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Inducing Selective Starvation of Cancer Cells Through Synergistic Inhibition of Glycolysis Using Mannoheptulose And 2-Deoxy-D-Glucose

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Abstract

The unique opportunity lies in targeting tumor cells through glycolysis, a pathway that compensates for decreased ATP production by increasing sugar consumption. This strategy allows for the development of a novel therapeutic approach, targeting malignant cells with selective pharmacological inhibitors of cancer metabolism without harming normal cells. In this study, hexokinase, glycolytic enzyme, was targeted to selectively inhibit energy production pathways in cancer cells without harming normal cells. The anticancer mechanisms of 2 Deoxy-D-Glucose (2DG) and Mannoheptulose (MH) in esophagus adenocarcinoma (SK-GT-4) and (HBL-100) normal cell lines were investigated using MTT and wound healing assays to assess their cytotoxic effects and cellular proliferation. The apoptosis effect was detected using the AO/EB assay. To confirm the effect of agents on cell energy metabolism, the levels of ATP, pyruvate, and hexokinase were assessed as glycolysis parameters. The results showed significant cytotoxic effects of 2DG and MH on the growth of cancer cells, with a strong synergistic effect when used in combination, especially in SK-GT-4 cells. The IC_{50} values for 2DG and MH were 278 and 440 µg/mL, respectively, in SK-GT-4 cells, while HBL-100 cells did not reach IC_{50} values. Wound healing outcomes indicated a decrease in proliferation and invasion of cancer cells. Measurements of ATP and pyruvate levels showed that the combined use of the two drugs more effectively cellular ATP depletion and Pyruvate accumulation compared to using them independently, suggesting a synergistic effect of 2DG and MH against cancer cells and potential for a more effective treatment approach in the future.

Keywords: Cancer Metabolism, Mannoheptulose, 2-Deoxy-D-Glucose, ATP Depletion, Pyruvate Accumulation.

Introduction

Cancer is a complex disease that poses a significant global health threat, ranking as the second leading cause of death worldwide [1, 2]. The diverse nature of cancer at the cellular, histological, and physiological levels presents challenges in diagnosis, treatment efficacy, and side effects [3]. Several studies have used natural products as alternative therapies with few side effects. However, many of these products have not been successful in clinical settings due to low selectivity, high toxicity, and adverse effects [4,5]. Targeted therapies, such as starvation and phytotherapy, along with combination treatments, offer promising solutions to address these challenges. The drawbacks of current cancer chemotherapy highlight the need for more effective treatment approaches.

Normal mammalian cells primarily obtain ATP from glycolysis in low oxygen conditions, while cancer cells heavily rely on glycolysis even in the presence of oxygen, a phenomenon known as Warburg effect [6]. Changes in cellular metabolism are a well-known characteristic of cancer, driven by oncogenic signals that promote metabolic alterations supporting tumor growth. These changes support resistance to treatment, and spread to other parts of the body [7]. The fate of pyruvate is determined by oxygen availability; with aerobic conditions favoring the conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase (PDH), while anaerobic conditions lead to the production of lactate using lactate dehydrogenase (LDH) [8]. In normal cells, acetyl-CoA enters the mitochondria to participate in the citric acid cycle, generating NADPH and FADH2 for ATP production through oxidative phosphorylation (OXPHOS) [9]. However, cancer cells develop this pathway to promote tumor growth [10]. Cancer cells rapidly divide by utilizing aerobic glycolysis instead of citric acid cycle for ATP generation , even in the

presence of oxygen [11]. These metabolic changes can be targeted for therapy as they differentiate cancer cells from normal cells [12].

Previous studies have found that cancer cells typically reduce oxidative phosphorylation and increase glycolysis as part of their metabolic adaptations [13]. This metabolic shift presents potential targets for therapy by exploiting the differences in energy metabolism between cancer cells and normal cells. Oncogenic mutations promote glucose transporters like GLUT 1 and GLUT 3, allowing cancer cells to consume more glucose and accelerate its metabolism. Enzymes involved in glycolysis, such as pyruvate kinase (PK)-M2 isoform, lactate dehydrogenase (LDH), hexokinase II (HKII), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), also play roles in non-glycolytic processes like transcriptional regulation and histone phosphorylation [14, 15]. Some glycolytic enzymes, such as hexokinase and GAPDH, can control apoptosis by affecting levels of apoptotic proteins such as Bak, Bad, and Bax [16, 17].

2-Deoxyglucose, a hexokinase inhibitor, is a glucose molecule that hinders glycolysis by substituting the 2-hydroxyl group with hydrogen. This glucose analog inhibits hexokinase and glucose-6-phosphate isomerase activity, causing the accumulation of 2-deoxy-d-glucose-6-phosphate (2-DG6P) within cells. Most cells rely on hexokinase to convert glucose to 2-deoxyglucose-6-phosphate, which competes with glucose-6-phosphate. The accumulation of phosphorylated 2-deoxyglucose leads to ATP depletion and cell death [18]. In this study, Mannoheptulose and 2-Deoxy-D-Glucose were used as a hexokinase inhibitor to disrupt the Warburg effect in treated cancer cells, and control cancer cells.

Materials and Methods

Cell culture

SK-GT-4 (esophageal adenocarcinoma) cancer cell line and the HBL-100 (human breast epithelial) normal cell line were obtained from the cell bank, department of Biology, University of Basrah, Iraq. The cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 g/mL streptomycin, following the common protocol, and incubated them at 37°C and 5% CO2 until the cell culture reached (80%–90%) confluence [19].

Cytotoxicity assay

The cytotoxicity of Mannoheptulose (MH) (Santa Cruz, USA) and 2 Deoxy-D-Glucose (2DG) (Santa Cruz, USA) were assessed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Bio World, USA). Cells were seeded in a 96-well microplate at a density of 1×10^4 cells/well and incubated at 37 °C for 24 h [20]. The cells were then exposed to different concentrations of MH (110, 275, 440, 1100, and 1760 µg/ml) and 2DG (75, 150, 300, 450, and 750 µg/ml), with three replicates for each concentration. Untreated cells, cells treated only with 0.1% dimethyl sulfoxide (DMSO) medium and serum-free medium served as negative control.

The MTT assay was used to determine cell inhibition after 72 h of incubation. Cells were incubated with 100 µl/well of MTT solution (10 µl MTT, 5 mg/mL of final concentration, and 90 µl serum-free media) for 2 h to converting the yellow insoluble tetrazolium salts to cyan or blue formazan by active cells, following by added 120 µL of DMSO in each well for 15 min in dark conditions. Then, absorbance was measured by a microplate reader at 490 nm. The cytotoxicity percentage (%), was calculated as follows [21].

$$Inhibition\ rate = \frac{ODcontrol\ -\ ODsample}{ODcontrol}\ \times 100$$

Where; $OD_{control}$: mean optical density of untreated wells;

OD_{sample}: optical density of treated wells.

The IC_{50} values were calculated by GraphPad Prism v 10.1.2 (324) (CombuSyn Inc., Paramus, NJ, USA).

The combination effect of MH and 2DG

The IC_{50} determination results from the cytotoxicity assay were used to select the doses for this study. Cell lines were seeded at a density of 1×10^4 cells/well in 96-well plates and incubated overnight. Five different concentrations of MH (110, 275, 440, 1100, and 1760 µg/ml) and 2DG (75, 150, 300, 450, and 750 µg/mL) were added in triplicate to the cells. A control group consisted of untreated cells. The treated and untreated cells were then cultured for 72 hours at 37°C with 5% CO2 in a humidified incubator.

