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MULTIFACETED ASSESSMENT OF METFORMIN'S IMPACT ON CERVICAL CANCER CELL LINES: CYTOTOXICITY, REACTIVE OXYGEN SPECIES MODULATION, AND GENE EXPRESSION

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Abstract:
Metformin, a widely used anti-diabetic medication, has shown potential anti-cancer effects in various studies. This research aims to comprehensively assess the impact of metformin on different cancer cell lines, focusing on cytotoxicity, modulation of reactive oxygen species (ROS), and gene expression analysis. The study includes HeLa (human cervical cancer), HBL100 (normal human cell),
In vitro experiments involved the treatment of cells with metformin at various concentrations, and assessments were conducted after 48 and 72 hours. Cytotoxicity was evaluated using the MTT cell viability assay, revealing a significant dose-dependent decrease in cell survival in HeLa cells. Morphological analysis highlighted metformin-induced cytomorphological changes, including atrophy, irregular shapes, and apoptotic features in HeLa cells.
Reactive oxygen species (ROS) assays demonstrated a significant reduction in ROS levels in HeLa cells treated with metformin at a concentration of 130 µM, indicating a potential antioxidant effect. The study also explored the gene expression patterns in HeLa cells exposed to half-lethal concentrations (IC50) of metformin. Results showed a slight increase in PIK3CA gene expression, a significant down-regulation of mTOR gene expression, and a significant up-regulation of AKT1 gene expression, suggesting modulation of the PIK3CA/AKT1/mTOR signaling pathway.
This multifaceted assessment provides valuable insights into the diverse effects of metformin on different cancer cell lines, emphasizing its potential as a cytotoxic agent against cervical cancer cells. The findings contribute to understanding the complex mechanisms underlying metformin's anti-cancer properties and may pave the way for further research on its cell- or tissue-specific therapeutic effects

Keyword: Metformin Cytotoxicity,) Gene Expression, HeLa, PIK3CA/AKT1/mTOR Signaling Pathway.

Introduction

Metformin, a member of the biguanide group, is commonly used in the treatment of diabetes due to its ability to inhibit hepatic glucose output, decrease gluconeogenesis, and enhance glucose uptake. Additionally (1), metformin improves insulin sensitivity by reducing circulating insulin levels Reactive oxygen species (ROS), including superoxide anion and hydrogen peroxide, are generated during aerobic respiration in mitochondria. While ROS are known for their harmful effects on DNA, proteins, and lipids, they also play crucial roles in cellular pathways, such as maintaining redox homeostasis and participating in various cellular signaling processes (2).

Cervical cancer is the fourth most common cancer in women globally, imposing a significant financial and medical burden on society. Conventional treatments, including surgery, chemotherapy, and radiotherapy, often exhibit limited effectiveness (3).Chemotherapeutic drugs like bevacizumab, topotecan hydrochloride, and gemcitabine/cisplatin are commonly used for cervical cancer treatment, but adverse effects and drug resistance are frequent challenges (4)

Metformin, primarily recognized for its anti-diabetic properties, has recently gained attention for its potential anti-cancer effects. Studies suggest its impact on cell proliferation, involving stimulation of AMPK, suppression of mTOR signaling, and targeting mitochondria complex I. However, its specific mechanisms in the context of cancer, as well as its cell- or tissue-specific therapeutic effects, remain unclear (5).

Generally, metformin's anti-diabetic actions and its impact on cell proliferation such as stimulation of AMPK suppression of mTOR signaling, and targeting of mitochondria complex I, common mechanism, such as the change of cellular energy state, even though the particular cause is however unclear. Moreover, several studies showed that the usage of metformin is not linked to a drop occurrence of certain types of human cancers or an improvement in their prognosis Future research is desired to determine whether the therapeutic impact of metformin is cell- or tissue-specific.(6)

Owing to its pleiotropic special effects, The commonly used drug metformin has gained attention among medical researcher(7)s. Metformin is mainly used to treat diabetes, but recent studies suggest it may also be useful in treating cancer and aging

2- Material & Method

Maintenance of cell line

In this study, diverse cell lines, encompassing HeLa (human cervical cancer), HBL100 (normal human cell), were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin (Ajenta Pharm, India), and 100 g/mL streptomycin (Ajenta Pharm, India). Cell passaging occurred bi-weekly at 50% confluence using Trypsin-EDTA, with an incubation temperature of 37°C and 5% CO2 to ensure optimal cell growth.(8)

Cytotoxicity assays employed the MTT cell viability assay on 96-well plates, treating cells, including Metformin (Samara company, Molecular weight =165.62, Melting point 222 C0to

226 C0) at various concentrations (0,5,10,25,35,45,65,130 µM), for 48 and 72 hours. The IC50 was calculated using non-linear regression(9)

Reactive Oxygen Species (ROS) assays were conducted using an (ELISA kit Human ROMO1 ELISA Kit (Catalog No: E-EL-H5430) - Elabscience®, USA Detection Range: 0.16~10 ng/mL Sensitivity: 0.10 ng/mL,) to evaluate Metformin's impact on ROS production in HeLa cells(10,11).

