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Sialidase activity in Glioblastoma cells treated with thyme oil

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Abstract

Background: Cancer is a genetic disease that causes aberrant cells to grow and spread uncontrollably throughout the body, where cerebral glioblastoma is one of the brain tumors. Thyme oil has many therapeutic properties such as antioxidants and anticancer.
Aim of study: Estimate sialidase enzyme in human glioblastoma cancer cell line (AMGM5) and rat embryo fibroblasts cell line (REF) in supernatant and suspension cells treated with thyme oil extract and cells without it. Methods: Sialidase activity was measured by ELISA (Enzyme-linked immunosorbent assay) in supernatant and suspension cells of AMGM5 and REF cells treated and untreated with thyme oil extract. Results: Sialidase showed a high level in the Glioblastoma cell line (AMGM5), while a low level in cells after exposure to diluted thyme oil (10µl/ml) after incubation for 24 hours, also showed a lower level in the Rat Embryo Fibroblast after being treated with thyme extract.
Conclusions The sialidase enzyme showed increased in glioblastoma cells but decreased in its level in these cells after exposure to thyme oil extract, which means that thyme oil inhibited cancer cell growth.

Keywords: *AMGM5, Glioblastoma, REF, Sialic acid, Sialidase, Thyme oil*

Introduction

Cancer is a complicated genetic disease that causes aberrant cells to grow and spread uncontrollably throughout the body as a result of age, changing lifestyles, hormone fluctuations, and exposure to environmental toxins. These factors are important signaling pathways that cause cancer to activate in humans (1). Glioblastoma is one of the common types of malignant primary brain tumors; glioblastomas are characterized by molecular heterogeneity and are believed to originate from neuroglial stem or progenitor cells (2). The enzyme sialidase (EC 3.2.1.18) also known as neuraminidases releases sialic acid, binding primarily to oligosaccharides or glycolipids. Sialic acid is present in cytosolic and membrane-bound enzymes, among other cellular locations. Sialidases have been linked to the pathophysiology of viral diseases by stimulating the removal of terminal sialic acid residues from various glycoconjugates (3).

The catalytic characteristics, kinetic parameters, and substrate selectivity of sialidases vary significantly. A glycosidase known as neuraminidase catalyzes the breakdown of α-glycosidic linkages, which connect sialic acid residues to the carbohydrate groups of glycolipids and glycoproteins. There are four varieties of sialidase NEU1 - NEU2 - NEU3 - NEU4, NEU1 called lysosomal sialidase, the four types differ in terms of their location, expression patterns, optimal pH, and functions (4). NEU1: human neuraminidase-1, located in lysosomes, is involved in immune system function, exocytosis, and elastic fiber collection, sialic acid belongs to the family of derivatives of neuraminic acid, it is an acid monosaccharide made up of nine C atoms, with an N-acetyl group in atom C number 5 and an (OH) group in atom C number 2, sialic acid is present in the structure of oligosaccharide chains at the terminal site, where it encodes a significant amount of data that is thought to be crucial for controlling transmission of signals between cells (5). There are two main reasons why sialic acids are thought to be significant in different biological processes: one is due to their hydrophilic and acidic characteristics, which have physicochemical impacts on the glycoconjugates to which they are linked, while the other functions as masking or recognition sites, respectively (6). Sialic acids play a role in resistance to brain tumor medications. Gangliosides, the predominant sialic acid transporters in the brain, are of special significance in this

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regard, and while sialic acid concentrations can vary depending on the particular tissue, evidence suggests that the brain has a comparatively high sialic acid content compared to other organs.

Furthermore, compared to tissues, the brain has more complicated ganglioside structures. N-glycolyl neuraminic acid is significant given the role sialylated glycolipids play in brain cancer, the brain's rich and intricate ganglioside expression may indicate biological processes unique to the brain involved in cancer development (7).

During different phases of carcinogenesis, NEU1 is involved in critical molecular functions at the cancer cell surface, these functions include controlling the activity of several receptor tyrosine kinases (RTKs) and TOLL-like receptors (TLRs), as well as their downstream signaling pathways. NEU1 is linked to RTKs and TLRs in the ectodomain, where it forms a complex with matrix metalloproteinase-9 (MMP-9) and G-protein-coupled receptors (GPCRs) when NEU1 is activated by binding of epidermal growth factor (EGF) to RTKs, it breaks down a glycoside linkage of Neu5Acα in the N-glycans attached to RTKs in the ectodomain (8), when compared to normal tissues, bladder cancer tissues have higher levels of NEU3 (sialidase3) expression, which enhances the invasiveness of bladder malignancies. Inhibition of NEU3 may be a novel therapeutic target for resistant bladder cancer and can suppress the invasion of invasive bladder cancer (9). The thyme plant (Thymus vulgaris L.) belongs to the Lamiaceae family, depending on the chemotype, it is made up of α-pinene, thymol, caryophyllene, and carvacrol, due to the presence of bioactive components, particularly carvacrol and thymol, essential oils of thyme have potent antibacterial, antiviral, antifungal, antiparasitic, and antioxidant activities, as well as some anti-inflammatory and hepatoprotective qualities (10). Thymol's primary anticancer action involved stopping the cell cycle at the G0/G1 junction, which worked in concert with the medication temozolomide (11).