The inhibition rate following exposure was determined using the MTT assay. The combination index (CI) of MH and 2DG was calculated using CompuSyn software v1 to analyze their interaction, with CI values of 0.9–1.1 indicating additive effects, <0.9 indicating synergism, and >1.1 indicating antagonism [22].

Apoptosis evaluation

Staining with Acridine Orange and Ethidium Bromide (AO & EB) was used to identify SK-GT-4 and HBL-100 cells death. The cells were seeded in a 6 well plate and incubate at 37° C with 5% of CO2 in a humidification incubator until confluence 80-90% achieved. Cells were treated with the IC_{50} concentration of MH, 2DG as alone or combined for 72 h.

Following treatment, cells were dyed with 50 µg/mL AO/EB for 2min at room temperature in dark condition and then fluorescent images were observed under inverted fluorescent microscope (Leica Microsystems, Germany) [23].

Wound healing (cell migration) assay

SK-GT-4 and HBL-100 cells were seeded in a 6-well plate at a density of 1x105 cells per well and allowed them to reach 80-90% confluence. The monolayer cell cultures were then scratched with a sterile pipette tip and exposed to IC50 concentrations of MH and 2DG, either alone or in combination, followed by a 72-hour incubation at 37°C with 5% CO2.

Images of the scratched wells were captured at regular intervals (0, 24, 48 and 72 h) using a digital camera (Cannon, Japan) connected to an inverted microscope (MIJEI, Japan). The wound healing rate was measured using ImageJ software. The wound healing rate (%) was then calculated using the following formula
([wound width at 0 h − width at 24 h]/ width at 0 h) x 100% [24].

Glycolysis assessment

HBL-100 and SK-GT-4 cells were used to analyze ATP and pyruvate content. The cells cultured in RPMI-1640 media with 10% FBS and incubated in a 37.5 ºC incubator with 5% CO2 for two to three days. After cells reached 80–90% confluence, were washed twice with PBS and the old media was removed. Following a 72-hour incubation, the cell lines were treated with the IC50 dose of MH and 2DG either alone or in combination.

After the treatment period, 750 µL of 0.25 percent trypsin was added to the cells, which were then rinsed and detached from their flasks. The cells were incubated for two to four minutes before being suspended in 5 mL of M.W.S. with gentle shake. Then, the suspension was centrifuged for three minutes at 1500 rpm to form a pellet, which was stored at -86 ºC until needed [25].

ATP content assay

An ATP colorimetric assay kit Catalog No: E-BC-K157-S (ElabScience Biotechnology Inc. USA) was used. The ATP content was determined by measuring the optical density (OD) value at 636 nm according to manufacturer's instructions.

Pyruvate content assay

A pyruvate colorimetric assay kit Catalog No: E-BC-K130-S (ElabScience Biotechnology Inc. USA) was used. The pyruvate content was calculated by measuring the OD value at 505 nm based on manufacturer's recommendation.

Statistical analyses

All data from the study were analyzed using ANOVA test in SPSS version 16, presenting means ± SD. IC_{50} values, curves, and histograms were generated using GraphPad version 8 and Origin Pro version 8.5. The synergy analysis of MH and 2DG combination was assessed using CompuSyn software program. CI was calculated using Chou-Talalay method The CI is a quantitative representation of pharmacological interactions (CI < 1, synergism; CI = 1, additivity; and CI > 1, antagonism) [26].

Results and Discussion

Anti-tumor suppressant potential

To assess the effects of different doses of 2DG and MH, MTT cytotoxicity assay was conducted on both cancer and normal cells. Results showed that effect of 2DG and MH on the cancer cell line SK-GT-4 was dose-dependent manner that inhibit the proliferation of the SK-GT-4.

The higher concentrations of these compounds increase effects on SK-GT-4 cells, especially, MH showed the highest inhibition rate (67 %) on SK-GT-4 cells at dose 1760 µg/ml with significant inhibition ($p < 0.05$), while, the lowest inhibition rate was 20 % at dose 110 µg/ml, but still significant inhibition ($p < 0.05$). In contrast, MH has no observed effects, but significant inhibition ($p < 0.05$), on HBL-100 cells at any concentration as shown in Figure 1 and Table 1.

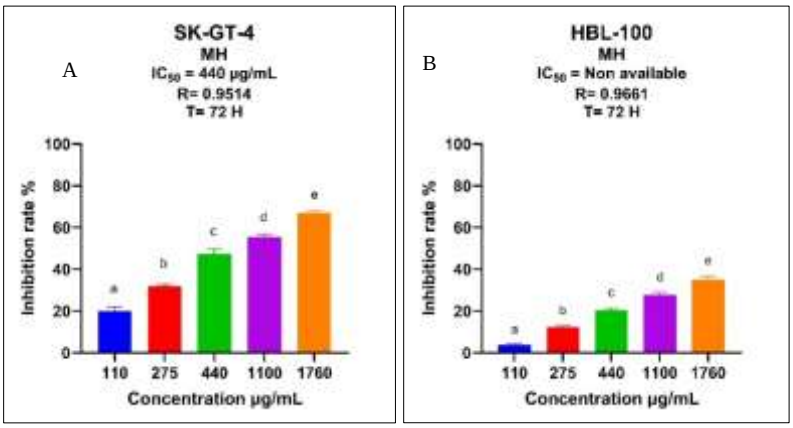


Figure 1. MTT assay reveals IC_{50} values of MH in SK-GT-4 and HBL-100, with a mean ± SD, $p<0.05$, N=4.

Table 1. The cytotoxic effect of MH on SK-GT-4 (A) and HBL-100 (B) cells after 72 h of treatment

(A) Concentration µg/ml	Inhibition rate %	(B) Concentration µg/ml	Inhibition rate %
110	20.05	110	3.97
275	31.79	275	12.36
440	47.42	440	20.25
1100	55.48	1100	27.72
1760	67.12	1760	35.12

2DG showed inhibition effects with significant inhibition ($p < 0.05$) only on SK-GT-4 cells, which reached in the highest inhibition rate with significant inhibition ($p < 0.05$) was 66 % at 750 µg/ml, while the lowest inhibition rate 18% at dose 75 µg/ml as shown in Figure 2 and Table 2. 2DG has no significant effect on HBL-100 cells in all test concentrations.

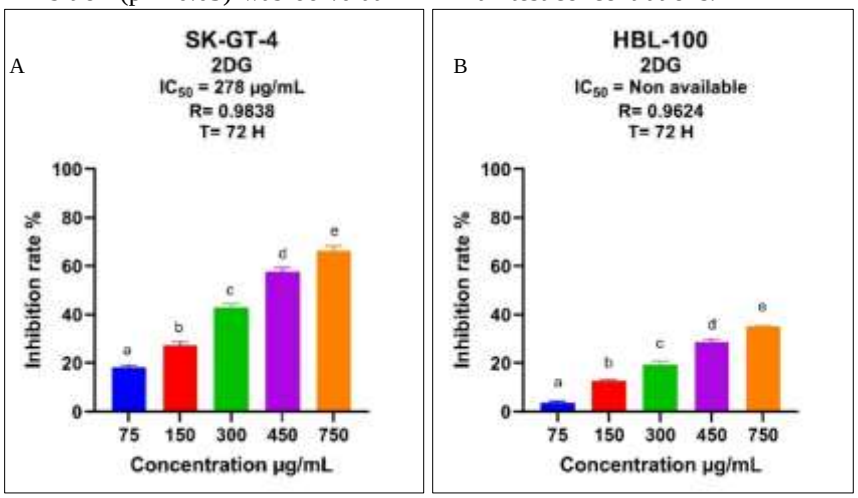


Figure 2. MTT assay reveals IC_{50} values of MH in SK-GT-4 and HBL-100, with a mean ± SD, $p<0.05$, N=4.

