) cell line cancerous cells' proliferation resulted in those differences. Breast cancer cells demonstrated enhanced sensitivity to TZB since the change in CT% was between 18% and 54% of the (MCF-7) cell line after exposure with MH.

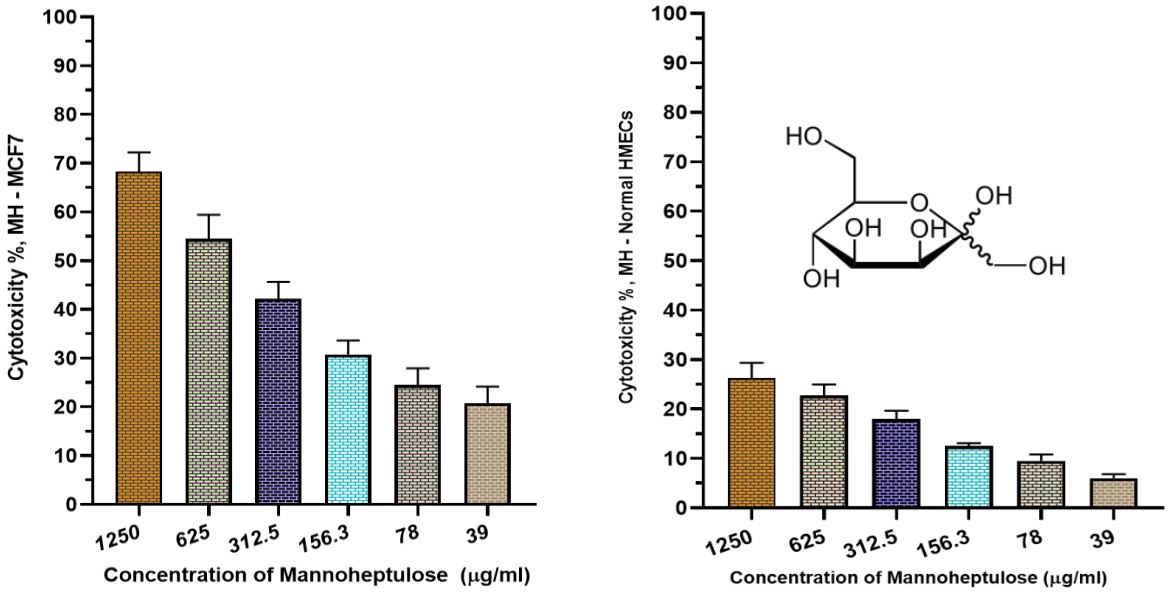


Figure 2. The D-Mannoheptulose (MH) breast cancer cell line exhibits cytotoxicity while the normal HMECs cell line does not show cytotoxicity. Cell therapy using MH was conducted at seriated concentrations (µg/ml) over 72 hours. The results were reported as mean value ± standard value.