This study was proposed to evaluate sialidase activity on the Glioblastoma cell line and the REF cell line after treatment with thyme extract.

Materials and Methods

Preparation of Thyme Oil Extract

Using a Clevenger apparatus, 30 grams of dried thyme (leaves were purchased from the local market in Baghdad) with 1 liter of distilled water, this extraction procedure was repeated several times over five hours.

Thymus vulgaris L. was classified by (Department of Biology - College of Sciences - University of Baghdad) according to code number (1570) on (16/ 7/ 2024).

Preparation of Diluted Plant Extract

Thyme oil was dissolved by DMSO (Dimethyl sulfoxide), and the mix was diluted with serum free media and thyme oil dilutions were made as (1, 2.5, 5, 10 µl/ml).

Cell culture

Cancer cell lines (AMGM5, Ahmed-Majeed-Glioblastoma-Multiforme-2005)(12)(13) and the REF cell line (Rat Embryo Fibroblast) were supplied by (Experimental Therapy Department) at the Iraqi Center for Cancer and Medical Genetic Research / Mustansiriyah University (Baghdad, Iraq). AMGM5 and REF cells were cultured in culture vessels (Santa Cruz Biotechnology, California, USA) that contain media (RPMI-1640) with 10% fetal bovine serum (FBS), 1% L-glutamine, penicillin / streptomycin and incubated at 37°C, 5% CO2 for 24, 48, 72 H. In AMGM5, REF vessels, attached monolayer cells were separated using 1 ml of trypsin/versine to produce a cell suspension, and 10 ml of prepared media were added to the vessel. A 96-well sterile microtiter plate was used to culture around 200 µl of the cells.

Cytotoxicity Assay

The AMGM5 cell line was used to investigate the cytotoxicity of thyme oil extract. After incubation of cells for 24 hours to form a monolayer in a microtiter plate, the medium was emptied from the cells and 200 µl of thyme oil dilutions (1, 2.5, 5, 10 µl/ml) were added, the microtiter plates were incubated for 24 h, the control cells in the wells are serum-free media-containing cells without extract. Butler (2004) provided instructions for preparing the procedure for handling and treating cells (14).Crystal violet stain was used to test cytotoxicity, after removal of the contents of each well, 50 µl of crystal violet stain was applied and left for 20 minutes at 37 ° C. The viable cell nuclei will be stained by the crystal violate stain. The plates were then examined at 492 nm using an ELISA reader (15).

Preparation of treated cancer cells with Thyme oil extract

Diluted thyme extract (10 µl\ml) was added to each cell vessel of AMGM5 and REF cell lines and incubated for 24h at 37°C. The cell supernatant of each vessel was collected after being separated by centrifuge (at 2000-3000 rpm) for 20 minutes. The cell suspension was collected by scraping the cells in vessels and diluting it into media-free serum then separated by centrifuging at (2000-3000 rpm) for 20 minutes, then the cell suspension was lysed through repeated freeze-thaw cycles to release cell components.

Enzyme-linked immunosorbent assay (ELISA)

The supernatant and suspension cells of (AMGM5 -REF cell lines and AMGM5 - REF cell lines treated with thyme extract) were seeded in a coated ELISA plate where Sialidase enzyme (NEU1) was measured using the ELISA assay following the manufacturer's instructions (Human Sialidase-1(NEU1) ELISA Kit Catalog No: YLA2402HU).

Image morphology

A digital color camera and an inverted light microscope with a 200 X magnification (Leica Microsystems, Germany) were used to capture four randomly selected fields were used to collect treated and untreated cells .

Statistical analysis

Statistical analysis was done by the unpaired T-Test using GraphPad Prism (version 9.5.1; Institute Inc., Cary, NC, USA). A difference that was deemed statistically significant was defined as $P \leq 0.05$.