Table 2. The cytotoxic effect of MH on SK-GT-4 (A) and HBL-100 (B) cells after 72 h of treatment

(A) Concentration µg/ml	Inhibition rate %	(B) Concentration µg/ml	Inhibition rate %
75	18.20	75	3.77
150	27.34	150	12.68
300	42.95	300	19.39
450	57.72	450	28.71
750	66.37	750	35.16

These results indicated that both 2DG and MH has inhibition effects on cancer cell proliferation. This study is considered as a novel approach that not only inhibit enzyme HK in glycolysis in cancer cell, but also minimize adverse impacts on normal cells by disrupting the Warburg effect pathway, ATP needed for cancer cell proliferation [27].

Hexokinase phosphorylates 2DG form 2-deoxyglucose-6-phosphate, which is not be utilized by cells. Consequently, 2-deoxyglucose-6-phosphate can accumulate inside cells, then inhibit HK and reduce glucose uptake [28]. The 2DG can block the ability of enzyme HK to phosphorylate glucose by mimic glucose deprivation.

Additionally, 2DG inhibit glycolysis demonstrated to enhance the responsiveness of cisplatin in the treatment of human head and neck cancer xenograft tumors. Targeting glycolysis is a logic treatment pathway in cancer therapy. Analysis of the reactions of tumor cells to anticancer medications showed that 2DG can increase apoptotic by suppression of glycolysis [29].

The IC_{50} value calculation

The results indicated that the IC50 of MH was 440 µg/ml, and the IC_{50} of 2DG was 287 µg/ml on SK-GT-4 cells. For HBL-100 cells, IC50 has not been calculated due to not showed any observed impact, as shown in Figure 1 and Figure. 2. Previous studies showed that 2DG has effects on BxPc-3, and PC-3 cancer cell lines [22,26].

The synergistic effect

In this study, the assessment of the effects of the combination of MH and 2DG on cancer cells using the Chou-Talalay formulas indicated unique results that could play a role in cancer treatment in future.

Our finding showed a synergistic effect between MH and 2DG on cancer cells at the lowest concentration using compuSyn Isobologram software analysis as shown in Figure 3.

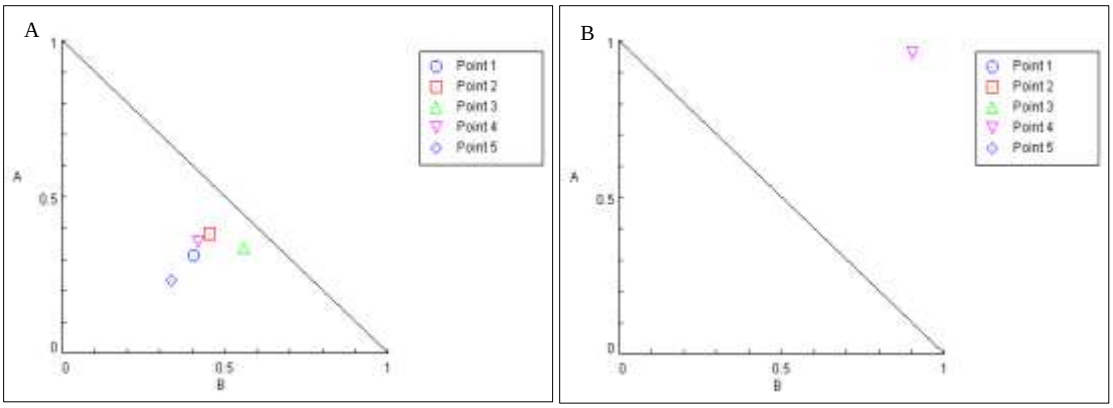


Figure 3. Illustration normalized isobologram of non-constant ratios combination. (A) Effect on SK-GT-4 cell line; (B) Effect on HBL-100 cell line.

The CI (combination index) value was CI <0.9 indicated a synergistic effect between the two compounds. The explanation of this finding is the 2DG and MH compete with glucose to bind with HK enzyme, leading to inhibiting the glycolytic [30]. The results also indicated that the combination of 2DG and MH could block the conversion of pyruvate into lactate that leading to reduce ATP required for the proliferation of the cancer cells as shown in Table 3 and Figure 4A and B.

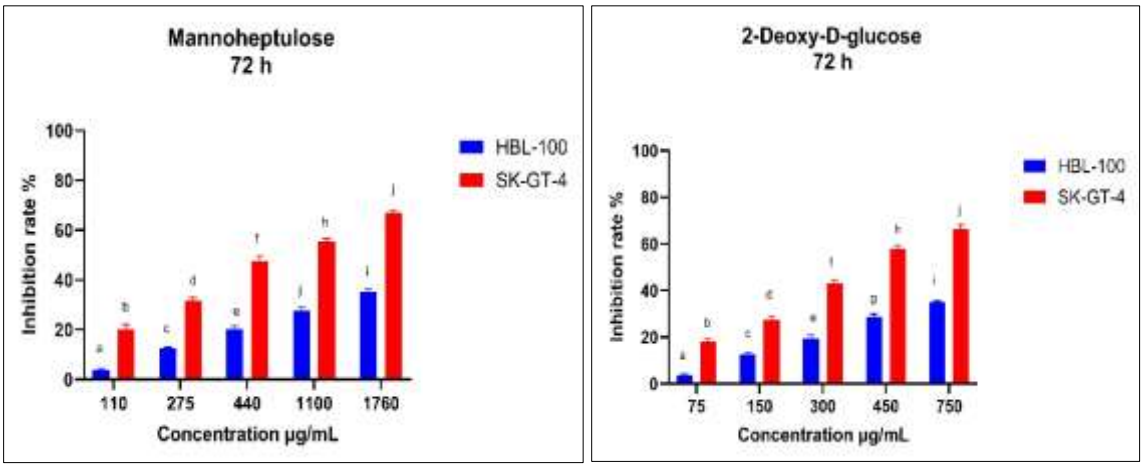


Figure 4. Comparison of MH and 2DG in HBL-100 and SK-GT-4 cells after 72 h of treatment.

Table 3. SK-GT-4 cells treated with combination of MH and 2-DG for 72 h

Points	Mannoheptulose µg/ml	2-Deoxy-D-Glucose µg/ml	Effect	Combination Index (CI)	Description
1	110	75	0.33	0.6163	Synergism
2	275	150	0.48	0.7377	Synergism
3	440	300	0.61	0.6569	Synergism
4	1100	450	0.77	0.5730	Strong Synergism
5	1760	750	0.88	0.4205	Strong Synergism

HBL100 cells showed antagonistic results at all doses, and the CI value was CI >1 as showed in Table 4 and Figure 5A and B. This finding could be considered safer, less toxic, and has high selectivity more than the previous study that used same compounds [31].