The experimental procedure included incubation, washing, and measurement of optical density at 450 nm. This comprehensive approach integrated visualizations of cell culture conditions and assay results, providing a multifaceted analysis of Metformin's effects on cell lines. Additionally, morphological analysis involved seeding 1x104 cells, exposing them to Metformin for 72 hours, and staining with crystal violet. Apoptosis assessment utilized acridine orange (AO) staining and ethidium bromide (EB) with fluorescence microscopy. In this study, gene expression analysis through Quantitative PCR (qPCR) was conducted on the Hela cancer cell line. Cells were cultured at a density of 100 x 104 cells per well in 12-well plates and exposed to half-lethal concentrations (IC50) of Metformin. RNA extraction was performed using the GENEzol™ TriRNA Pure Kit (Geneaid GENEzol™ TriRNA Pure Kit - Geneaid / Taiwan AccuPower® RocketScriptTM RT PreMix - “Bioneer” US) according to the manufacturer’s protocol. This included on-column DNase I digestion (10 units/column) to eliminate potential contaminating DNA from the samples. The RNA purification protocol involved sample homogenization, RNA binding, washing, and elution steps. Optional DNA digestion using DNase I in solution was performed, and the RNA sample was repurified using GENEzolTM. RNA concentration was determined using an Implennano photometer N60, and the eluted RNA was stored at -80°C. First-strand cDNA synthesis was carried out using 1 µg of RNA and AccuPower® RocketScriptTM RT Premix (Bioneer) according to the manufacturer’s instructions. The synthesized cDNA was diluted 5-fold in nuclease-free water and stored at -20°C for subsequent analysis.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was conducted using a GoTaq® qPCR Master Mix kit and an Agilent thermal cycler (USA). Each reaction included GoTaq® qPCR Master Mix and forward/reverse primers as per the manufacturer’s protocol. A 5 µl aliquot of template cDNA was added to each reaction. The cycling conditions comprised a pre-denaturation step at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. A dissociation curve was generated to assess the specificity of the PCR products, to analyze the gene expression in the Hela cancer cell line.patterns in response to Metformin exposure(12)

In the statistical analysis of the study, data organization and coding were executed using Microsoft Excel-2010. Subsequent descriptive and analytic statistical analyses were performed using the SPSS statistical software program, Version 23. Prior to conducting statistical tests, the normal distribution of data in all groups was assessed through normality tests, including the Kolmogorov-Smirnov and Shapiro-Wilk tests. If the p-value exceeded 0.05, indicating normal distribution, parametric tests were applied; otherwise, non-parametric tests were employed.

Descriptive statistics were presented as mean ± Standard Error of Mean (SEM) for measurable variables. The One-way Analysis of Variance (ANOVA) test, coupled with Duncan's multiple comparison test, was utilized to analyze the data, with a significance threshold set at a p-value less than 0.05, considering less than 0.01 as highly significant.

For comparisons between two different groups, an Unpaired t-test was employed,.

Additionally, the half-maximal inhibitory concentration (IC50) was calculated using Graph Pad Prism version 8, providing a comprehensive and rigorous statistical approach to evaluate the outcomes of the study.(10) .

Result

The impact of Metformin on cancer cell lines.

Evaluation of Cytotoxicity of Metformin on Human Cervical Cancer Cell Lines (Hela) After 48 and 72hr.

Based on the results of MTT tests, our study demonstrates a notable cytotoxic effect of metformin on the human cervical cell line over a 48-hour period, as illustrated in Figure 1. The data indicates a significant decline in cell survival rates (86.093%, 49.206%, 26.466%, 25.097%, 24.869%, 24.185%, and 23.044%) with increasing metformin concentrations in the HeLa cell line (5, 10, 25, 35, 45, 65, 130 M) compared to the control group with 0 concentration prior to metformin addition.

Furthermore, after a 72-hour exposure to metformin, there is a substantial reduction in cell survival rates, reaching 73.132%, 43.057%, 22.452%, 15.649%, 15.305%, 15.279%, and 15.081% at varying concentrations in the HeLa cell line. The significant decrease in cell survival is particularly pronounced at higher metformin doses, as detailed in figure (1). These findings underscore the pronounced cytotoxic impact of metformin, suggesting its potential as a cytotoxic agent against the HeLa cell line, with a dose-dependent effect over the tested duration.

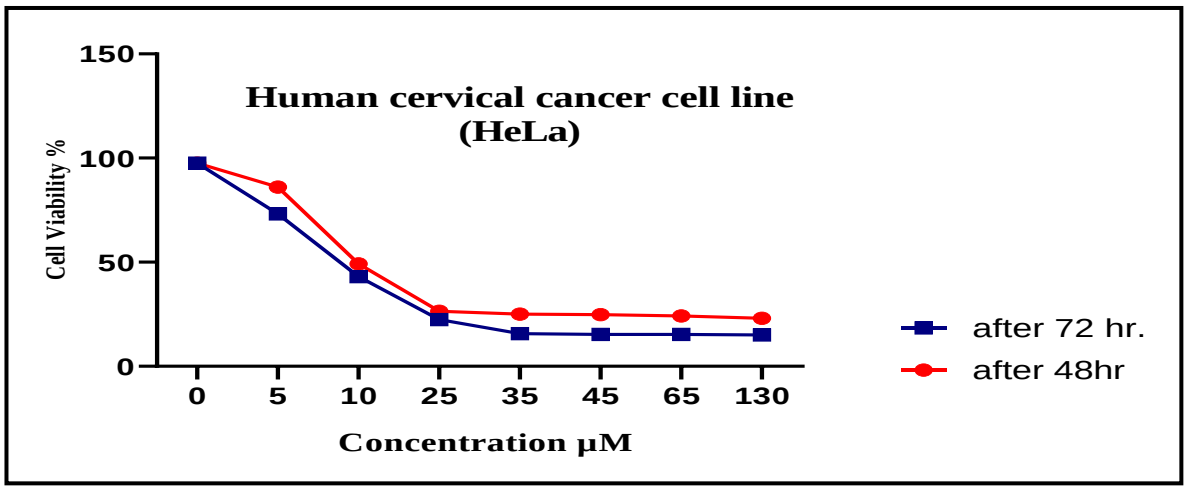

Figure (1):Divergent Impact on Viability: A Comparative Analysis of Normal and Cancerous Cells under Metformin Exposure

Table (1) presents the effect of metformin on HeLa (human cervical cancer) and HBL100 (normal human cell) cell lines after a 72-hour exposure, with concentrations measured in micromolar (µM). The table includes mean cell viability percentages and standard deviations for each metformin concentration, along with corresponding statistical significance values (P-Value).