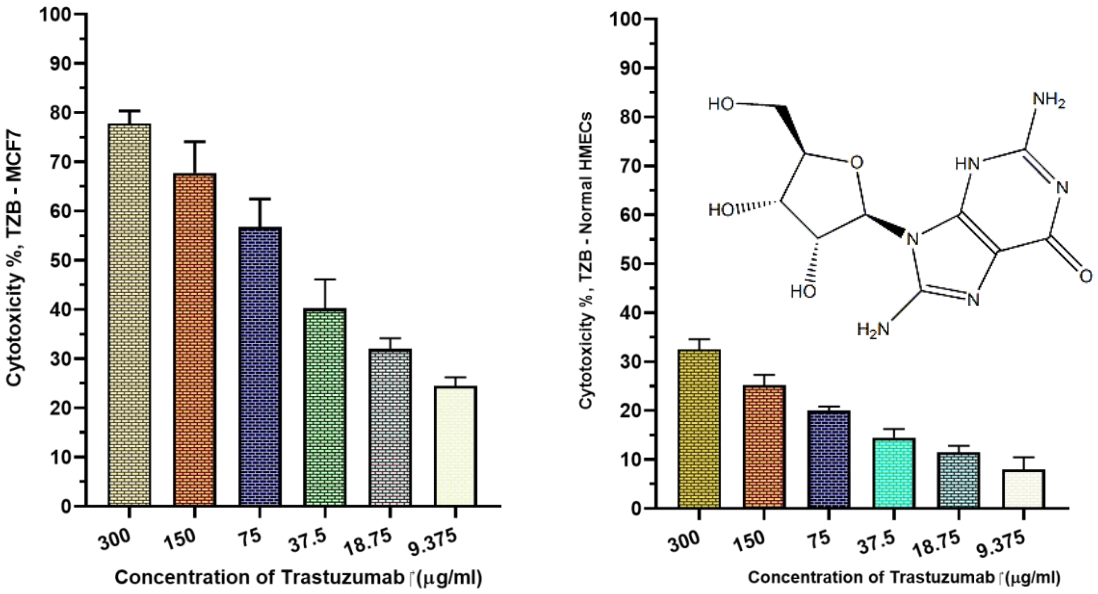


Figure 3. Data indicate the cytotoxicity of Trastuzumab (TZB) towards MCF-7 breast cancer cell lines, while demonstrating non-cytotoxicity to normal HMEC cell lines. Cells were treated with TZB at several doses (µg/ml) for 72 hours. Results were expressed as mean values ± standard deviation.

Determination of IC50

The half maximum inhibitory concentration (IC50) value was assessed in the MCF-7 breast cancer cell line and the normal HMEC cell line to assess the effect of each therapy on cell growth. The normal HMEC cell line had an IC50 value of 975.1 µg/mL for D-Mannoheptulose (MH), but the breast cancer MCF-7 cell line had an IC50 value of 263.3 µg/mL. This suggests a significant variation in the amounts needed to prevent 50% of the cell multiplication. Similarly, (Figure 4) illustrates that the IC50 -value for trastuzumab (TZB) in the normal HMEC cell line was 255.5 µg/ mL, but it was 70.43 µg/mL in the breast cancer MCF-7 cell line. An inhibitor's or drug's potency in preventing a certain cell line from proliferating is indicated by its

IC50 value. It shows the amount of the inhibitory material (TZB or MH) needed to stop 50% of the cells from proliferating. As shown in (Figure 5), these results imply that, after treatment with MH and TZB, there are notable changes in the IC50 values between the normal HMEC cell line and the breast cancer cell line (MCF-7).