Results

The inhibition rate of thyme extract oil at different concentrations in the in vitro cell line (AMGM5) was evaluated by crystal violet dye where the highest percentage of inhibition rate was observed in the extract diluted 1µl/ml and the lowest inhibition rate in the extract diluted at 5 µl/ml as shown in Table (1).

Table 1: Inhibition rate of thyme extract

Thyme extract diluted	Inhibition rate of thyme extract diluted in the AMGM5 cell line
1 µl/ml	70.75949 %
2.5 µl/ml	69.746835 %
5 µl/ml	59.91561 %
10 µl/ml	67.46835 %

Thyme extract diluted at 10 µl/ml was used to evaluate its effect on sialidase activity. This dilute was used because it contains a high concentration of thyme, and it could be said that it is an experimental study with this concentration in which it caused growth inhibition of AMGM5 - REF cell lines for each vessel, after incubation for 24 hours at 37 °C, as shown by the microscopic images in Figures (1)(2).

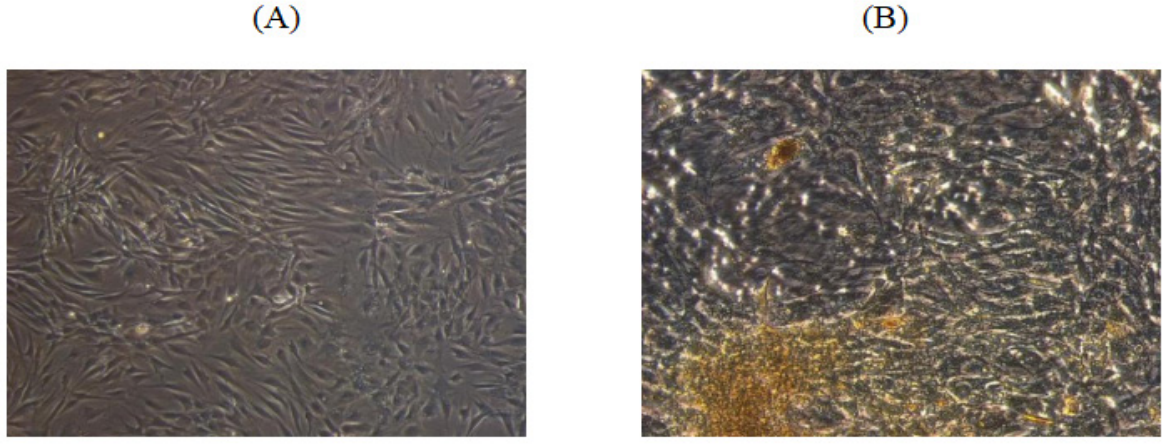

Figure 1: (A) Microscopic image of AMGM5 cell line. (B) Microscopic image of AMGM5 cell line exposed to thyme oil at 10 µl/ml for 24 Hours at 37°C. (Microscope magnification power 200 X) .

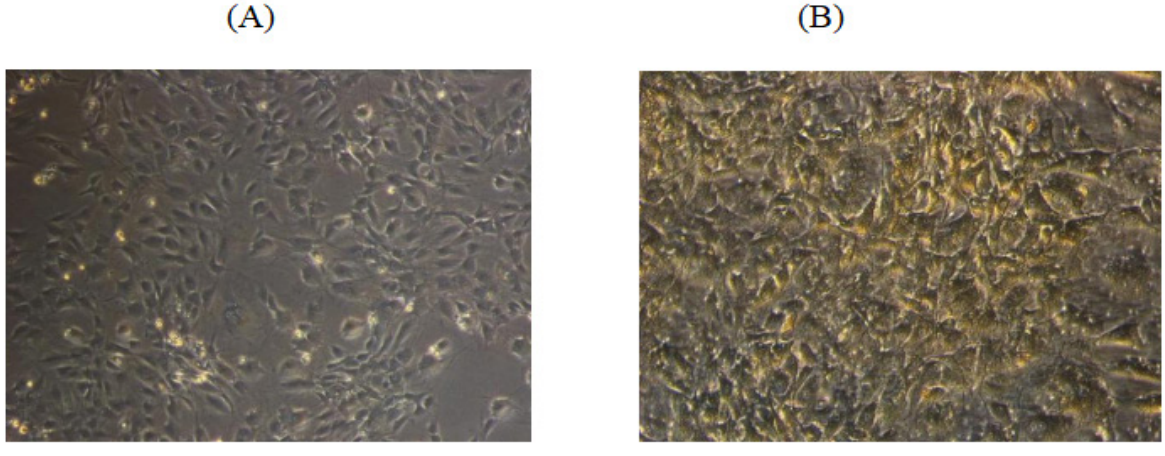

Figure 2: (A) Microscopic image of REF cell line. (B) Microscopic image of REF cell line exposed to thyme oil at 10 µl/ml for 24 Hours at 37°C. (Microscope magnification power 200 X) .