Table (1): Effect of metformin on HeLa, and HBL100 normal cell lines after 72 hours of exposure to metformin

Met. Conc. µM	**HBL100**	**HeLa**	***P*-Value**
0	99.636±0.0869 A	97.563±0.3703 A	0.034
5	98.848± 3.721 B	73.132±1.372 A	**0.003***
10	98.219 ±4.539 E	43.057± 1.602 C	**0.001****
25	95.547 ±1.886 D	22.452±0.754 A	**0.000****
35	94.329 ±1.735 E	15.649±0.272 A	**0.000****
45	94.132 ±2.322 E	15.305 ±0.135 A	**0.000****
65	93.688 ±1.373 D	15.279 ±0.251 A	**0.000****
130	86.518±2.498 E	15.081 ±0.167 A	**0.000****

- Unpaired t-test
- Different letter mean significant less than 0.05,similar letter mean non-signficant more than 0.05

Determination of Hela IC50 &HBL100

Metformin has a dose-dependent cytotoxic effect on HeLa cells, meaning that the higher the concentration of metformin, the lower the viability of HeLa cells.

Metformin has minimal cytotoxic effects on HBL100 cells, even at the highest concentration. figure 2 .A showed the IC50 of Hela cancer cell (2)showed the IC50 of HBL100 cancer cell (2) as figure 2B

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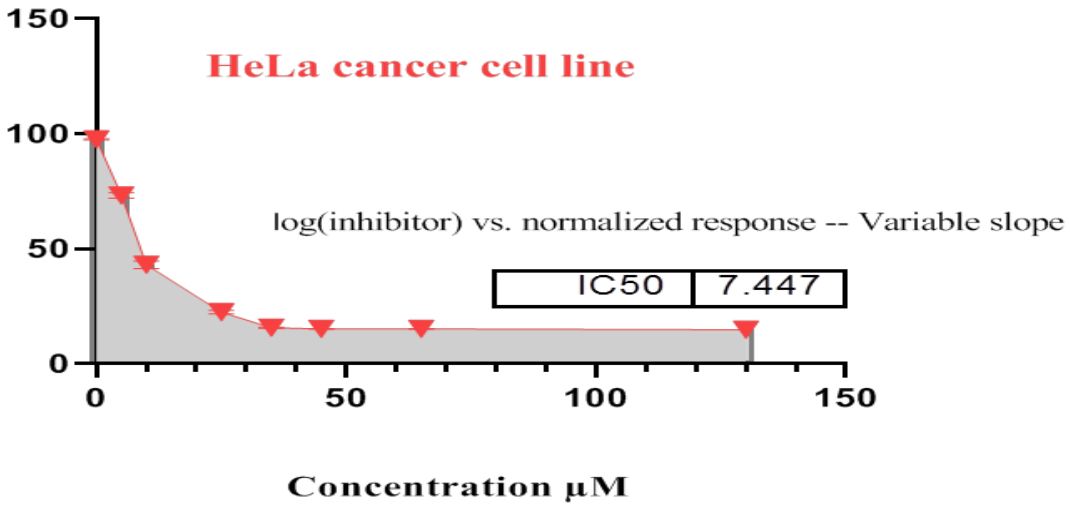
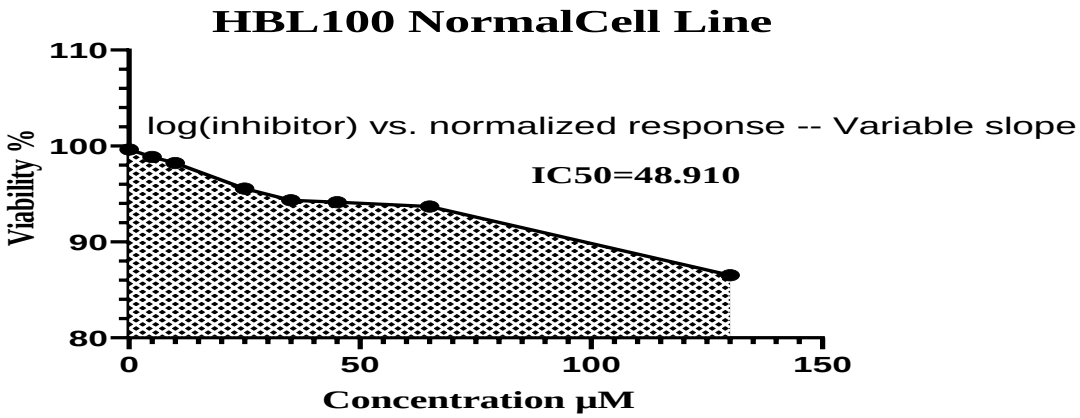


(A)



(B)

Figure(2): Determination of Hela IC50 And HBL100

Morphological Analysis.

Figure (3) illustrates untreated HBL100 cells grown in a monolayer, while Figure (4) depicts untreated Hela cells. Microscopic examination, utilizing Hematoxylin and Eosin (H&E) staining, revealed distinct cytomorphological changes in cervical cancer cell lines (Hela) after 72 hours of exposure to metformin. Characteristic alterations, such as atrophy, spherical and irregular shapes, were observed—an indicative hallmark of the cytopathic effect where cells

undergo transformations to maintain their structure. The cytoskeleton's role is pivotal in preserving cell shape, and any deviation in its organization results in structural changes.

Metformin-induced changes manifested as large spaces in cell cultures due to cell atrophy, shrinkage, and decomposition. Karyopyknosis, observed in Figures (5), s a characteristic feature of apoptotic cells. Some cells exhibited hyperchromy, while others showcased nuclei atrophy alongside vacuolative degeneration, signifying cytoplasmic vacuolation. Nucleic fragmentation and the formation of apoptotic bodies, common apoptotic features, were also evident.