Table 4. HBL-100 cells treated with combination of MH and 2-DG for 72 h

Points	Mannoheptulose µg/ml	2-Deoxy-D-Glucose µg/ml	Effect	Combination Index (CI)	Description
1	110	75	0.031	3.7003	Strong Antagonism
2	275	150	0.10	2.8084	Strong Antagonism
3	440	300	0.18	1.9059	Moderately Antagonism
4	1100	450	0.28	1.8660	Moderately Antagonism
5	1760	750	0.33	2.7537	Strong Antagonism

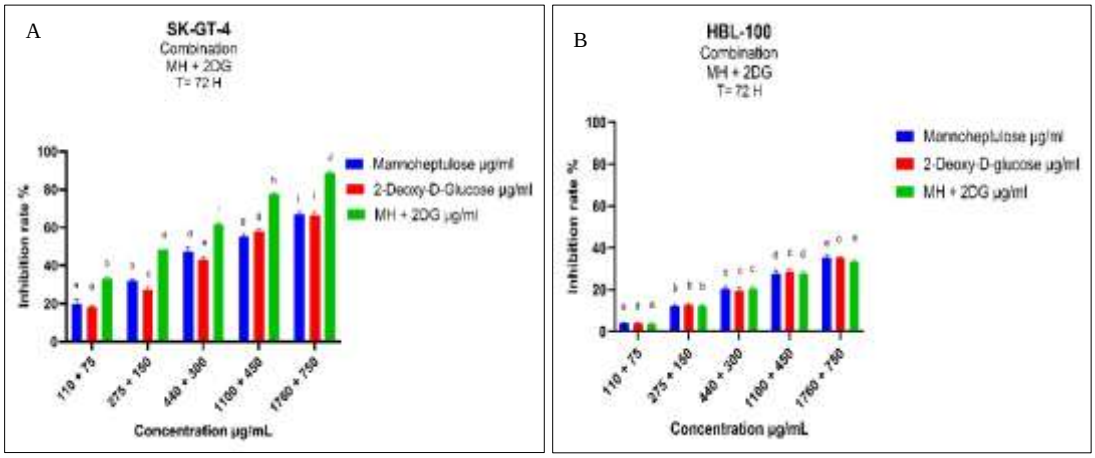


Figure 5. Synergistic effect of combined doses of MH and 2DG on SK-GT-4 cells (A) and HBL-100 cells (B).

Apoptotic cell death in treated cells

Acridine orange/ethidium bromide double stain method was used to evaluate the MH and 2DG ability to induce apoptosis in SK-GT-4 and HBL-100 cells. The results showed a strong cytopathic impact of the combined treatment. The treated cells with MH, 2DG and combined together appeared yellow-orange (early apoptosis) or red (late apoptosis or dead cells), while untreated cells remained green (viable cells) when observed under a fluorescence microscope using the AO/EB assay.

However, 2DG and MH have no effects on normal cells. The results also indicated that SK-GT-4 cells treated with MH and 2DG together increased red fluorescence emission significantly compared to monotherapies, indicated apoptosis as shown in Figure 6. This approach of using 2DG and MH, either alone or in combination considered a promising approach in cancer treatment due to targeting the altered metabolism in cancer cells and led to ATP depletion cancer cells deaths.

Many studies showed that efficient energy production required c-Sc activity through oxidative phosphorylation, and for the suppression of reactive oxygen species (ROS) production. Specifically, c-Src phosphorylates NADH dehydrogenase (ubiquinone) flavoprotein 2 (NDUFV2) at Tyr193 in respiratory complex I (CxI), which is indispensable for NADH dehydrogenase activity and ATP production, and succinate dehydrogenase A (SDHA) at Tyr215 in CxII, which is required for the prevention of ROS production and for cell survival. Proteolytic proteins within the cell may have been stimulated as a result of the depletion of energy due to the suppression of the glycolysis process [(32)]. Activation of c-Src in mitochondria may prevent the generation of free radicals, and since the mitochondrial membrane has been damaged, this gives justification for the accumulation of free radicals and their increased damage [(33)].

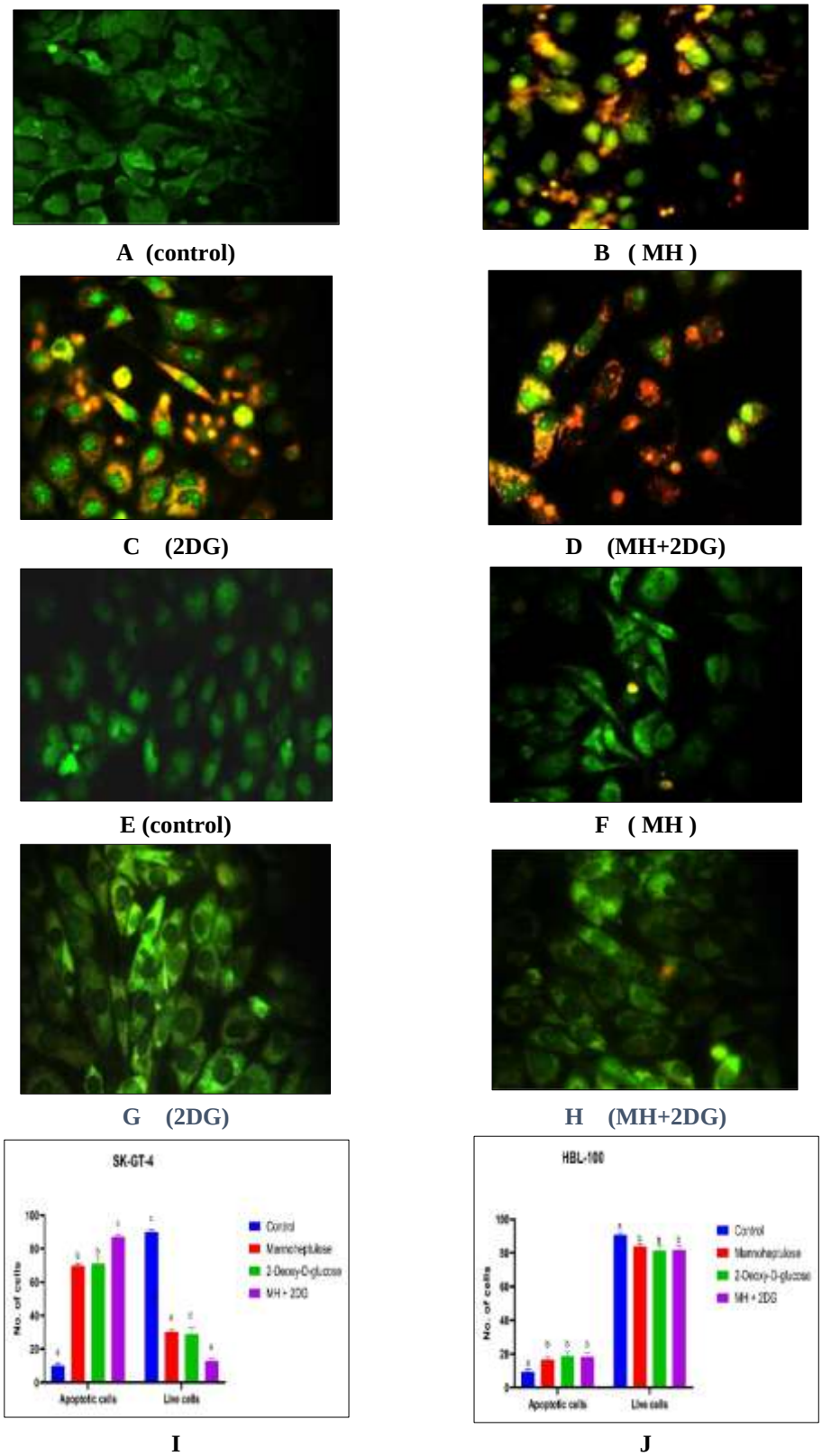


Figure 6. fluorescent microscopy images of the treated and untreated cells at 72 h. magnification (40 x). (A) Untreated and (B, C, and D) treated SK-GT-4 cells. (E) Untreated and (F, G, and H) treated HBL-100 cells. (I and J) The number of live and apoptotic cells in SK-GT-4 and HBL-100 cell lines respectively treated with MH, 2DG and MH + 2DG for 72 h.

