

# Evaluation of Cytotoxic Effect of Metformin on a Variety of Cancer Cell Lines

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## Abstract

Summary Metformin is an oral hypoglycaemic and anti-diabetic medication. Metformin suppresses cancer cell development and reduce the amounts of stimuli that promote cell proliferation. Cancer is a category of diseases marked by the uncontrollable development and spread of abnormal cells. This study was aimed to determine the efficacy of metformin cytotoxicity on a variety of cancer cell lines. In this study methods used serious metformin concentrations (0, 5, 10, 15, 25, 35, 65, 130  $\mu$ M) on a variety cells (Hela, AMJ3, HCAM, A172, HBL100) were incubated for 48,72 hours, We utilize the MTT assay to determine the viability of cancer cells, which is a typical test for determining the cytotoxic effects. Analysis indicated that populations of cancer cells was lowering the results revealed significant reduce viability of cancer cell line had highly significant cytotoxic effect at levels ( $P < 0.01$ ) with in HeLa significantly than, AMJ3, HCAM, A172 when compare with normal cell HBL00 in dose 130  $\mu$ M. The potential cytotoxic effect of metformin in Hela than other cancer cell line and normal cell  $15.081 \pm 0.167$  and IC 50  $\mu$ M for metformin on Hela (7.492  $\mu$ M). We concluded of this study metformin had a highly significant selective cytotoxic effect on HeLa, and lesser effect on AMJ3, HCAM, and A172 cells than on normal cells.

**Keywords:** Antitumor • Hela • Breast cancer • Cell lines

## Introduction

Cancer is a dangerous disease induced by environmental influences producing mutation in genes involved in cell growth regulation it is defined by an inability to control. Cell proliferation happens as a result of uncontrolled cell activity which affects the surrounding tissues, resulting in cell proliferation [1]. Metformin's anti-diabetic effects have been studied extensively, and this includes interactions with pathways in tissues that lead to insulin resistance [2]. Metformin mechanism as anticancer function suggested cancer cell proliferation is inhibited when mammalian target of rapamycin complex 1 is suppressed (mTORC1). The mTOR protein kinase and the raptor scaffolding protein make up mTORC1, amulti protein complex [3]. Metformin's anticancer activity is mediated by the activation of Adenosine Monophosphate Protein Kinase (AMPK), which inhibits the mTORC1 signaling pathway, according to the anticancer function of metformin. Metformin reduces endogenous ROS production, oxidative stress, DNA damage, and mutagenesis in normal somatic cells or their variants expressing activated oncogenes provide a novel mechanism to explain the reduced cancer incidence associated with metformin therapy and increase the possibility of novel applications, according to a variety of studies [4]. Metformin also decreases inflammation, which is a key factor in the initiation and progression of carcinogenesis [5]. In the antineoplastic effect of metformin, described a new mechanism involving the regulation of Adenosine A1 Receptor (ADORA1) expression in human colorectal and breast cancer cells. Metformin's antitumor activity is attributed to a reduction in mitochondrial gluconeogenesis [6]. High levels of IGF-1 and IGF-2 are linked to the growth of cancer or with cancer recurrence in cancer survivors, IGF mediated signaling has important roles in regulating cellular proliferation and apoptosis (role as circulating hormone and a tissue growth factor) apart from their increased levels in various cancers [7].

Aim of the study is to assess the percentage of viability to identify the cytotoxic effects of metformin in different cell growth lines (Hela, HCAM, AMJ13) and *in vitro* on normal cell line (HBL100) and to Compare the IC50 of metformin in cancer cells to the IC50 in normal cells.

## Materials and Methods

### Maintenance of cell cultures

Lines of normal and cancerous cells the IRAQ Biotech Cell Bank provided human cervical cancer cell lines (HeLa), liver cancer cell lines (HCAM), normal human HBL cell lines (HBL100), glioma cancer cell (A172) Glioma, malignant brain tumor, and a new breast cancer cell line (AMJ13) All cell kept in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 g/mL streptomycin. Trypsin-EDTA was used to passage the cells. Reseeded at 50% confluence twice a week and incubated at 37°C and 5% Co<sub>2</sub>.

### Cytotoxicity assays

To test the cytotoxic effect, the MTT cell viability assay was performed on 96-well plates. Cell lines were planted at a density of  $1 \times 10^4$  cells per well. After 24 hours and when a confluent monolayer was produced, cells were treated with the tested compound at varying concentrations with Metformin (Samara company Iraq) (0, 5, 10, 25, 35, 45, 65, 130  $\mu$ M). After 48 and 72 hours, cell viability was measure by removing the medium, adding 28  $\mu$ L of 2 mg/mL solution of MTT (and incubating the cells for 2 h at 37°C Following removal of the MTT solution, the crystals in the wells were solubilized by adding 100 L of DMSO (Dimethyl Sulphoxide) and incubating at 37°C for 15 minutes with shaking [8]. The absorbency was measured using a micro plate reader at 620 nm (test wavelength), and the assay was done three times. The assay was performed in triplicate.

Cell viability % = Mean OD/Control OD \*100%.

The half maximal Inhibitory Concentration (IC50) is values were calculated from survival-concentration curves using non-linear regression. The IC50 was calculated using Graph Pad Prism (version 8) for metformin after 72 hours for all cancer and normal cell line.