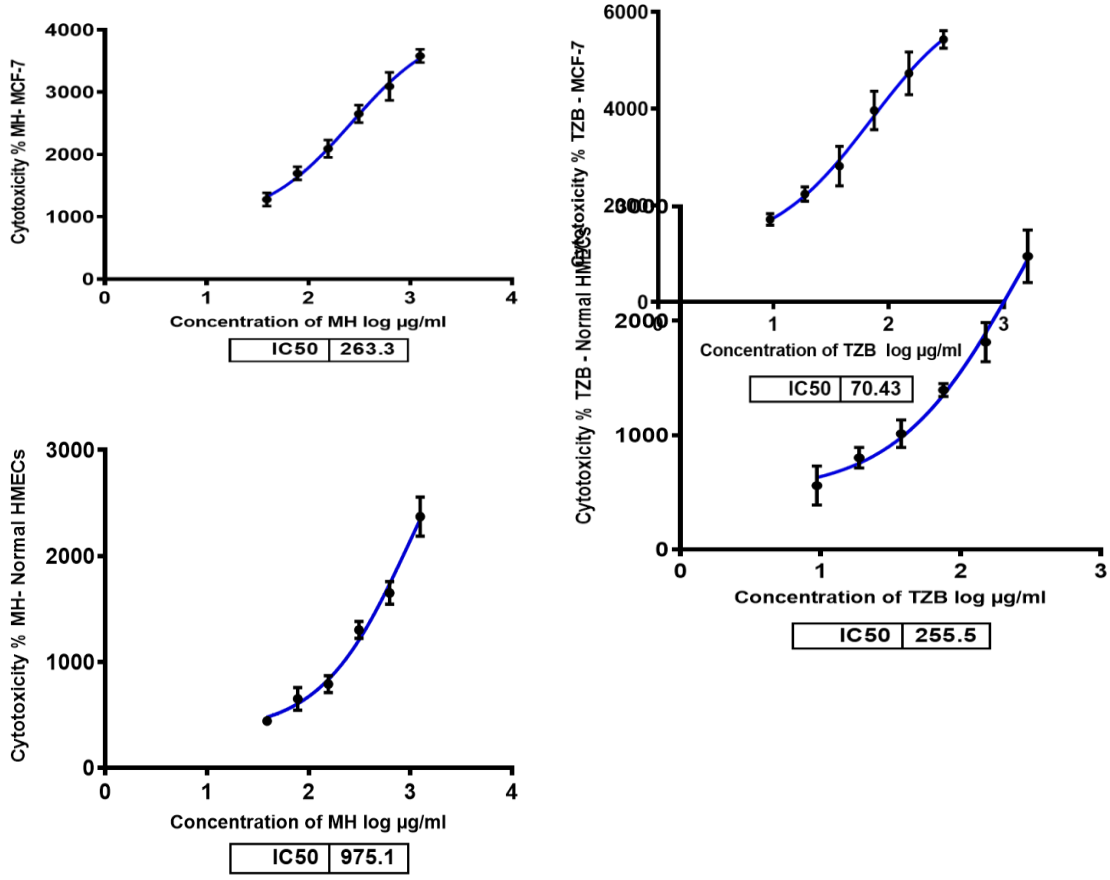


Figure 4. IC50- values after treating MCF-7 cell lines and normal HMECs with MH and trastuzumab

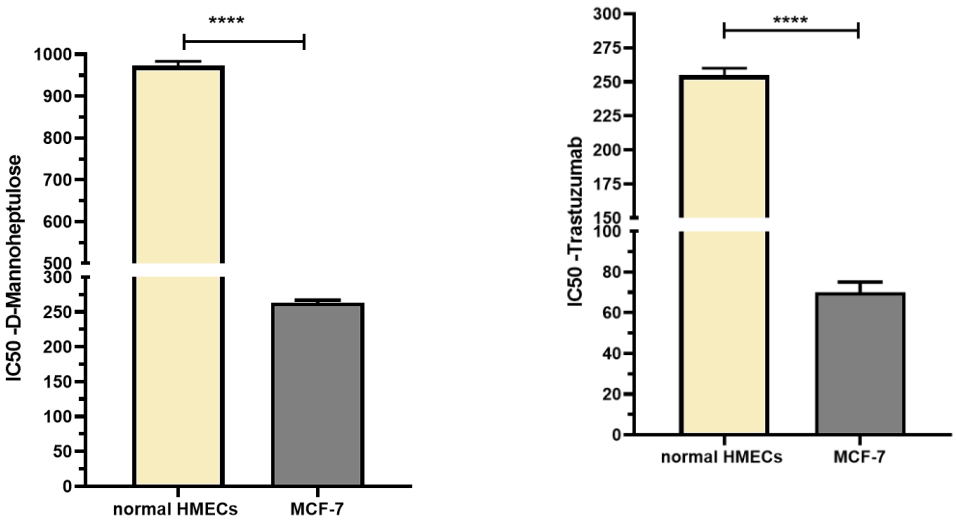


Figure 5. Comparative analysis of IC50 -values between MCF-7 cell lines and HMECs post-treatment with MH and TZB over a period of 72 hours.

Assessment of the predictive performance of TZB and MH using the ROC Curve

In this case, diagnostic accuracy and discriminant ability was evaluated by means of the ROC curve. The results of the study concerning estimating values for MH and TZB for both breast cancer and normal cell lines were quite illuminating. It is possible to assess the aid of ROC curves in estimating their possible diagnostic power by calculating the area under the curve (AUC), the sensitivity and specificity of MH and TZB, the cutoff values (Figure 6) and many more.