Moreover, multinucleation was observed in certain cells, accentuating the diverse cytomorphological alterations in Hela cells, as depicted in Figure (6). These findings underscore the profound impact of metformin on the structural integrity of cervical cancer cells, revealing apoptotic signatures and significant morphological variations.

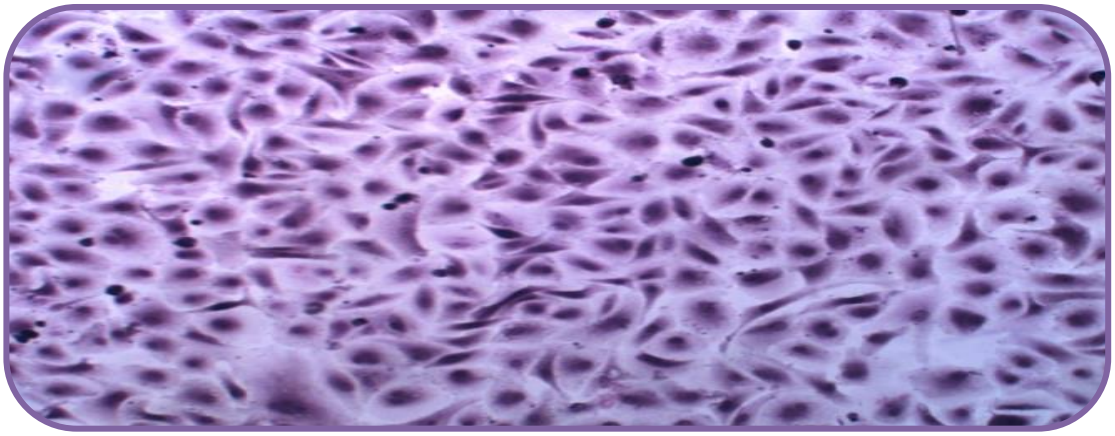
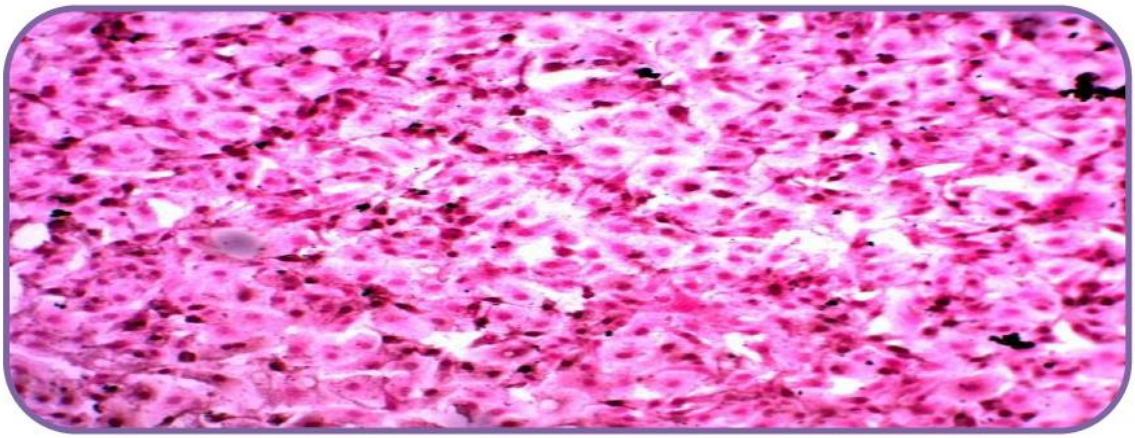


Figure (3): Untreated HBL100 cells were grown in monolayer with normal cell lines HBL100 morphology 10 X.



Figure(4): Untreated cells were grown in monolayer with cervical cancer cells Lines (Hela) morphology 10 X.

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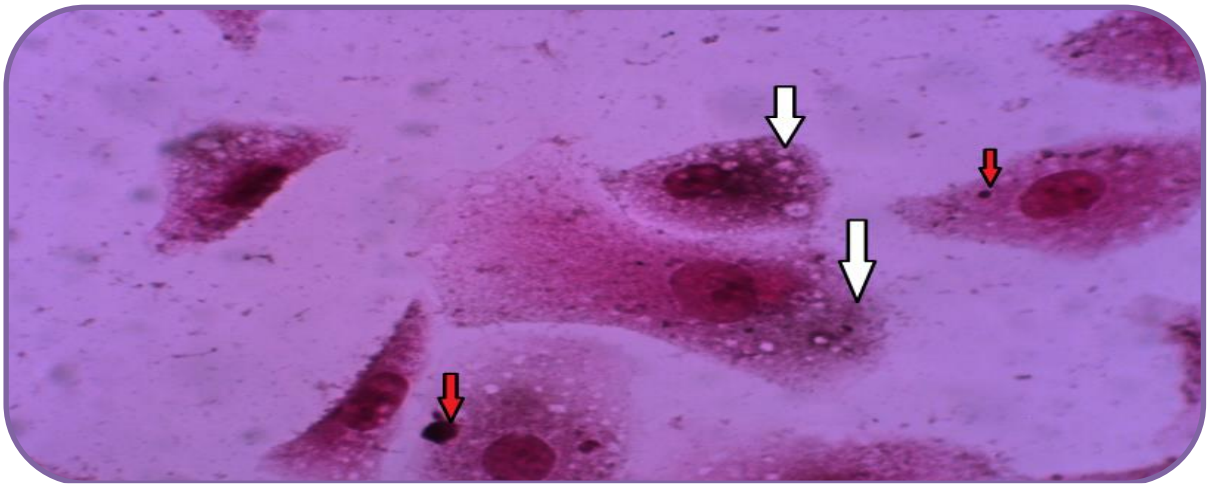

Hela Cells Vacuolation: Figure (5)

Treated cells vacuolated degeneration (white arrows change the cell shape (black arrows) necrosis (red arrows) cells decomposition karyopyknosis nuclei degradation, large spaces between cells was observed and cells decomposition formation of apoptotic bodies100X.