Sialidase activity in the AMGM5 cell line

Sialidase activity level demonstrated differences between the AMGM5 extracellular (cell supernatant) and the AMGM5 extracellular treated with thyme oil, where the variation is significant (P>0.001). The sialidase activity of AMGM5 intracellular (cell suspension) showed significant differences (P> 0.001) with AMGM5 intracellular treated with thyme oil, as shown in Figure (3).

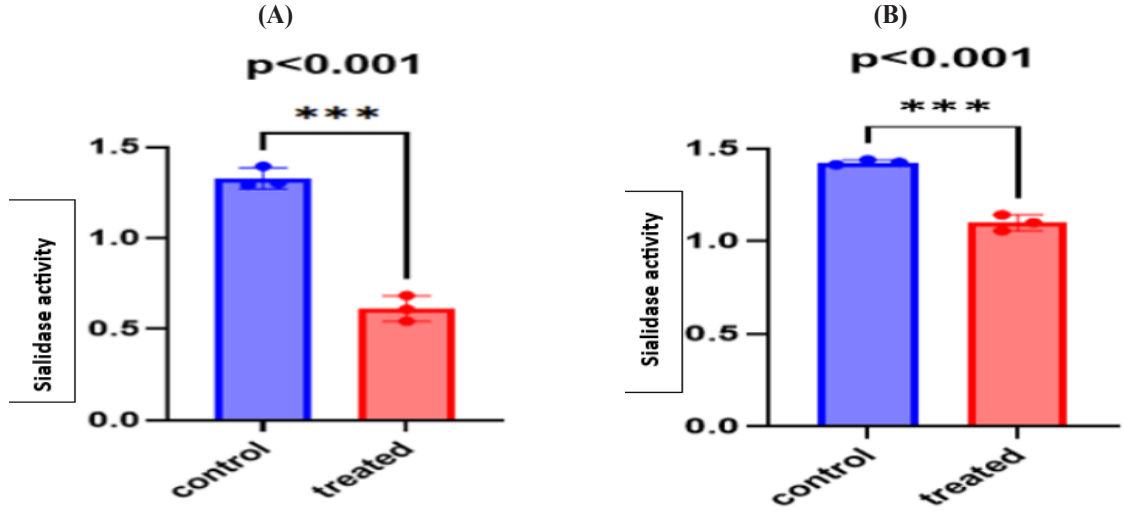

Figure 3: (A) Sialidase activity in the supernatant of the AMGM5 cell line, with and without treatment with thyme extract. (B) Sialidase activity in the suspension of the AMGM5 cell line, with and without treatment with thyme oil extract.

Sialidase activity in REF cell line

The levels of sialidase activity in the extracellular supernatant of REF cells show a significant variation (P>0.001) compared to the extracellular supernatant of REF treated with thyme oil.

Furthermore, the results of sialidase activity in REF intracellular cells show a significant difference (P>0.01) with intracellular cells treated with thyme oil, as shown in Figure (4).