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## Data Analysis

All results expressed by version 17.1 of the Statistical Software for Social Sciences (SPSS) to conduct the statistical analyses. The mean and standard error calculated using traditional statistical procedures. P-values were calculated using analysis of variance (ANOVA) and the Duncan test post hoc to find significant differences between and within groups. The statistical tools Graph Pad prism version 8 used to perform and display the results as means SEM. P values \* means  $p < 0.05$  mean significant, \*\* means  $p < 0.01$  highly significant [9] (Table 1).

## Results

Metformin's cytotoxic effect against the human cervical cell line has been assessed for 48 hours determined by MTT testing as in Table 1. The statistical assignment of three replicates shows a significant difference as actual points. The cells were treated for 48 hours with a series of metformin concentrations. The results showed the decline in the cell survival rate (26.466, 25.097, 24.869, 24.185, 23.044%) with the increasing concentrations of metformin in Hela cell line (25,35,65,130  $\mu\text{M}$ ) that different significantly to 0 concentration (before add metformin). The results demonstrate which Hela cell proliferation inhibitory style is already in conformity with the dose dependent method.

Means with different superscript different small letter (a, b, c, d) are significantly difference between different group in same period (Duncken Test) \* $p < 0.05$  mean significant. \*\* mean  $p < 0.01$  highly significant. In 72

results showed that the cell survival rate declines with the increasing concentrations of metformin in Hela cell line reach to (17.442, 15.649, 15.305, 15.279, 15.081  $\mu\text{M}$ ) significantly decline cell survival rate than 0 before add the metformin and 5,10  $\mu\text{M}$  as in Table 2. Means with slandered error of mean different superscript different small letter (a, b, c, d) are significantly difference between different group in same period (Duncken Test) \* $p < 0.05$  mean significant \*\* mean  $p < 0.01$  highly significant (Table 2).

In Figure 1 show that metformin reduces cell viability in the cancer cell lines (Hela) in a time- and concentration-dependent manner. Cell viability as determined by the MTT assay in Hela (incubated for 48 and 72) hours with (0-130  $\mu\text{M}$ ) concentrations of metformin that agree with [10].

Metformin's cytotoxic effect on the viability of a cancer cell line breast cell line (AMJ3) in order to assess metformin's cytotoxic effect on (AMJ3). MTT was used to determine the vitality of AMJ3 cells. The viability of AMJ3 was obviously reduced in a dose-dependent manner, with viability reducing to 31.130% when the concentration was increased to 130  $\mu\text{M}$  after 48 hours compare statically of conc. 0 before adding metformin that agree with as in Table 3 [11].

Means with different superscript different small letter (a, b, c, d, e) are significantly difference between different group in same period (Duncken Test) \* $p < 0.05$  mean significant \*\* mean  $p < 0.01$  highly significant. In 72 hours, time depended manner reduced cell viability reach to 21.453 at dose 130  $\mu\text{M}$  in the Table 4. Means with different superscript different small letter (a, b, c, d) are significantly difference between different group in same period (Duncken Test) \* $p < 0.05$  mean significant \*\* mean  $p < 0.01$  highly significant (Table 4).

Met.Conc $\mu\text{M}$	0	5	10	25	35	45	65	130	P -Value
Mean Viability	97.517 <sup>d</sup>	86.093 <sup>c</sup>	49.206 <sup>b</sup>	26.466 <sup>a</sup>	25.097 <sup>a</sup>	24.869 <sup>a</sup>	24.185 <sup>a</sup>	23.044 <sup>a</sup>	0.000**
SEM	0.324	1.089	1.747	0.235	0.235	0.294	0.256	0.176	

Note: Effect Of Metformin On Human Cervical Cancer Cell Line(Hela) After 48 hours.

\*\*: $p < 0.01$ : a,b,c,d significantly difference between different group in same period.

Table 1. Cytotoxicity of metformin on viability of Hela cell lines after 48 hours cell viabilit was measured with MTT assay.

Met.Conc $\mu\text{M}$	0	5	10	25	35	45	65	130	P -Value
Mean Viability	97.517 <sup>d</sup>	73.132 <sup>c</sup>	43.057 <sup>b</sup>	17.442 <sup>a</sup>	15.649 <sup>a</sup>	15.305 <sup>a</sup>	15.279 <sup>a</sup>	15.081 <sup>a</sup>	0.000**
SEM	0.324	1.372	1.602	0.754	0.272	0.135	0.251	0.1677	

Note: Cytotoxic Effect Of Metformin On Human Cervical Cancer Cell Line (Hela) Cell Lines After 72 hours.

\*\*: $p < 0.01$ : a,b,c,d significantly difference between different group in same period.

Table 2. Cytotoxicity of metformin on viability of Hela cell lines after 72 hours cell viability was measured with MTT assay.