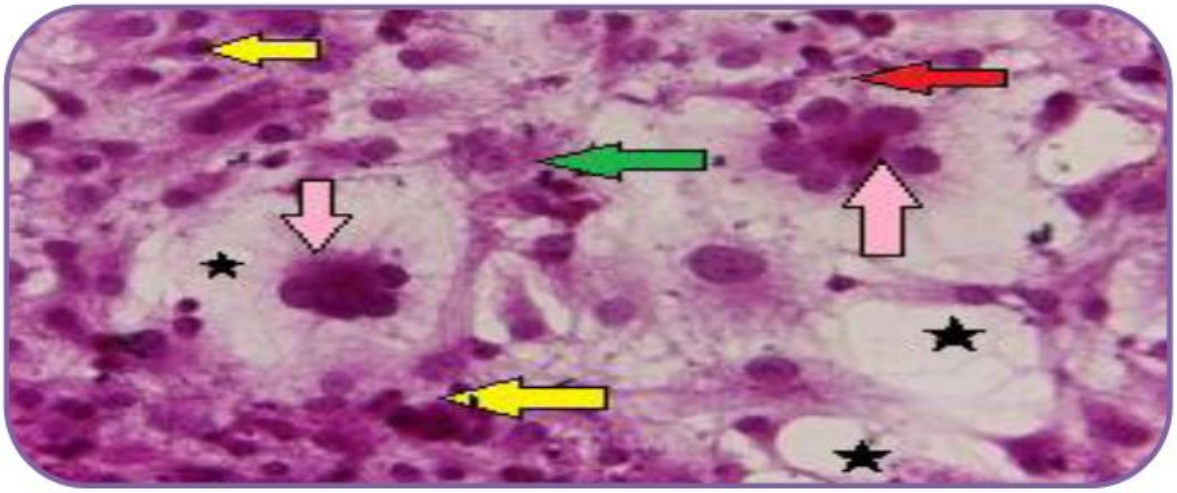

Figure (6): Hela Necrotic & Degradation

(B)Treated cells showing karyopyknosis (yellow arrows) multinucleated (pink arrows) and necrosis (green arrow), showing degradation (red arrow) large spaces

Cytomorphological Death Regarding to AO/EB Apoptosis Assay

Cell morphological death associated with AO/EB apoptosis assay When the HeLa and HBL-100 cell lines are stained with AO/EB dye, it can be seen under the microscope that the untreated cells are stained green by AO, indicating that the cells are intact. HBL-100 Figure (.6). Metformin-treated cells were stained yellow or red with EB dye, indicating cell death, as shown in Figure (7). The AO/EB assay was used to observe differential uptake of the fluorescent DNA-binding dye AO/EB. Cell morphology analyzed by the AO/EB method at

least suggested, if not absolutely guaranteed, the mechanism of cytotoxic effects on the cell lines and confirmed all cell lines in which necrosis occurred Red staining revealed a lack of permeability in necrotic and apoptotic cells, preventing EBs from crossing the nuclear and plasma membranes and binding to nuclear material. Yellow cells promote apoptosis or pro-necrosis and die. Untreated cells were stained with AO dye, which gives them a green hue and demonstrates the integrity of their HB100- and Hela-associated membranes, as shown in Figures 8 and 9 (associated with Hela)

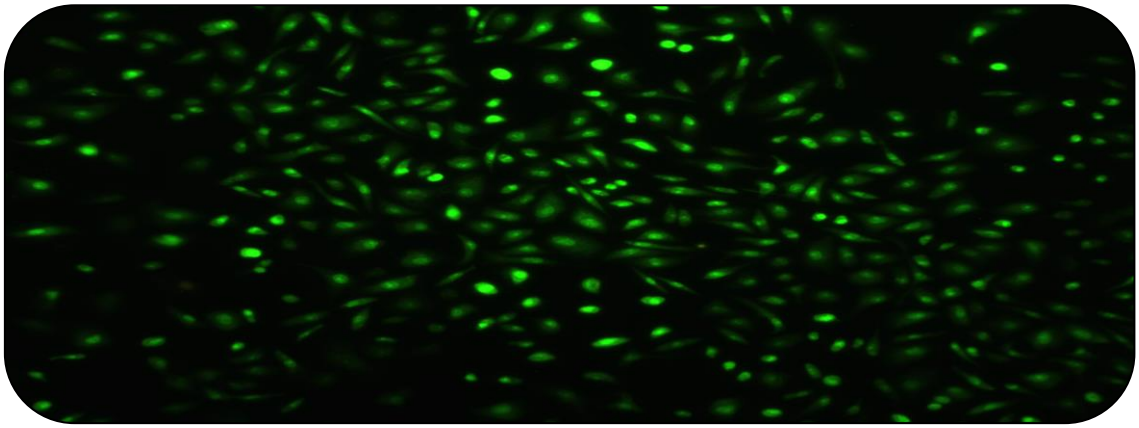

Figure (6): Untreated HBL-100 cells 10 X.