The suppressive of malignant cells invasion and migration

Cell migration is a complex process for tissue development, cancer invasion and wound repair in multicellular organisms [34]. The effects of 2DG and MH alone or together were examined on the ability of the migration on SK-GT-4 and HBL-100 cells using inverted microscopy with a digital camera. The results showed that the treatment with 2DG and MH alone or in combination at IC_{50} dose has a remarkable inhibition on cells proliferation and migration as shown in Figure 7.

In contrast, the 2DG and MH alone or together have no observed effects or slightly effects on the migration of HBL-100 cells, both cells are increased as shown in Figure 8.

On the other hand, the exposure on SK-GT-4 cancer cell line upregulated inhibition at 48 hours compared to the control cells that showed no invasion inhibition to the wounded area. The growth halted because of the compounds inhibit glycolysis leading to apoptosis This is evidence that HK can bind with Mannoheptulose that indicated cancer cells have one isomer hexokinase enzyme (II), but normal cells have five isomers of HK enzyme [35]. The treated HBL-100 cells were very active, with large migration area, however, control cells have larger area of migration as shown in Figure 9.

Additionally, cancer cells have a large quantity of non-metabolizable glucose analog 2DG that presented as accumulation toxic waste. The results clearly demonstrated that combined treatment of 2DG and MH have highly synergistic impacts in inhibiting cancer cells through effecting on several biological processes such as invasion, metastasis, and migration, eventually leading to cell death.

SK-GT-4 cells exposed to MH or 2DG alone for 72 hours showed abnormal cells appearance, impaired migration and halted growth. While HBL-100 cells were not affected by either compounds or together after 72 hours as shown in Figure 10.

Most cells remain active and area size reduced significantly. Untreated normal cells showed the largest invasion rate and the cells able to repair wounded area. Our data clearly showed that treatment with MH and 2DG or in combination caused a significant inhibition of cell migration in a time-dependent manner. One of the most possible reasons for poor migration of cancer cells could be the activation of cell death pathways treated with MH and 2DG. Rapid tumor growth usually outstrips oxygen supply, leading to areas of hypoxia. It is known that hypoxia affects the reliance of cancer cells on glycolysis to produce energy and survive, leading to cells death [36, 37].

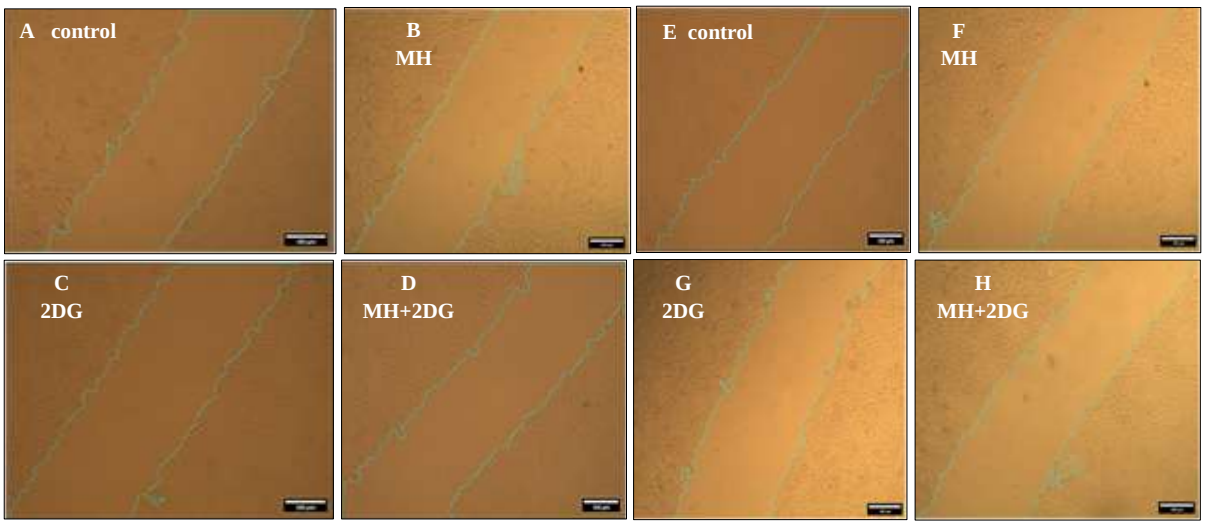
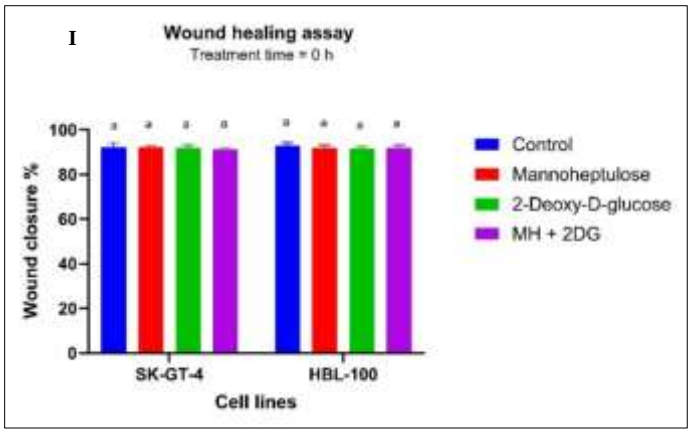
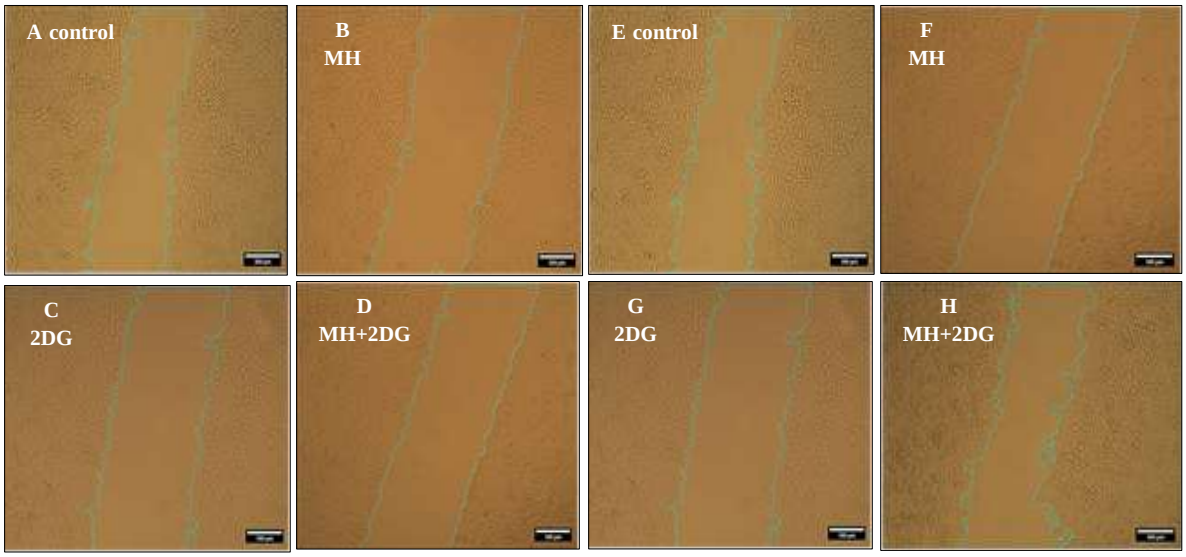


Figure 7. Wound healing assay analysis of SK-GT-4 and HBL-100 cells migration in vitro at 0 h (scale bar = 300 µm) magnification 40 x. (A) Untreated and (B, C, and D) treated SK-GT-4 cells. (E) Untreated and (F, G, and H) treated HBL-100 cells.