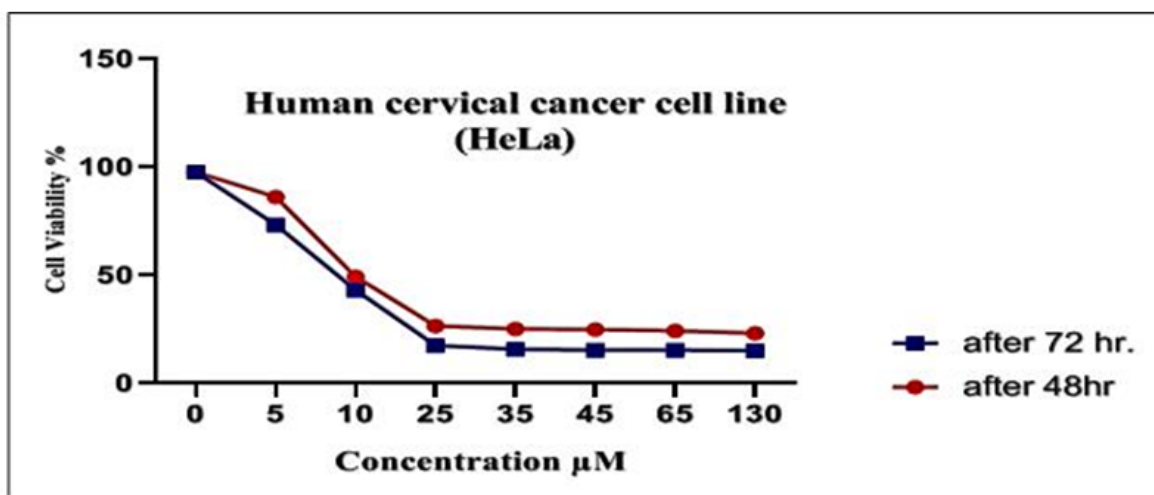


Figure 1. Metformins cytotoxic effect on human cervical cancer cell lines (HeLa)after 48 and 72 hrs.

Met.ConcµM	0	5	10	25	35	45	65	130	P -Value
Mean Viability	97.835 <sup>e</sup>	95.547 <sup>de</sup>	95.373 <sup>de</sup>	90.205 <sup>c</sup>	84.657 <sup>c</sup>	47.219 <sup>b</sup>	45.102 <sup>b</sup>	31.130 <sup>a</sup>	0.000**
SEM	0.885	1.886	1.937	0.753	2.075	1.787	2.144	1.1425	

**Note:** Metformin's the cytotoxic effect on the breast cell lines 48 hours of AMJ3.

\*\*: $p < 0.01$ : a,b,c,d,e significantly difference between different group in same period.

**Table 3.** Cytotoxicity of metformin on viability of breast cell lines after 48 hours cell viability was measured with MTT assay.

Met.ConcµM	0	5	10	25	35	45	65	130	P -Value
Mean Viability	97.835 <sup>e</sup>	93.967 <sup>de</sup>	33.022 <sup>de</sup>	22.400 <sup>c</sup>	22.295 <sup>c</sup>	22.084 <sup>b</sup>	21.979 <sup>b</sup>	21.453 <sup>a</sup>	0.000**
SEM	0.885	2.971	0.297	0.128	0.196	0.223	0.074	0.128	

**Note:** Cytotoxic effect of Metformin on breast cancer cell line AMJ3 after 72 hours.

\*\*: $p < 0.01$ : a,b,c,d,e significantly difference between different group in same period.

**Table 4.** Cytotoxic effect of Metformin on breast cancer cell line AMJ3 after 72 hours cell viability was measured with MTT assay.

In the Figure 2 mentioned the Cytotoxic effect of Metformin on Breast cancer cell line (AMJ3) cell lines after 48 hours. and 72 hours, Metformin's cytotoxic effect on the HCAM cell line after 48 hours. In 48 hours show high concentration reduced viability to 44.466% significantly than 99.320 % at 0 concentrations before added the metformin to plate contain HCAM cell line as in Table 5.

Means with different superscript different small letter (a, b, c, d, e) are significantly difference different group in same period (Duncken Test) \* $p < 0.05$  mean significant \*\* mean  $p < 0.01$  highly significant.

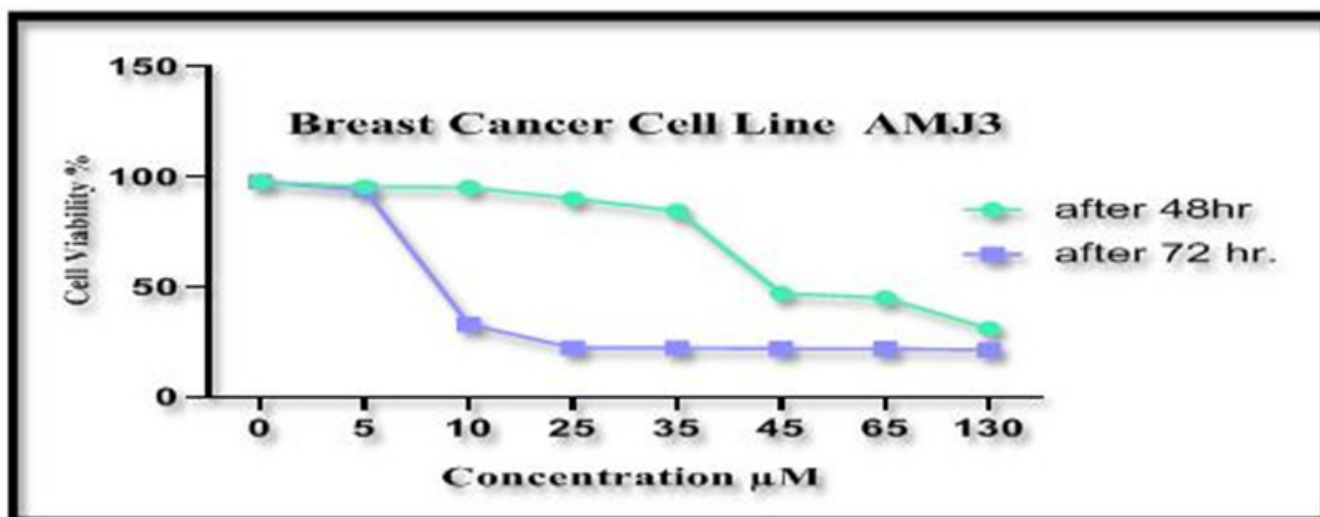
In 72 hours. HCAM viability was clearly reduced in a dose-dependent manner since the viability decreased to 36.38% when the concentration was increased to 130 µM. Metformin also inhibited liver cancer cell proliferation in a time dependent manner (Table 6).