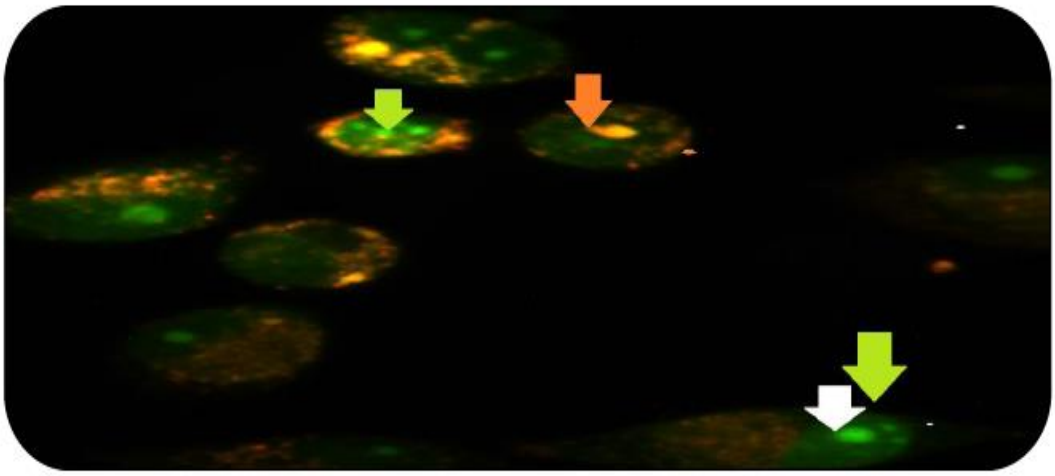

Figure (7): Treated HBL-100 cells with metformin green arrow of cell live, red arrow dead cell, white arrow related to nucleus 40X.

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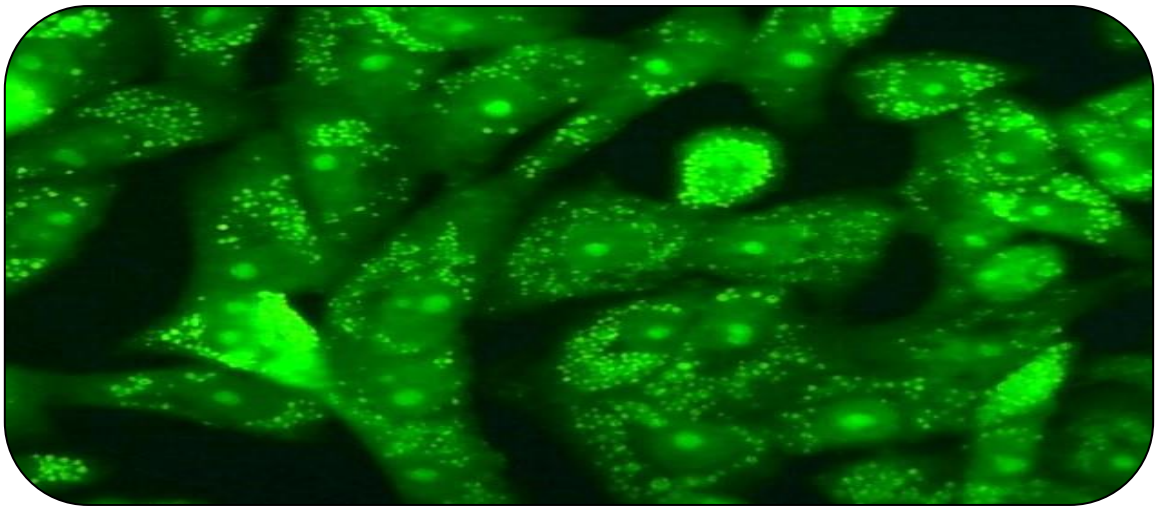

Figure (8): Untreated Hela 40X

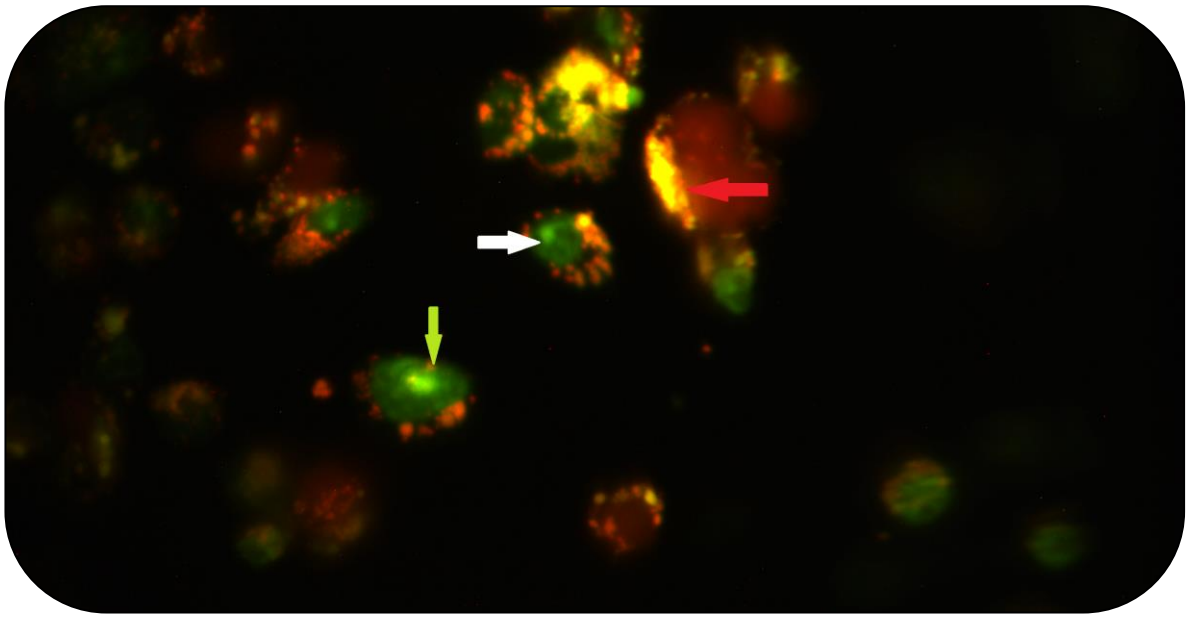

Figure (9): Hela Cells Treated with Metform 40X.

Assessment of reactive oxygen species (ROS)

Table (2) shows a highly significant difference (p = 0.000) in the reduction of ROS in Hela cervical cells at a concentration of 130 µM

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Table (2): Effect of Metformin on ROS production on Cervical Cancer Cells Lines (Hela) after 72Hrs.