Continued figure 7 . (I) wound closure (%) of the total area in µm^2.



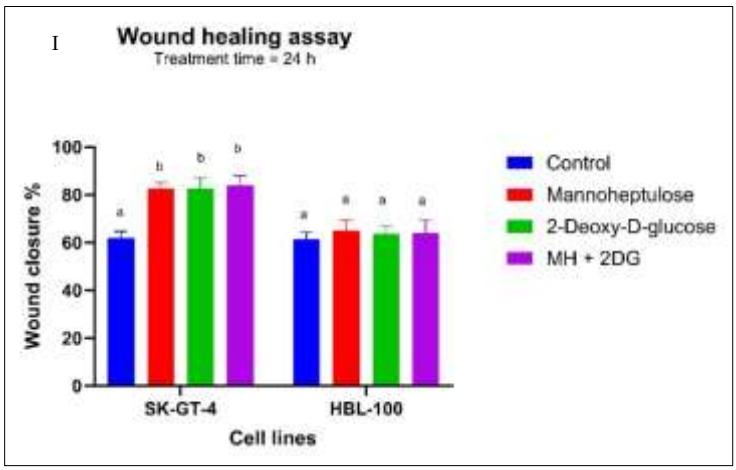


Figure 8. Wound healing assay analysis of SK-GT-4 and HBL-100 cells migration in vitro at 24 h (scale bar = 300 µm) magnification 40 x. (A) Untreated and (B, C, and D) treated SK-GT-4 cells. (E) Untreated and (F, G, and H) treated HBL-100 cells. (I) wound closure (%) of the total area in µm^2.

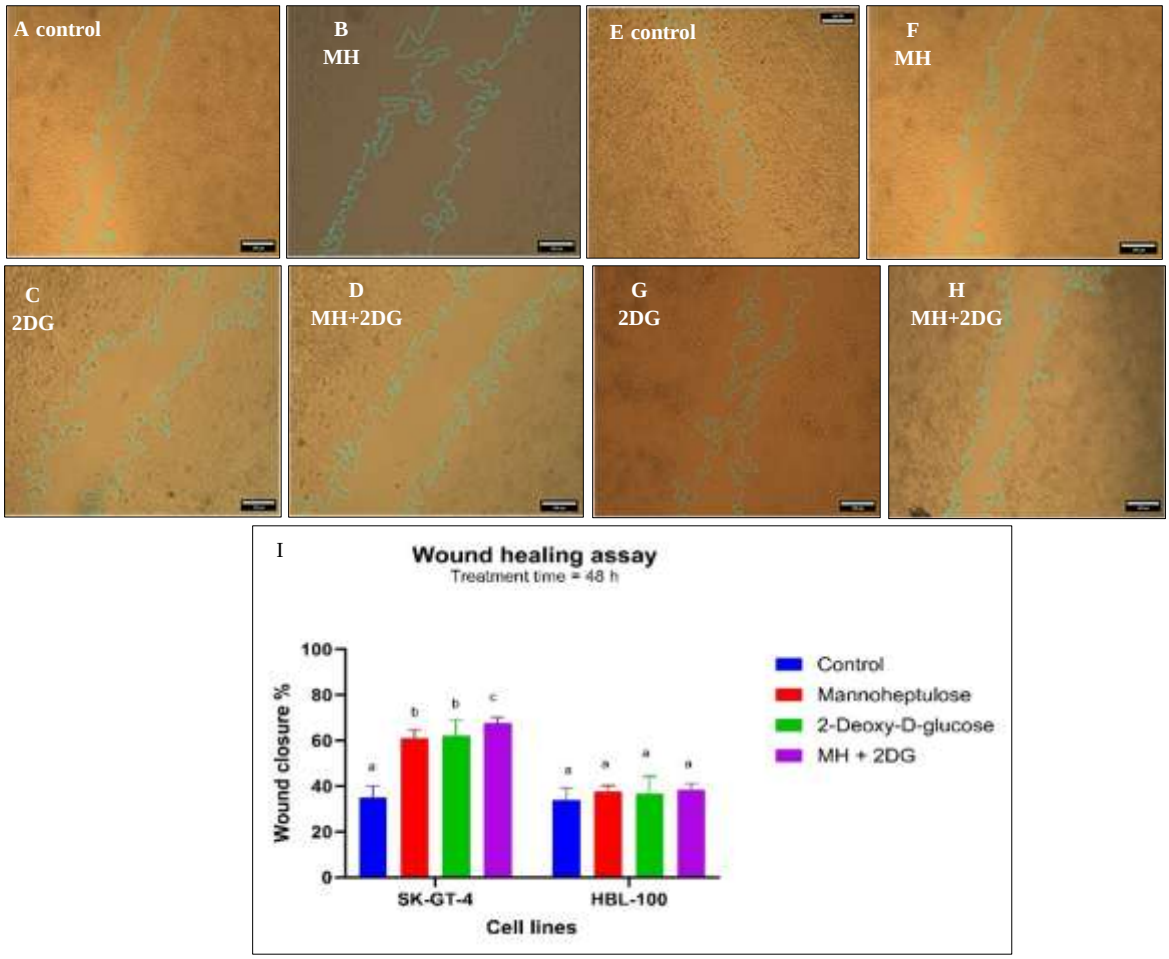


Figure 9. Wound healing assay analysis of SK-GT-4 and HBL-100 cells migration in vitro at 48 h (scale bar = 300 µm) magnification 40 x. (A) Untreated and (B, C, and D) treated SK-GT-4 cells. (E) Untreated and (F, G, and H) treated HBL-100 cells. (I) wound closure (%) of the total area in µm^2.

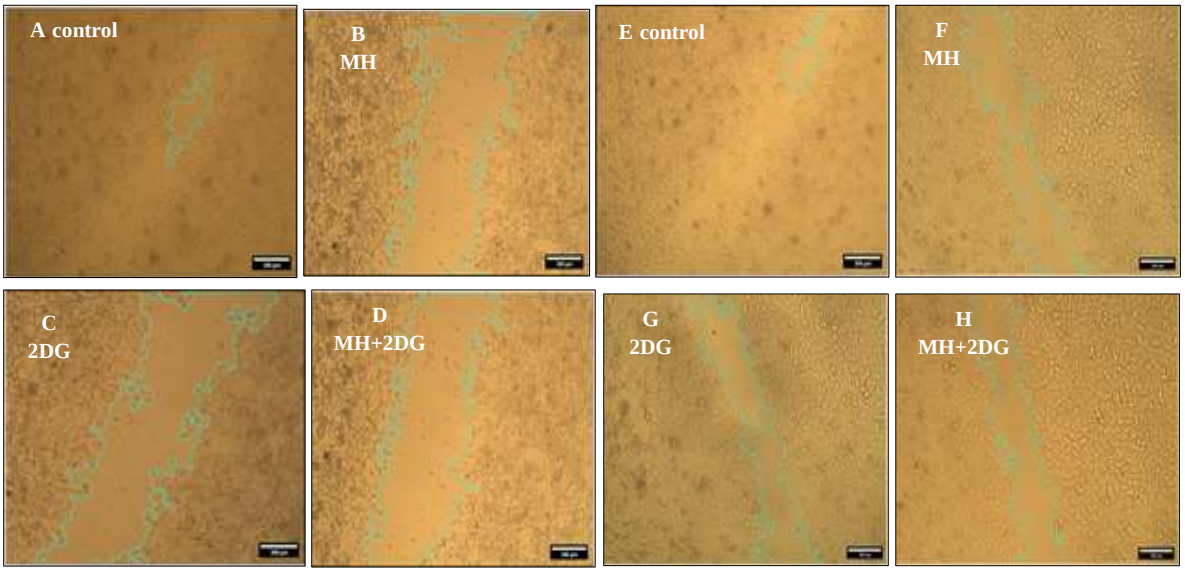
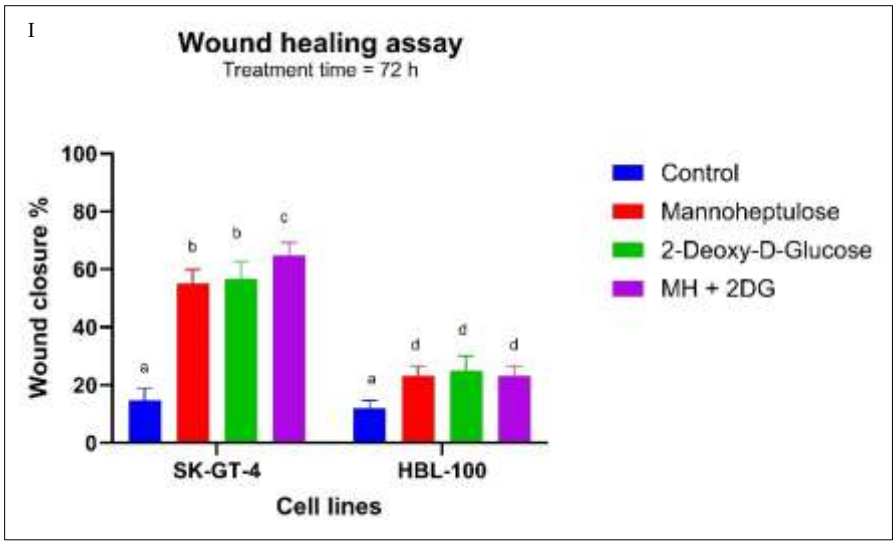