Means with different superscript different small letter (a, b, c, d) are significantly difference between different group in same period (Duncken Test) \* $p < 0.05$  mean significant \*\* mean  $p < 0.01$  highly significant (Table 7).

In Figure 3 show the viability cell line of metformin on HCAM for 48 and 72 hours incubation mHCAM viability was clearly reduced in a time-dependent manner.

Metformin's cytotoxic effect on glioma cancer cell (A172) after 48 hour metformin (130 µM) resulted in a significant decrease in cell viability ( $P < 0.01$ ) at a 48 h reach to (74.258µM) show in Table 7, while higher concentrations of Metformin demonstrated highly significant decreases in viability (47.820) at 72 hours compared with the control before added metformin ( $P < 0.01$ ) (Table 8).

Means with different superscript different small letter (a, b, c, d, e) are significantly difference between different group in same period (Duncken Test) \* $p < 0.05$  mean significant \*\* mean  $p < 0.01$  highly significant. Means with different superscript different small letter (a, b, c, d, e, f) are significantly difference between different group in same period (Duncken Test) \* $p < 0.05$  mean significant \*\* mean  $p < 0.01$  highly significant.



**Figure 2.** Metformin cytotoxic effects in the 48 and 72-hour breast cancer cell lines (AMJ3).

Met.ConcµM	0	5	10	25	35	45	65	130	P -Value
Mean Viability	99.3201 <sup>e</sup>	98.004 <sup>e</sup>	92.941 <sup>e</sup>	78.463 <sup>d</sup>	73.503 <sup>cd</sup>	69.655 <sup>c</sup>	56.089 <sup>b</sup>	44.446 <sup>a</sup>	0.000**
SEM	0.490	0.874	2.257	2.482	1.388	2.825	3.061	2.170	

**Note:** Cytotoxic effect of Metformin on liver cancer cell line HCAM after 48 hours.

\*\*: $p < 0.01$ : a,b,c,d,e significantly difference between different group in same period.

**Table 5.** Cytotoxic Effect Of Metformin On Liver Cancer Cell Line (HCAM) After 48 Hours cell viability was measured with MTT assay.

Met. Conc $\mu$ M	0	5	10	25	35	45	65	130	P -Value
Mean Viability	99.320 <sup>d</sup>	71.890 <sup>e</sup>	70.814 <sup>c</sup>	55.058 <sup>b</sup>	54.513 <sup>b</sup>	53.641 <sup>b</sup>	52.224 <sup>b</sup>	36.838 <sup>a</sup>	0.000**
SEM	0.490	0.295	1.988	1.582	0.708	1.918	0.712	1.462	

Note: Cytotoxic effect of Metformin on liver cancer cell line (HCAM) after 72 hours.

\*\*p<0.01: a,b,c,d significantly difference between different group in same period.

Table 6. Cytotoxic Effect Of Metformin On Liver Cancer Cell Line (HCAM) After 72 Hours cell viability was measured with MTT assay.