Concentration (µM)	ROS (ng/ml)		*p*- value
	Mean	SEM	
Control	0.593	0.027	0.000**
Metformin 130µm	0.370	0.010	
* (P≤0.05) Significant, ** (P<0.01) highly significant Independent sample t –test			

Gene expression results related to the effect of metformin on the PIK3CA/AKT1/mTOR signaling pathway.

The expression of PIK3CA gene increased slightly, but not significantly. Compared with the PIK3CA gene, the expression of the mTOR gene was significantly down-regulated, while the expression of AKT1 was significantly up-regulated. This suggests that metformin treatment has an effect on both the mTOR and AKT1 genes but not on PIK3CA. As shown in Figures 10

Data consist of the mean and standard error of the mean. Calculate the normalized transcript abundance of the PIK3CA, mTOR, and AKT1 genes using the formula 2-ΔCt, where ΔCt is the Ct value of the housekeeping gene (GAPDH), with the target gene (PIK3CA, mTOR, or AKT1) subtracted.

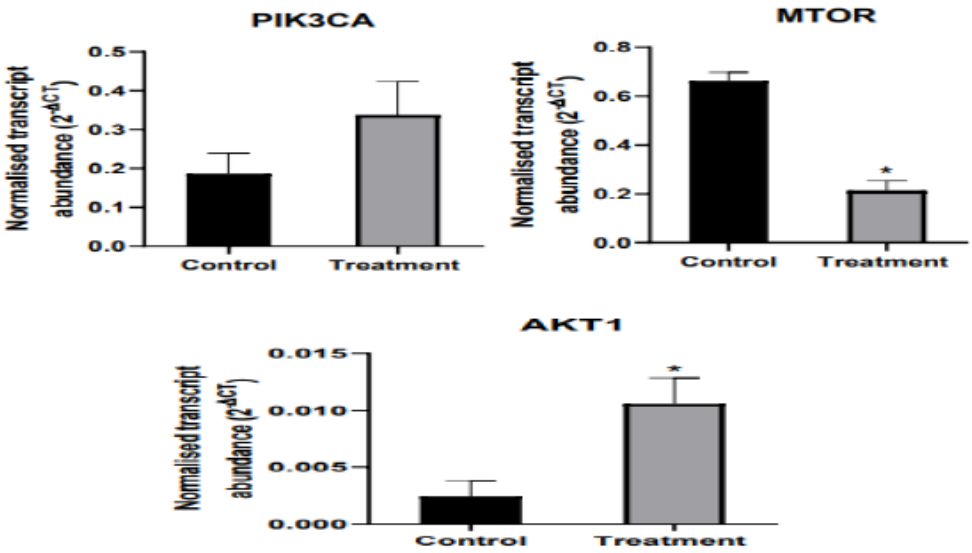

Figure (10): The Expression of PIK3CA, mTOR and AKT1 genes in HeLa cells treated with metformin.

Discussion:

The observed cytotoxic effects of metformin on HeLa cells, as evidenced by the significant reduction in cell viability, underscore its potential as a promising agent in cancer treatment. The dose-dependent response, as indicated by the IC50 values, further solidifies the notion that metformin exerts a pronounced and specific impact on cervical cancer cells(13).

The comparison between HeLa and HBL100 cell lines provides valuable insights into the selectivity of metformin towards cancer cells. The significant decrease in cell viability in HeLa cells, in contrast to minimal effects on HBL100, suggests a potential therapeutic window where metformin exerts its cytotoxicity preferentially on malignant cells. This selectivity aligns with previous literature highlighting metformin's differential impact on cancerous versus normal cells, making it a compelling candidate for targeted cancer therapies(14,15)

Moreover, the determination of IC50 values not only quantifies the potency of metformin but also allows for a nuanced understanding of its concentration-dependent effects. The establishment of IC50 values for both HeLa and HBL100 cells serves as a crucial metric for future studies and facilitates comparisons with other anti-cancer agents(15) This quantitative approach enhances the robustness of the study, providing a foundation for further investigations into the optimal therapeutic dosage(15)

In support of these findings, existing literature has demonstrated metformin's ability to disrupt cellular pathways crucial for cancer cell survival. For instance, metformin has been shown to activate AMP-activated protein kinase (AMPK) and inhibit the mammalian target of rapamycin (mTOR) signaling pathway, both of which play pivotal roles in cell proliferation and survival (16).

Additionally, metformin's impact on reactive oxygen species (ROS) reduction in HeLa cells aligns with emerging evidence suggesting a multifaceted mode of action. Elevated ROS levels are often associated with cancer progression, and metformin's ability to mitigate these oxidative stressors may contribute to its anti-cancer effects (14)

The ROS assessment adds a layer of complexity to the discussion, hinting at potential mechanisms beyond direct cytotoxicity Epithelial-to-Mesenchymal Transition (EMT): High ROS levels can promote EMT, where epithelial cells transform into mesenchymal cells, acquiring increased motility and invasive potential. By reducing ROS, metformin might inhibit EMT, hindering cancer cell spread and colonization in distant organs(17).

Cancer stem cells (CSCs): ROS contribute to CSC self-renewal and survival. Metformin's ROS-reducing effect might decrease CSC populations, limiting their ability to initiate and sustain metastasis. (18)

Vascular Endothelial Growth Factor (VEGF): ROS upregulates VEGF expression, a key player in tumor angiogenesis. Reducing ROS levels through metformin could downregulate VEGF, impeding blood vessel formation and limiting tumor growth(19).

Signaling pathways: Metformin interacts with the AMPK/mTOR pathway, which can negatively regulate angiogenesis. Additionally, it might influence pro-angiogenic pathways like PI3K/Akt, further restricting new blood vessel growth(20).