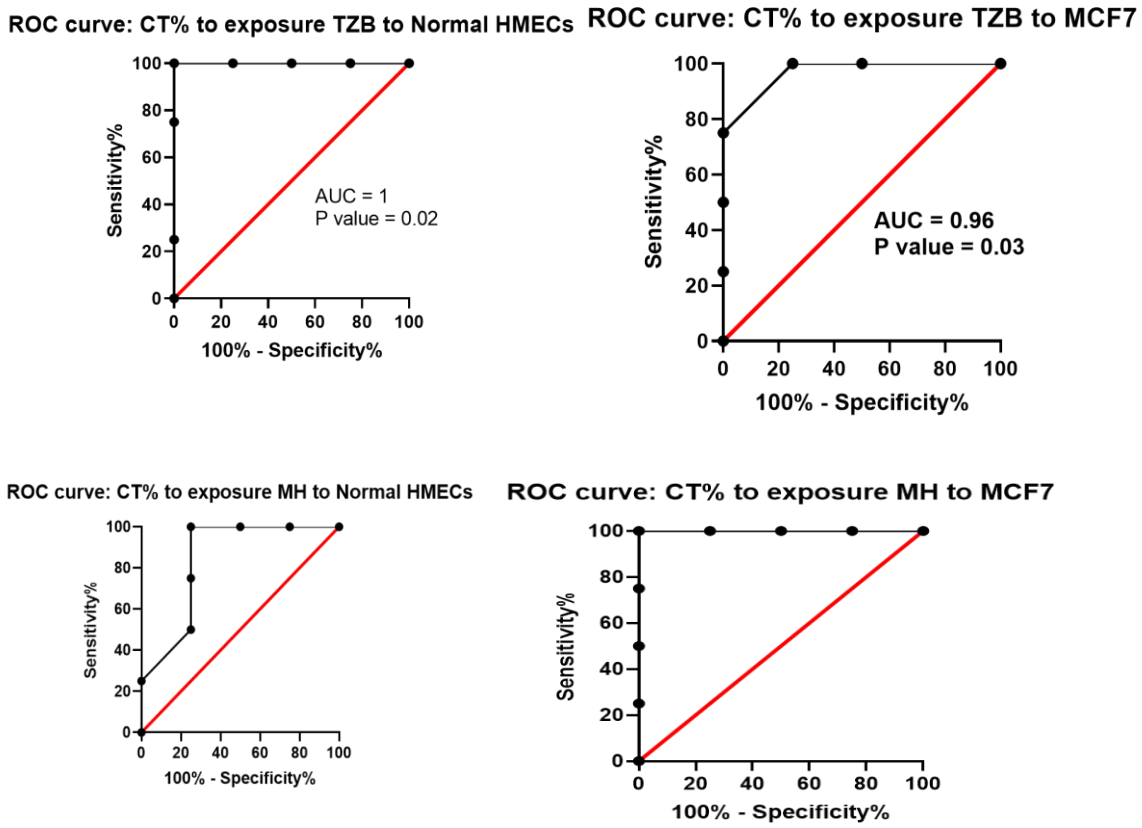


Figure 6: The ROC curve MH and TZB in MCF-7 and normal HMECs cell lines, demonstrate the sensitivity and specificity of breast cancer detection as well as its rate of progression.

Variation of IC50

A large variation in the IC50 value (half-maximal inhibitory concentration) of the monoclonal antibody trastuzumab (Herceptin) has been found between normal cells and breast cancer cells, and high IC50 values have been observed in both normal HMECs and MCF7 cells line. Therefore, lower concentrations and gradient will be used. The differences between normal cells and breast cancer cells depend on the degree of toxicity and IC50 values (Figure 7).

Narrowing of the concentration gradient

The IC50 values for Trastuzumab in normal HMECs and MCF-7 breast cancer cells was determined in narrowing of the concentration gradient as follow:

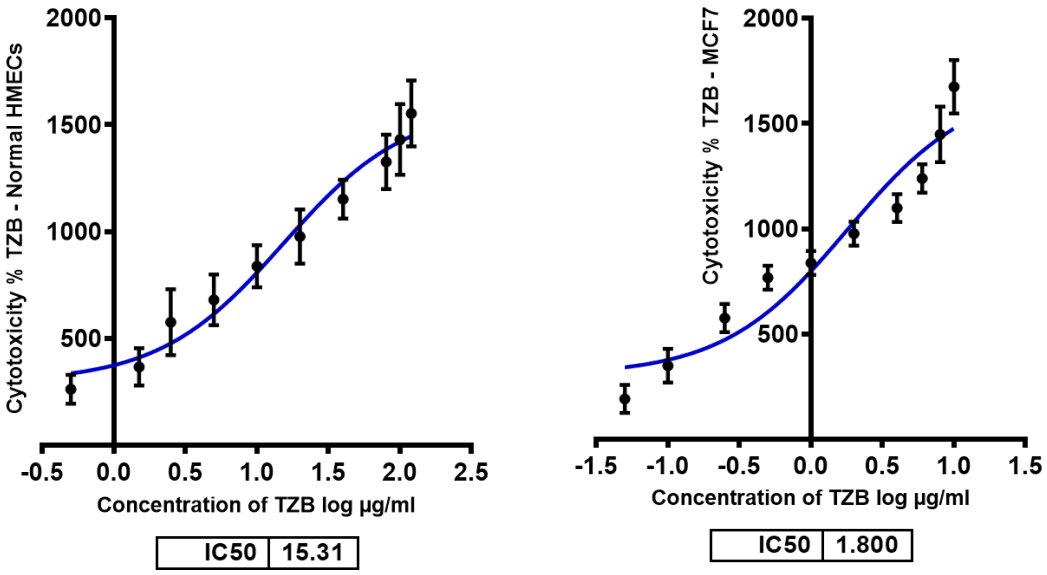


Figure 7: IC50 values following the exposure of normal HMECs and MCF7 cell lines with Herceptin at the reduction of the concentration gradient

Discussion

This study focuses on analyzing the effects of D-Mannoheptulose (MH) together with the monoclonal antibody Trastuzumab (Herceptin) (TZB) on MCF7. The main focus was to evaluate (i) the anticancer efficacy of MH and TZB and (ii) the possible clinical applications of MH and TZB. It has been noted that the findings of the study support the position that Herceptin and TZB are effective in countering the actions of MCF7 [Yakes FM., et al., 2002]. These results correlate with previous studies focusing on the strategy of targeting cancer cell metabolism in order to slow down malignant cell progression. Results concerning the inhibition of glycolysis [Warburg O.,1956] also support these findings. Tumor cells have higher metabolic rates because of their increased need for energy, which leads to ATP synthesis

through glycolysis (the Warburg effect). Advanced medicines targeting breast cancer cells were shown to have therapeutic effects when the glycolysis pathway was inhibited in these cells [Al-Ziaydi AG., et al.,2020].

This study shows that MH was able to inhibit glycolysis and thus reduce the production of pyruvate and ATP, which aligns with previous studies. Besides MH, TZB shows some other potential to act on breast cancer cell lines. The principal action of trastuzumab appears to be the direct binding of its Fab fragment to tumor cell-expressed HER2 protein (Slamon DJ., 2001). Trastuzumab preferentially targets the HER2 (human epidermal growth factor receptor 2) protein which is overexpressed on about 20-30 % of the breast cancer cells. HER2 is a receptor tyrosine kinase that has a significant function in the ability of a cancer cell to grow and live without being destroyed by the immune system. Apart from this, trastuzumab also prevents cancer cells from growing and multiplying as they interact with the HER2 receptors; its action is especially directed at the deactivation of the HER2 signaling pathway that controls cell division and growth. HER2-positive cancer cells' growth and development are reduced when the activity of HER2 is suppressed. Science can do this and so it can attract immune cells like the natural killer cells to detect and eradicate the cancer cells. Known as this procedure enhances the immune system’s efficiency in eradicating cancer cells with a HER2-positive connotation through a process labeled as Antibody-Dependent Cell-Mediated Cytotoxicity or ADCC (Clynes RA., 2002). Also, trastuzumab inhibits the growth and metastasis of tumors due to suppression of angiogenesis which is the development of new blood vessels necessary for their nourishment. This is a very important aspect of its mechanism of action because by causing cell cycle arrest trastuzumab can prevent cancer cells from dividing and multiplying (Yakes FM., 2002). Moreover, it is capable of tying down HER2-positive most cancer cells and leaving them trained to die. Trastuzumab is hence a useful targeted treatment available for the management of HER2-positive breast cancer and other malignancies. In comparison with other studies, the findings of this research suggest that several scholarly documents have detailed the anticancer properties of MH and trastuzumab in various cancer types. Ding et al. [Ding H., 2009] showed that some phytochemical constituents of avocado like MH