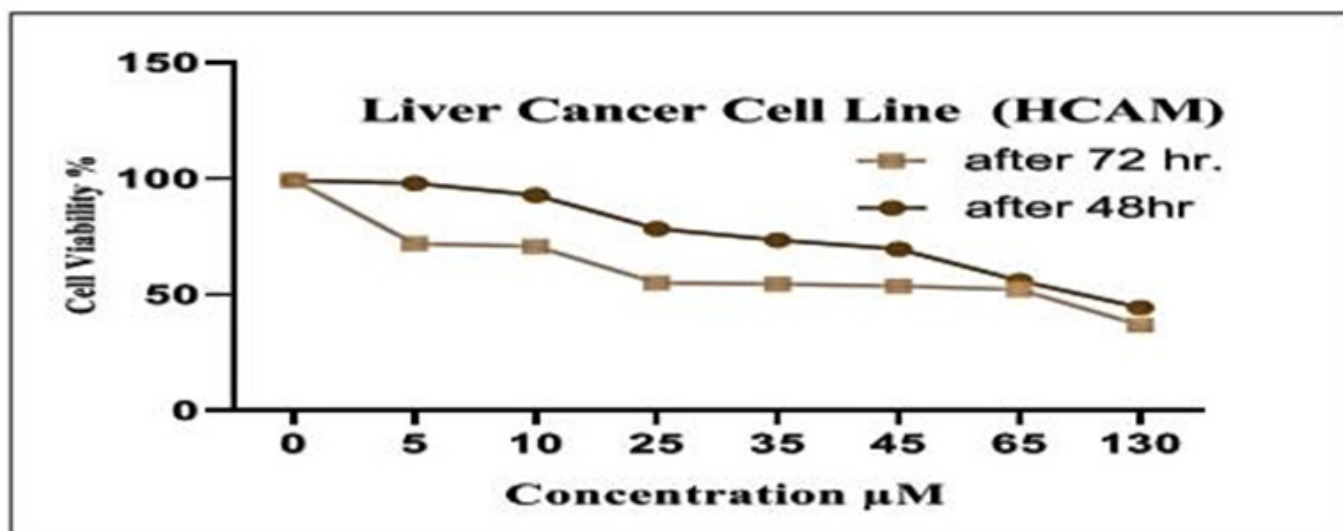


Figure 3. Cytotoxic effect of metformin on liver cancer cell line (HACM) cell lines after 48 and 72 hours.

Met. Conc $\mu$ M	0	5	10	25	35	45	65	130	P -Value
Mean Viability	97.761 <sup>e</sup>	96.892 <sup>e</sup>	93.175 <sup>d</sup>	91.345 <sup>d</sup>	87.301 <sup>c</sup>	86.437 <sup>b</sup>	83.988 <sup>b</sup>	74.258 <sup>a</sup>	0.000**
SEM	1.022	0.727	0.664	0.305	1.197	0.404	1.001	1.054	

Note: Cytotoxic effect of Metformin on A172 cell line after 48 hours.

\*\*p<0.01: a,b,c,d,e significantly difference between different group in same period.

Table 7. Metformin's cytotoxic effect on the A172 cell line after 48 hours cell viability was measured with MTT assay.

Met. Conc $\mu$ M	0	5	10	25	35	45	65	130	P -Value
Mean Viability	98.375 <sup>f</sup>	95.873 <sup>f</sup>	86.006 <sup>e</sup>	75.031 <sup>d</sup>	75.645 <sup>d</sup>	64.500 <sup>c</sup>	55.305 <sup>b</sup>	47.820 <sup>a</sup>	0.000**
SEM	0.911	0.865	1.401	0.361	1.366	1.855	1.195	0.618	

Note: Cytotoxic effect of Metformin on A172 cancer cell line after 72 hours.

\*\*p<0.01: a,b,c,d,e,f significantly difference between different group in same period.

Table 8. Show Cytotoxic effect of Metformin on A172 cell line after 72 hours cell viability was measured with MTT assay.

In Figure 4 Metformin demonstrated an inhibitory effect on A172 human glioma cells, and cell viability was decreased more significantly with greater Metformin doses and longer treatment durations [12]. To be ready for testing the cytotoxic effect of metformin on cancer cell lines, each line of cancer cell must have more than 90% cell viability each line of cancer cell must take 0 concentration to compare the effect after adding metformin, all of the above is cancer cell should be evaluated if this drug effect on normal cell as effect on cancer cell (to compare the safety and specificity of drug to cancer tissue) in Table 9. After 72 hours of exposure, the effect of varying Metformin concentrations on the development of a normal cell line was investigated result concluded a slight effect on the viability of a normal cell line. Table 9 illustrates that the inhibition rates have a non-significant effect (0.0624).

Means with different superscript different small letter (a, b) are significantly difference between different group in same period (Duncken Test) \*p<0.05 mean significant \*\* mean p<0.01 highly significant. After 72 hours of exposure to metformin, the viability and cytotoxic effect of metformin on Hela, AMJ3, HCAM, A172 cancer cell line, and HBL100 normal cell lines are shown in Table 10. The results of this study on the effect of metformin at the maximum concentration (130  $\mu$ M) on the proliferation of cancer cells line are as follows: Hela<AMJ3< HCAM<A172 and no significant effect on the HBL100 normal cell lines as in Table 10.

Means with different superscript different small letter (a, b, c, d, e) are significantly difference between different group in same period (Duncken Test) \*p<0.05 mean significant\*\*\* mean p<0.01 highly significant.

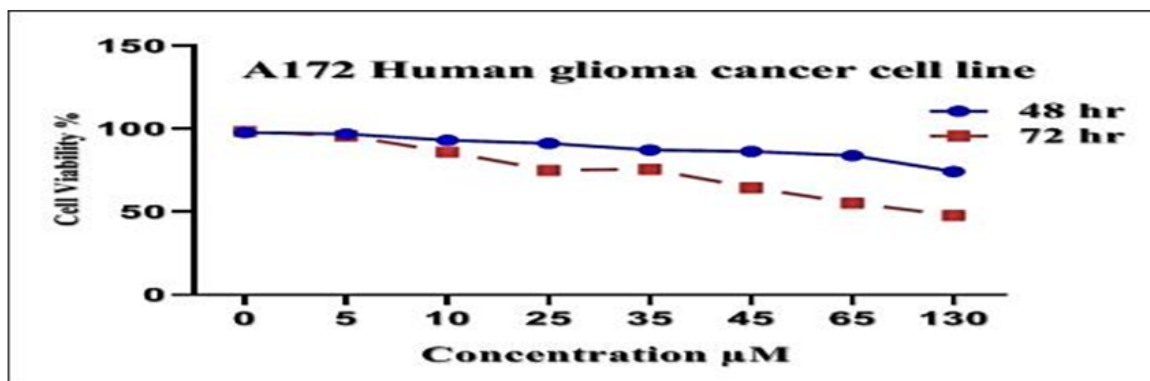


Figure 4. Cytotoxic effect of metformin on A172 cancer cell line after 48 and 72 hours.