Figure 10. Wound healing assay analysis of SK-GT-4 and HBL-100 cells migration in vitro at 72 h (scale bar = 300 µm) magnification 40 x. (A) Untreated and (B, B, and D) treated SK-GT-4 cells. (E) Untreated and (F, G, and H) treated HBL-100 cells. (I) wound closure (%) of the total area in µm^2.



Continued figure 10 . (I) wound closure (%) of the total area in µm^2.

ATP depletion and pyruvate accumulation through Warburg effect selective inhibition

To study the inhibition of ATP depletion and pyruvate accumulation, SK-GT-4 and HBL-100 cells were exposed to MH and 2DG either alone or in combination. ATP and pyruvate levels in both cells were measured for 72 hours. The results found the combination of MH and 2DG significantly ($p < 0.05$) decreased ATP and pyruvate levels in SK-GT-4 cells compared to monotherapy exposure as shown in Figure 11.

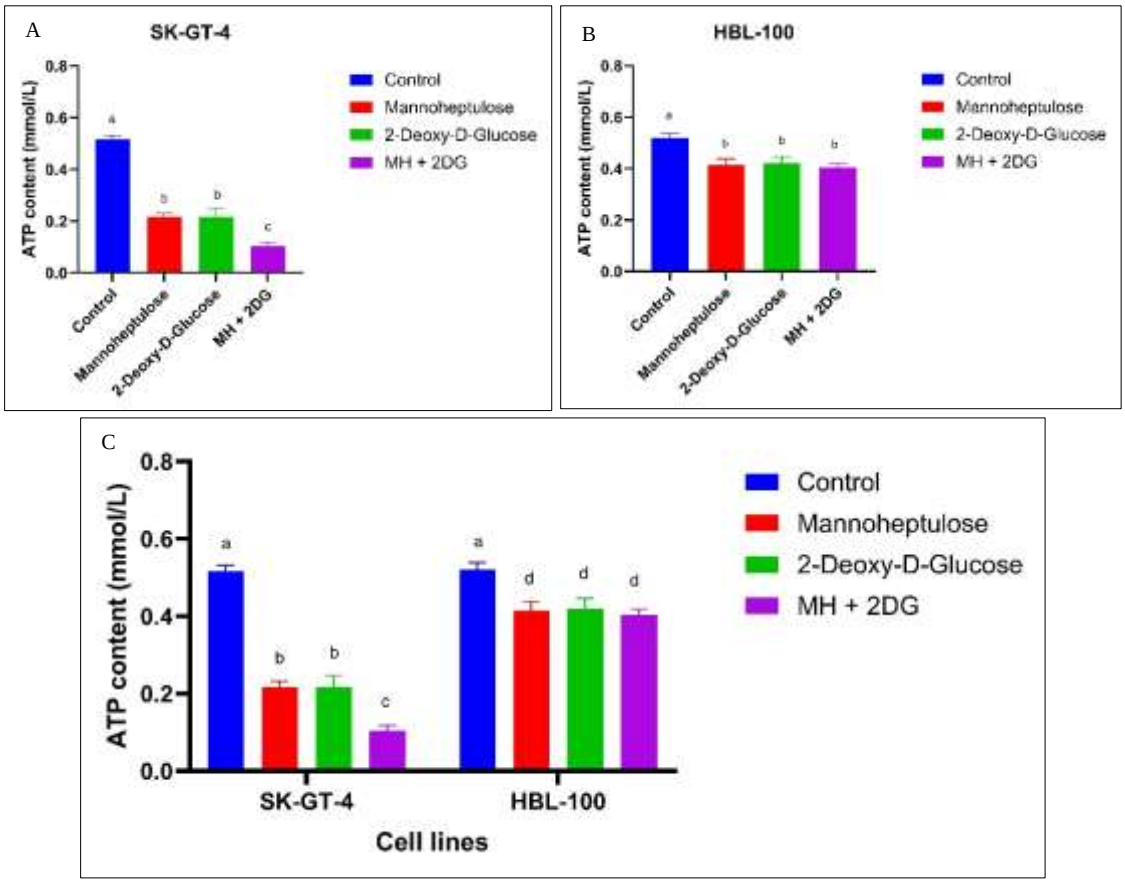


Figure 11. Comparison of the effect of monotherapies and combination of MH + 2DG on ATP production on treated cells (A) SK-GT-4 cells (B) HBL-100 cells (C) MH + 2DG combination therapy (*P < 0.05)

Furthermore, blocking glycolysis with MH and 2DG may cause cancer cells to undergo apoptosis due to the mitochondrial pathway [38]. The levels of pyruvate in SK-GT-4 cells are significantly ($p < 0.05$) reduced by MH and 2DG together, but not in normal HBL-100 cells as shown in Figure 12.

The metabolic inhibitor 2DG plays multiple roles in metabolism energy. Firstly, it competes with glucose for GLUTs, which enhance glucose diffusion inside cell decreasing glucose uptake by cancer cells. Secondly, the non-selective inhibition of HK traps glucose inside the cell by phosphorylation, further decreases the overall glycolytic flux. Once phosphorylated by HK, the 2DG is a dead-end metabolite that is not involved in downstream metabolism.

Theoretically, 2DG is a promising candidate for inhibiting the Warburg effect, however, 2DG has not been successful in clinical trials alone. Previous studies have focused on combination strategy with conventional chemotherapeutics or radiotherapy. The energy deficiency observed in this study may be attributed to the ability of MH and 2DG to bind to the active site of the HK enzyme and compete with the glucose molecule [39].

However, both MH and 2DG are non-metabolic substances, this leads to a decrease in ATP and accumulation of pyruvate as a result of incomplete glycolysis. This can impact cell vitality and activity of cells. The inhibition of glycolysis may lead to energy depletion, which in turn stimulates protein-degrading pathways within the cell, causing damage to organelle membranes, including mitochondria [40].