induced apoptosis selectively in human oral epithelial cancer cell lines and did not affect normal cells. The results validate the notion that MH selectively kills breast cancer cells while leaving healthy cells unharmed. This is because of the Warburg effect, which causes cancer cells to produce ATP more quickly by primarily relying on glycolysis as opposed to normal cells' more effective utilization of oxidative phosphorylation. Despite the seeming paradox of the cancer cells' high energy requirements for growth, the Warburg effect enables them to generate significant amounts of ATP through glycolysis far more quickly than through the slower process of oxidative phosphorylation. This gives the cancer cells the energy they need to continue growing and proliferating quickly [28]. The findings show that by inhibiting the activity of the enzyme hexokinase (HK), MH can suppress the glycolytic flow in MCF-7 breast cancer cells. This causes ATP to be depleted, which in turn causes an energy shortage that prevents the cancer cells from proliferating and going through mitosis.

This result is consistent with the earlier findings published by Mathupala et al. suggesting that the mitochondrial location of HK helps suppress the death of cancer cells. By inhibiting HK, MH disrupts this protective mechanism, leading to energy insufficiency and ultimately impeding the cancer cells' ability to divide and proliferate. [Al-Ziaydi AG., et al.,2020] According to the research, hexokinase (HK), an enzyme, is located in the mitochondria and may have a role in inhibiting the death of cancer cells. The cancer cells depend on this crucial pathway to survive. All things considered; by highlighting the potential of TZB and MH as therapeutic agents for the treatment of breast cancer, this study contributes to the body of current research. The development of tailored medicines can benefit from the knowledge provided by the powerful anti-tumor activities of TZB and the specific cytotoxic effects ratio of MH on cancer cells.

To better understand these medications' underlying the mechanisms of action and determine the ideal dosage schedules, more study is necessary. This would reduce any possible detrimental side effects while maximizing their efficacy in treating breast cancer. The MTT assay results demonstrate that both MH and TZB display considerable concentration-dependent cytotoxicity toward the MCF-7 breast cancer cell line. Conversely, even at

elevated doses of the inhibitors/drugs, there was no observable effect on the normal HMEC line. The Warburg effect idea posits that MCF-7 cells exhibit increased cytotoxicity (Warburg O., 1056).

Conclusion

The study has described the impact of TZB, a monoclonal antibody against HER 2/neu oncogene and MH on MCF-7 breast cancer cell line and HMEC. The results revealed that concentration-dependently both MH and TZB enhanced their cytotoxic and antiproliferative potential on the breast cancer cell line MCF-7. The normal HMEC cells are also unaffected by the cytotoxic impacts of MH and TZB, which was not observable with the treated cells. Thus, the ability of MH and TZB to inhibit glycolysis and induce apoptosis was the chief factor for their amplified toxicity effects on the MCF-7 breast cancer cells. MH inhibited the glycolysis of breast cancer cells resulting in the reduction of hexokinase activity, pyruvate, and among them, ATP. In conclusion, these results raise the conclusion that MH phytochemical and TZB monoclonal antibodies are effective targets in HER2-positive MCF-7 breast cancer cell lines in terms of cytotoxic and anti-tumor activity involving the inhibition of glycolysis-dependent apoptosis actions. This comparative overview shows that both TZB and MH have the prospects of being used as therapeutic drugs in the treatment of breast cancer, particularly the HER 2-positive type. Thus the lowered IC50 of Trastuzumab in the MCF-7 breast cancer cells (1. 8 μg/mL) compared to that of the normal HMECs (15.31 μg/mL) ensures that the present breast cancer cells bear higher sensitivity of the cytotoxicity of Trastuzumab. This is a good indication that relative to MCF-7 breast cancer cells, Trastuzumab is more effective and selective in eliminating the survival of breast cancer cells and more harmless to HMECs. Therefore, one could conclude that the decrease in the difference between the two IC50 values means that, Trastuzumab has a superior therapeutic ratio and it needs to be applied for the selective killing of breast cancer cells without affecting the normal ones.

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Conflict of interest

The authors declare no conflicts of interest.

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