Met. Conc μM	0	5	10	25	35	45	65	130	P-Value
Mean Viability	99.636 <sup>b</sup>	98.848 <sup>b</sup>	98.219 <sup>b</sup>	95.547 <sup>ab</sup>	94.329 <sup>a</sup>	94.132 <sup>a</sup>	93.688 <sup>a</sup>	86.518 <sup>a</sup>	0.0624**
SEM	0.086	3.721	4.539	1.886	1.735	2.322	1.373	2.498	

Note: Cytotoxic effect of Metformin on HBL100 ( normal breast cell) after 72 hours.

\*\*p<0.01: a,b significantly difference between different group in same period.

Table 9. Cytotoxic effect of Metformin on HBL100 ( normal breast cell) after 72 hours cell viability was measured with MTT assay.

Met. Conc. μM	HBL100	HeLa	AMJ3	HCAM	A172	P-Value
0	99.636 ± 0.0869 <sup>a</sup>	97.563 ± 0.3703 <sup>a</sup>	97.835 ± 0.885 <sup>a</sup>	99.070 ± 0.233 <sup>a</sup>	98.375 ± 0.911 <sup>a</sup>	0.273
5	98.848 ± 3.721 <sup>b</sup>	73.132 ± 1.372 <sup>a</sup>	93.967 ± 2.971 <sup>b</sup>	71.876 ± 0.295 <sup>a</sup>	95.873 ± 0.865 <sup>b</sup>	0.000**
10	98.219 ± 4.539 <sup>c</sup>	43.057 ± 1.602 <sup>c</sup>	33.022 ± 0.297 <sup>a</sup>	70.814 ± 1.988 <sup>b</sup>	86.006 ± 1.401 <sup>d</sup>	0.000**
25	95.547 ± 1.886 <sup>e</sup>	17.442 ± 0.754 <sup>a</sup>	22.400 ± 0.128 <sup>b</sup>	55.058 ± 1.582 <sup>c</sup>	75.645 ± 1.366 <sup>d</sup>	0.000**
35	94.329 ± 1.735 <sup>e</sup>	15.649 ± 0.272 <sup>a</sup>	22.295 ± 0.196 <sup>b</sup>	54.513 ± 0.708 <sup>c</sup>	75.031 ± 0.361 <sup>d</sup>	0.000**
45	94.132 ± 2.322 <sup>e</sup>	15.305 ± 0.135 <sup>a</sup>	22.084 ± 0.223 <sup>b</sup>	53.641 ± 1.918 <sup>c</sup>	64.500 ± 1.855 <sup>d</sup>	0.000**
65	93.688 ± 1.373 <sup>d</sup>	15.279 ± 0.251 <sup>a</sup>	21.979 ± 0.074 <sup>b</sup>	52.224 ± 0.712 <sup>c</sup>	55.305 ± 1.195 <sup>c</sup>	0.000**
130	86.518 ± 2.498 <sup>e</sup>	15.081 ± 0.167 <sup>a</sup>	21.453 ± 0.128 <sup>b</sup>	36.838 ± 1.462 <sup>c</sup>	47.820 ± 0.618 <sup>d</sup>	0.000**

Note: Cytotoxic Effect Of Metformin On Different Cell Line After 72 Hr.

\*\*p<0.01: a,b,c,d,e significantly difference between different group in same period.

Table 10. Cytotoxic effect of metformin on HeLa, AMJ3,HCAM, A172 cancer cell line and HBL100 normal cell lines after 72 hours of exposure to Metformin.

Type cancer cell	Hela	AMJ 13	HCAM	A172	HBL100	P -value
Mean	7.492 <sup>a</sup>	9.168 <sup>b</sup>	10.150 <sup>b</sup>	28.299 <sup>c</sup>	48.910 <sup>d</sup>	0.000**
SEM	0.281	0.244	0.405	0.298	0.589	4.539

Note: IC50 of Metformin in cancer cell lines.

\*\*p<0.01: a,b,c,d significantly difference between different group in same period.

Table 11. In vitro sensitivity of the drugs with IC50 values metformin on the Cancer and normal cell lines.

### IC50 of Metformin in cancer cell lines

The activity of the metformin on cancer cell line was determined by its IC50 values compounds with IC50 values <10 μM were selected as active cytotoxic effect in this study IC 50 for metformin on (Hela 7.492)his may implicate a clinical antitumor effect with less toxicity to normal tissues as in Table 11.

Means with different superscript different small letter (a, b, c) are significantly difference between different group in same period (Duncken Test) \*p<0. 05 mean significant \*\* mean p<0.01 highly significant.

## Discussion

Metformin has been widely used as an anti-diabetic drug because of its relatively mild side effects and effective mechanism of action on sugar levels, Metformin is an approved drug for the treatment of T2DM that has few adverse effects [13]. The hydrophilic and cationic nature of metformin at physiological pH makes it highly unlikely that metformin rapidly diffuses through the cell membrane and exerts it effect on cell function [14].