This damage leads to an increase in free radical generation. Research indicates that the activation of c-Src in mitochondria can prevent the generation of free radicals. However, when mitochondrial membrane is damaged, it allows an accumulation of free radicals and an increase in harmful effects. This accumulation explains the observed decrease in cell proliferation, incomplete edge closure in wound repair, and the stimulation of cellular death. Reduced cell proliferation and inadequate wound healing may be attributed to inhibition of glycolysis, which can activate several cellular events including ATP depletion, enzymes stimulation, mitochondrial damage, and an excess of reactive oxygen species.

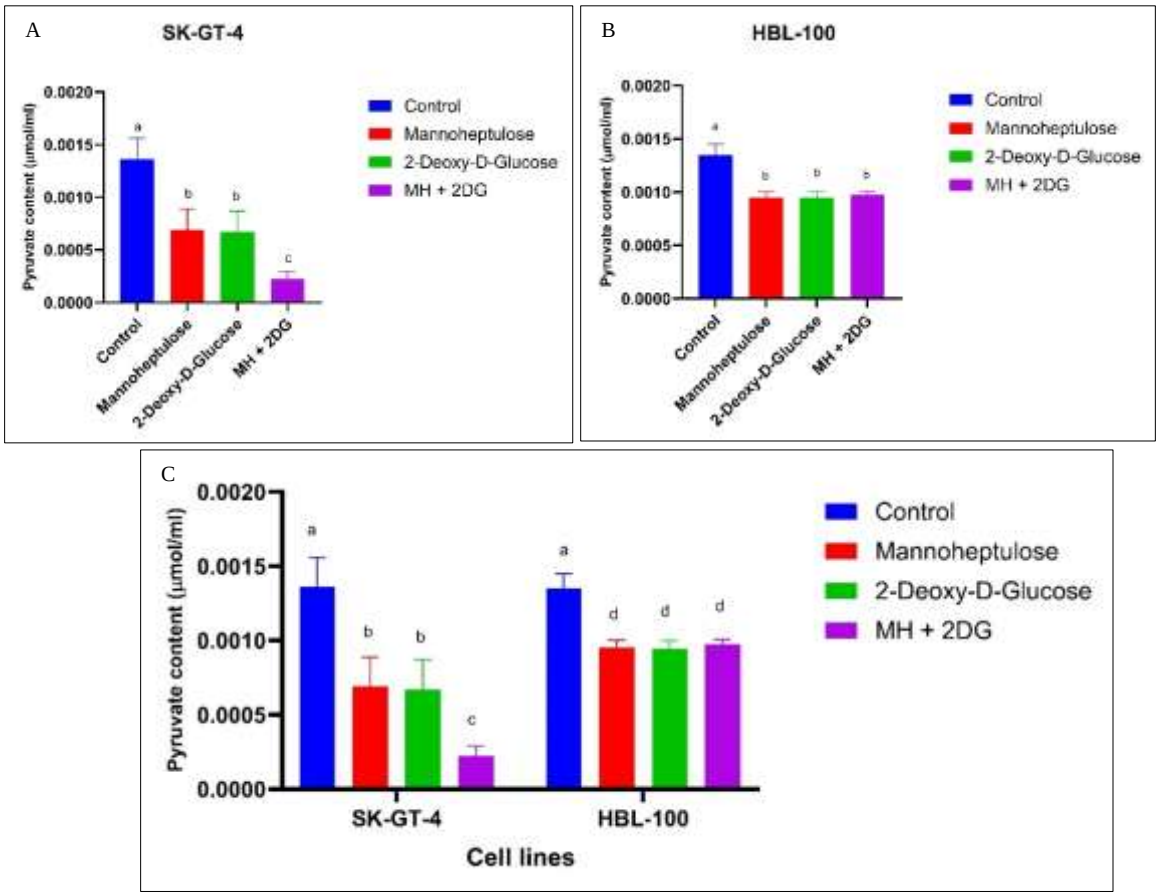


Figure 12. Comparison of the effect of monotherapies and combination of MH + 2DG on ATP production on treated cells (A) SK-GT-4 cells (B) HBL-100 cells (C) MH + 2DG combination therapy (*P < 0.05)

Conclusion

MH and 2DG, either alone or in combination, increase cytotoxicity by inhibiting glycolysis and inducing apoptosis, effectively preventing the growth and spread of SK-GT-4 cancer cells. The inhibition of glycolysis resulted in a decrease in pyruvate levels, which subsequently led to a reduction in lactate production and ATP synthesis. This reduction in ATP levels enhanced the cytotoxic effect on cancer cells while sparing normal cells. The combination of MH and 2DG, when used alone or in combination with other selective targeting agents, presents promising potential for cancer therapy.

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Conflicts of Interest

The authors declare no competing interests.

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Author Contribution

Ahmad Naeem Al-Khammas: conducting and visualizing experiments, data analysis and interpretation, wrote the manuscript. Ali Abd Allateef Al- Ali: designed and photographed the experiments; provided materials, analysis tools, or contributed data; Data analysis and interpretation, also wrote the first draft, project supervision, final draft approving.

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إستحثاث التجويع الإنتقائي للخلايا السرطانية من خلال التثبيط التآزري لمسار التحلل السكري بإستخدام مادتي المانوهيبتالوز و ٢-ديوكسي كلوكوز

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الخلاصة

تعتمد الخلايا السرطانية بشكل كبير على مسار تحلل السكر لإنتاج الطاقة، رغم كونه أقل كفاءة من الفسفرة التأكسدية، مما يجعلها تستهلك كميات كبيرة من السكر. يمكن استهداف هذا المسار بشكل انتقائي باستخدام مثبطات لإنزيم الهيكسوكيناز، مما يعطل إنتاج الطاقة في الخلايا السرطانية دون التأثير على الخلايا الطبيعية. تم التحقيق في الآليات المضادة للسرطان ل ٢ Deoxy-D-Glucose (2DG) و Mannoheptulose (MH) في خط خلايا سرطان المريء الغدي (SK-GT-4) و خط الخلايا الطبيعية (HBL-100) باستخدام MTT و إختبار التئام الجروح لتقييم الآثار السامة لمواد الدراسة ضد الخلايا والانتشار الخلوي. تم الكشف عن تأثير موت الخلايا المبرمج باستخدام تقنية AO / EB. لتأكيد تأثير العوامل على استقلاب طاقة الخلية، تم تقييم مستويات ATP والبيروفات و hexokinase كمعلمات تحلل السكر. أظهرت النتائج تأثيرات سامة عالية الإنتقائية ضد نمو و تكاثر الخلايا السرطانية ل ٢DG و MH، مع تأثير تآزري قوي عند استخدامها معا ، خاصة في خلايا SK-GT-4. كانت قيم IC_{50} لـ 2DG و 278 MH و 440 ميكروغرام / مل ، على التوالي ، في خلايا SK-GT-4 ، بينما لم تصل خلايا HBL-100 إلى قيم IC_{50}. أشارت نتائج التئام الجروح إلى انخفاض في تكاثر وغزو الخلايا السرطانية. أظهرت نتائج مستويات ATP والبيروفات أن الاستخدام المشترك للمادتين يقلل بشكل أكثر من الطاقة الخلوية و يكدس البيروفيت مقارنة مع استخدامهما بشكل منفرد، مما يشير إلى تأثير تآزري ضد الخلايا السرطانية وإمكانية اتباع نهج علاجي أكثر فعالية في المستقبل.

الكلمات المفتاحية: ايض الورم ، مانوهيبتالوز،٢-ديوكسي كلوكوز، أستنزاف الطاقة، تكدس البايروفيت.