Bcl-2/Bax balance: ROS can dysregulate the Bcl-2/Bax balance, promoting cell survival by increasing anti-apoptotic Bcl-2 protein levels. Metformin's ROS-reducing effect could tip the balance towards pro-apoptotic Bax, sensitizing cancer cells to apoptosis.(21)

PI3K/Akt pathway: This pathway, often activated in cancer cells, promotes cell survival and inhibits apoptosis. Metformin's interaction with AMPK can indirectly suppress PI3K/Akt, enhancing cancer cell susceptibility to programmed cell death(21)

These are just some examples, and the exact mechanisms might differ depending on cancer type and other factors. Additionally, remember that metformin interacts with diverse pathways beyond ROS modulation, potentially contributing to its effects on these hallmarks in multifaceted ways(22)..

While these findings are promising, it is crucial to acknowledge the limitations of in vitro studies. Cell culture conditions may not fully replicate the complexities of in vivo tumor microenvironments, and the translation of these results to clinical settings requires cautious consideration. Future studies should aim to validate these findings in preclinical models and eventually in clinical trials(23).

Conclusion

In conclusion, the presented data strongly supports metformin's selective and dose-dependent cytotoxicity on HeLa cells, providing a solid foundation for further exploration of its potential as an anti-cancer agent.

References

1. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017;60(9):1577–85.
2. Tirichen H, Yaigoub H, Xu W, Wu C, Li R, Li Y. Mitochondrial Reactive Oxygen Species and Their Contribution in Chronic Kidney Disease Progression Through Oxidative Stress. Front Physiol. 2021;12(April):1–12.
3. Zhang S, Xu H, Zhang L, Qiao Y. Cervical cancer: Epidemiology, risk factors and screening. Chin J Cancer Res. 2020 Dec;32(6):720–8.
4. Gadducci A, Cosio S. Pharmacological Treatment of Patients with Metastatic, Recurrent or Persistent Cervical Cancer Not Amenable by Surgery or Radiotherapy: State of Art and Perspectives of Clinical Research. Cancers (Basel). 2020 Sep;12(9).
5. Parchment RE, Doroshow JH. Pharmacodynamic endpoints as clinical trial objectives to answer important questions in oncology drug development. Semin Oncol. 2016 Aug;43(4):514–25.
6. Vancura A, Bu P, Bhagwat M, Zeng J, Vancurova I. Metformin as an anticancer agent. Trends Pharmacol Sci. 2018;39(10):867–78.
7. AM AR, Mahmood IH. Cytotoxic Effect of Metformin and Mechanism of Action Review. Eur Sch J. 2022;3(2):9–14.
8. Capes-Davis A, Freshney RI, Geraghty RJ, Nims RW. Freshney's culture of animal cells: a manual of basic technique and specialized applications. (No Title). 2021;

9. Rasool A, Mahmood IH, Mahmood, IsamRasool A, Hamo. Evaluation of Cytotoxic Effect of Metformin on a Variety of Cancer Cell Lines. Clin Schizophr Relat Psychoses. 2021;15(3).
10. Mansour A, Rasool A, Mahmood IH. Comparison Cytotoxicity effects of metformin and paclitaxel on cervical cells and normal cell. 2022;140(04):2323–31.
11. Rasool AMA, Mahmood IH. Metformin's antioxidant activity and protective nature. In: AIP Conference Proceedings. AIP Publishing; 2023.
12. Artika IM, Dewi YP, Nainggolan IM, Siregar JE, Antonjaya U. Real-Time Polymerase Chain Reaction: Current Techniques, Applications, and Role in COVID-19 Diagnosis. Genes (Basel). 2022 Dec;13(12).
13. Li X, Celotto S, Pizzol D, Gasevic D, Ji MM, Barnini T, et al. Metformin and health outcomes: An umbrella review of systematic reviews with meta-analyses. Eur J Clin Invest. 2021 Jul;51(7):e13536.
14. Yang H, Chen X, Li K, Cheaito H, Yang Q, Wu G, et al. Repurposing old drugs as new inhibitors of the ubiquitin-proteasome pathway for cancer treatment. Semin Cancer Biol. 2021 Jan;68:105–22.
15. Ling S, Song L, Fan N, Feng T, Liu L, Yang X, et al. Combination of metformin and sorafenib suppresses proliferation and induces autophagy of hepatocellular carcinoma via targeting the mTOR pathway. Int J Oncol. 2017 Jan;50(1):297–309.
16. Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA, et al. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Proc Natl Acad Sci U S A. 2004 Mar;101(10):3329–35.
17. Hamanaka RB, Chandel NS. Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. Trends Biochem Sci. 2010 Sep;35(9):505–13.
18. Liu S, Yang B, Hou Y, Cui K, Yang X, Li X, et al. The mechanism of STING autoinhibition and activation. Mol Cell. 2023;83(9):1502–18.
19. Thomas NL, Dart C, Helassa N. The role of calcium and calcium binding proteins in cell physiology and disease. Front Physiol. 2023;14:1228885.
20. Shen J, San W, Zheng Y, Zhang S, Cao D, Chen Y, et al. Different types of cell death in diabetic endothelial dysfunction. Biomed Pharmacother. 2023;168:115802.
21. Li D, Ueta E, Kimura T, Yamamoto T, Osaki T. Reactive oxygen species (ROS) control the expression of Bcl-2 family proteins by regulating their phosphorylation and ubiquitination. Cancer Sci. 2004;95(8):644–50.
22. Uprety B, Abrahamse H. Targeting breast cancer and their stem cell population through AMPK activation: Novel insights. Cells. 2022;11(3):576.
23. Cogels MM, Rouas R, Ghanem GE, Martinive P, Awada A, Van Gestel D, et al. Humanized Mice as a Valuable Pre-Clinical Model for Cancer Immunotherapy Research. Front Oncol. 2021;11:784947.