Metformin, with its low market price compared to other cancer treatment pharmaceuticals and its ability to be used with or without chemotherapeutic pharmaceuticals in cancer therapy, is predicted to provide economic benefits as well as improve the chances of cancer patients surviving [15]. TT is a colorimetric; enzyme-based method for determining the activity of mitochondrial dehydrogenase in cells because it is simple, safe, and sensitive, this approach is commonly employed is the most widely used method for determining the viability and cytotoxicity of cells [16]. Cervical cancer is the fourth most frequent malignancy in women and the fourth major cause of cancer death [17]. Metformin inhibits HeLa cell proliferation hypothesized that synthetic medicines with an IC50 (the potency of drug, in inhibiting cancer cell lines) of less than 10 μM could be used as anticancer medicines *in vitro*, IC50 is a quantitative measure that indicates the amount of a particular inhibitory substance Metformin needed to inhibit proliferation of cancer cell lines. Therefore, our results indicate that IC50 7.365 μM his result suggests that metformin is potentially Cytotoxicity [18] From an Iraqi breast cancer patient, a new breast cancer cell line (AMJ13) has been developed is unique in that it is the first of its kind for an Iraqi population, and it is expected to be useful in breast cancer research [19]. Several *in vitro* and *in vivo* studies have found significant evidence supporting the use of

metformin as breast cancer anti-cancer agent, both as a monotherapy and in combination with other commonly used chemotherapeutic drugs/radiation therapy occurring compounds with known anti-cancer potential with other drugs or therapeutic modalities is critical to achieve therapeutic efficiency in the treatment with minimal side effects then used orally in the treatment of diabetes, the anti-hyperglycemic effects of metformin have been reported at plasma concentrations ranging from around from 10–100  $\mu\text{M}$  this range considering metformin for its potential cancer preventive or cancer-treatment effect, adhering to a precision medicine approach [20,21]. A new liver cancer cell line (HCAM) has been identified and has been considered as a useful tool in the research of liver cancer [22]. Metformin is expected to relieve the hepatocyte of substrate overload due to its glucose-lowering impact the glucose-lowering effect of metformin is primarily attributed to its inhibition of hepatocyte gluconeogenesis. However, the underlying mechanism that once seemed to be coined as mediated by the direct inhibition of mitochondrial respiratory complex I by metformin, is still under active [23], We provide a new insight and therapeutic approach by targeting autophagy in the treatment of HCC (Hepatocellular Carcinoma). Metformin promotes apoptosis in hepatocellular carcinoma through the induced autophagy pathway [24]. Gliomas are being treated with a comprehensive therapeutic approach that includes surgery, chemotherapy, irradiation, and molecular targeted therapy [25], but the therapeutic efficacy is poor, with a low 5-year survival rate and a high mortality rate Finding effective and low-toxicity anti-glioma drugs remains one of the most important fields of study, with the objective of improving glioma prognosis and therapy [26], and the cell survival rate decreases as metformin concentrations rise [27].

## Conclusion

Metformin and a Possible Clinical Trials Pathway Metformin are currently being studied *in vitro* cell-based studies for its anti-cancer potential in cervical cancer cells, with a weaker effect in the breast, liver and glioma. Despite the fact that the end consequences are linked in terms of molecular mechanism of action, cell proliferation inhibition. It is true that focuses on a particular mechanism makes treatment a disease easier.

## Limitations and Future Studies

Metformin is a very good option for treating cancer in a complementary way et formin is an effective additional cancer therapy alternative et formin's properties have led to significant research because of its stability (lack of change), the fact that it has no or little side effects in the body, that it has no or minimal interactions with other treatments, and that it is inexpensive.