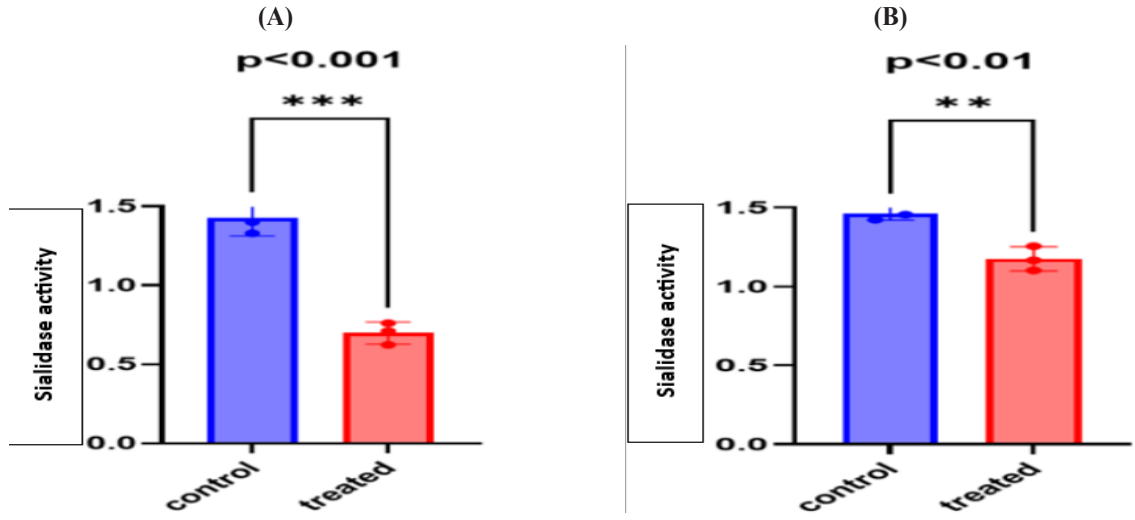

Figure 4: (A) Sialidase activity in the supernatant of the REF cell line, with and without treatment with thyme extract. (B) Sialidase activity in the suspension of the REF cell line, with and without treatment with thyme oil extract.

Discussion

In the present study, thyme oil was extracted to detect its effect on Glioblastoma cancer, physiologically active compounds of medicinal plants have been the subject of much research, to develop new, potentially nontoxic medications and treat and prevent specific types of cancer (16)(17), humans have used medicinal plants of the Lamiaceae family for thousands of years

and thyme essential oil has been recognized for its therapeutic benefits due to its in vitro antimutagenic and anticancer capabilities (18), where it contains active phenolic compounds (thymol 2-isopropyl-5- methyl phenol; C10H14O and carvacrol) (11), which are antioxidant agents and can affect the removal of free radicals and the decomposition of peroxide, it has anticancer properties (19) and has been shown to inhibit cell growth in cancer cell line (AMGM5) in this study after 24 hours when different dilutions of thyme oil about (1, 2.5, 5, 10 µl/ml) where inhibition rates were (70.7 % - 69.7% -59.9% -67.4%) sequentially. Deb et al. (2011) examined Thymol's anticancer properties using PBMC (normal peripheral blood mononuclear cells) and HL-60 (human acute promyelocytic leukemia) cells. After 24 hours of treatment, thymol showed dose-dependent cytotoxic effects on HL-60 cells in its investigation. However, thymol did not exhibit cytotoxic effects in PBMC (20).

This study also measured sialidase enzyme levels in AMGM5 cells which showed a high level in supernatant cells compared to supernatant cells treated with thyme oil extract. As sialidases catalyze the removal of sialic acid residues from glycoconjugates, the abnormal sialylation process in glycolipids and glycoproteins signs that sialidase has a role in cancer progression (21), another study by (Meuillet EJ et al) explained that the presence of sialic acid has an important role in the interaction of gangliosides with growth factor receptors, adding to the effect of sialidase enzyme in the proliferation process, where gangliosides are necessary for both cell-to-cell, cell-to-matrix interactions, and transmembrane signaling. According to ganglioside, it is considered one of the essential compositions in the brain; therefore, any alteration in the sialidase enzyme leads to changes in brain cells (22) as demonstrated in this study. Also in suspension cells, the enzyme estimation was high compared with its values in suspension cells exposed to thyme oil, where this oil can decrease cancer cell growth and subsequently decrease sialidase levels. One of the defining characteristics of cancer cells is aberrant glycosylation. Specifically, changes in sialylation during malignant transformation have been linked to a highly invasive malignant phenotype that can spread (6).

In the REF cell line, the level of sialidase in cells decreased with exposure to thyme oil extract in supernatant cells, but in suspension cells, the sialidase enzyme had little change in its value when measured in cells treated with oil extract, another study showed various percentages of inhibition in the REF cell line depending on thymol concentration where the thymol effect had slight toxicity in normal cells (23).

Conclusions

Sialidase shows high levels in cancer cells due to changes in the sialylation process during malignant transformation. Therefore, it is possible that, if the enzyme is inhibited, cancer cell growth can be controlled or prevented. Additionally, because thyme oil activates particular molecular targets that trigger cell death, it inhibits the growth of cancer cells without damaging healthy ones. Thyme oil is considered a novel class of anticancer medications that shrink tumors very well and have few side effects.

Authors' Contribution

Thyme oil extraction: Ola H Fadhil , Budoor Sattar Abbas , Bushra Shihab Hamad

Visualization: Ola H Fadhil and Bushra Shihab Hamad

Methodology: Amer T. Tawfeeq

cytotoxicity: Budoor Sattar Abbas.

ELISA technique for measuring enzyme: Ola H Fadhil

Software (statistical analysis): Abbas A. Mohammed.

Writing Paper: Ola H Fadhil, under supervision, Amer T. Tawfeeq.

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Conflict of interest

The authors disclosed no conflicts of interest.

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