## Acknowledgement

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## References

1. Siegel, RL and Miller, K. "Cancer Statistics." *CA Cancer J Clin* 70 (2020): 17–30.
2. Pernicova, Ida, and Marta Korbonits. "Metformin—Mode of Action and Clinical Implications for Diabetes and Cancer." *Nat Rev Endocrinol* 10 (2014): 143-156.
3. Laplante, Mathieu, and David M. Sabatini. "mTOR Signaling in Growth Control and Disease." *Cell* 149 (2012): 274-293.
4. Algire, Carolyn, Olga Moiseeva, Xavier Deschênes-Simard, and Lilian Amrein, et al. "Metformin Reduces Endogenous Reactive Oxygen Species and Associated DNA Damage." *Cancer Prev Res* 5 (2012): 536-543.
5. Hirsch, Heather A, Dimitrios Iliopoulos, Philip N Tschlis, and Kevin Struhl. "Metformin Selectively Targets Cancer Stem Cells, and Acts Together with Chemotherapy to Block Tumor Growth and Prolong Remission." *Cancer Res* 69 (2009): 7507-7511.
6. Shaw, Reuben J, Katja A. Lamia, Debbie Vasquez, and Seung-Hoi Koo, et al. "The Kinase LKB1 Mediates Glucose Homeostasis in Liver and Therapeutic Effects of Metformin." *Science* 310 (2005): 1642-1646.
7. Pollak, Michael. "Insulin, Insulin-Like Growth Factors and Neoplasia." *Best Pract Res Clin Endocrinol Metab* 22 (2008): 625-638.
8. Al-Shammari, Ahmed Majeed, Al-Esmee Wafeer Naser, Allateef, Hassan, and Aysar Ahmed. "Enhancement of Oncolytic Activity of Newcastle Disease Virus Through Combination with Retinoic Acid Against Digestive System Malignancies." *Molecular Ther* 27(2019):126-127.
9. Chou, Ting-Chao. "Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method." *Cancer Res* 70 (2010): 440-446.
10. Yudhani, Ratih Dewi, Indwiani Astuti, Mustofa Mustofa, and Dono Indarto, et al. "Metformin Modulates Cyclin D1 and P53 Expression to Inhibit Cell Proliferation and to Induce Apoptosis in Cervical Cancer Cell Lines." *Asian Pac J Cancer Prev* 20 (2019): 1667.
11. Menendez, Javier A, Rosa Quirantes-Piné, Esther Rodríguez-Gallego, and Silvia Cufi, et al. "Oncobiguanides: Paracelsus' Law and Nonconventional Routes for Administering Diabetobiguanides for Cancer Treatment." *Oncotarget* 5 (2014): 2344.
12. Xiong, Zhang Sheng, Song Feng Gong, Wen Si, and Taipeng Jiang, et al. "Effect of Metformin on Cell Proliferation, Apoptosis, Migration and Invasion in A172 Glioma Cells and its Mechanisms." *Mol Med Rep* 20 (2019): 887-894.
13. Liang, Xiaomin, and Kathleen M Giacomini. "Transporters Involved in Metformin Pharmacokinetics and Treatment Response." *J Pharma Sci* 106 (2017): 2245-2250.
14. Zhang, Hui-Hui, and Xiu-Li Guo. "Combinational Strategies of Metformin and Chemotherapy in Cancers." *Cancer Chemother Pharmacol* 78 (2016): 13-26.
15. Ahmad, Shama, Aftab Ahmad, B Kelly Schneider, and Carl W White. "Cholesterol Interferes with the MTT Assay in Human Epithelial-Like (A549) and Endothelial (HLMVE and HCAE) Cells." *Int J Toxicol* 25 (2006): 17-23.
16. Bray, Freddie, Jacques Ferlay, Isabelle Soerjomataram, and Rebecca L Siegel, et al. "Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries." *CA Cancer J Clin* 68 (2018): 394-424.
17. Al-Shammari Ahmed Majeed, Alshami A Mortadha, Mahfoodha Abbas Umran, and Asmaa AmerAlmukhtar, et al. "Establishment and Characterization of a Receptor-Negative, Hormone-Nonresponsive Breast Cancer Cell Line from an Iraqi Patient, Breast Cancer Targets and Therapy." 7 (2015): 223–230.
18. Samuel, Samson Mathews, Suparna Ghosh, Yasser Majeed, and Gnanapragasam Arunachalam, et al. "Metformin Represses Glucose Starvation Induced Autophagic Response in Microvascular Endothelial Cells and Promotes Cell Death." *Biochem Pharmacol* 132 (2017): 118-132.
19. Al-Musawi, HM, Al-Shammari, AM, and Al-Bazii SJ. Biological and Physiological Characterization of HCAM Hepatocellular Carcinoma Cell Line. *Prensa Med Argent* 106 (2010): 1-9.
20. Madiraju, Anila K, Yang Qiu, Rachel J Perry, and Yasmeen Rahimi, et al. "Metformin Inhibits Gluconeogenesis Via a Redox-Dependent Mechanism in Vivo." *Nat Med* 24 (2018): 1384-1394.
21. Tsai, Hsin-Hwa, Hong-Yue Lai, Yueh-Chiu Chen, and Chien-Feng Li, et al. "Metformin Promotes Apoptosis in Hepatocellular Carcinoma Through the CEBPD-Induced Autophagy Pathway." *Oncotarget* 8 (2017): 13832.
22. Carmignani, M Antonio, Volpe M Aldea, O Soritau, and A Irimie, et al. "Glioblastoma Stem Cells: A New Target for Metformin and Arsenic Trioxide." *J Biol Regul Homeost Agents* 28 (2014): 1-15.
23. Podhorecka, Monika, Blanca Ibanez, and Anna Dmoszyńska. "Metformin's Potential Anti-Cancer and Anti-Aging Effects." *Adv Hyg Exper Med* 71 (2017):170-175.

24. Li, Shu-Man Hsieh, Shu-Ting Liu, Yung-Lung Chang, and Ching-Liang Ho, et al. "Metformin Causes Cancer Cell Death Through Downregulation of p53-Dependent Differentiated Embryo Chondrocyte 1." *J Bio Sci* 25 (2018): 1-13.
25. Kahn, Barbara B, Thierry Alquier, David Carling, and D Grahame Hardie. "AMP-Activated Protein Kinase: Ancient Energy Gauge Provides Clues to Modern Understanding of Metabolism." *Cell Metabolism* 1 (2005): 15-25.
26. Lan, Bin, Jian Zhang, Peng Zhang, Weihong Zhang, and Shugang Yang, et al. "Metformin Suppresses CRC Growth by Inducing Apoptosis via ADORA1." *Front Bio sci* 22 (2017): 248-257.
27. Larsson, Dhana E, Henrik Lövborg, Linda Rickardson, and Rolf Larsson, et al. "Identification and Evaluation of Potential Anti-Cancer Drugs on Human Neuroendocrine Tumor Cell Lines." *Anticancer Res* 26 (2006): 4125-4129.

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