European Science Methodical Journal
ISSN (E): 2938-3641
Volume 3, Issue 5, May- 2025

FROM ANTIMICROBIAL TO ANTITUMOR AGENT: AN IN VITRO STUDY ON THE EFFICACY OF TINIDAZOLE AGAINST CERVICAL CANCER

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Abstract:
Background: Repositioning currently marketed drugs offers a promising strategy for discovering new anticancer agents. Tinidazole is one such medication that may potentially have an anticancer effect.
Objective: This study evaluated the anticancer effects of Tinidazole and its selective toxicity toward cervical cancer cell lines.
Methods: The MTT assay was employed to assess the anticancer activity of Tinidazole using the HeLa cancer cell line (human cervical cancer cell line). This assay involved two incubation periods of 24 and 72 hours, utilizing concentrations from 0.1 to 1000 µg/ml. For comparison, Cisplatin was included. A score of the selective toxicity index was estimated after assessing the cytotoxic impact of Tinidazole on human-derived adipose tissue (NHF) cell lines, along with the estimation of IC50 to evaluate the selective toxicity of Tinidazole towards cancer cells.
Results: The MTT assay demonstrated that Tinidazole inhibits cervical cancer growth in a concentration-dependent and time-dependent manner, showing greater growth inhibition compared to cisplatin. Regarding its impact on the NHF cell line, Tinidazole had a lesser effect than on cancer cells. Furthermore, Tinidazole displayed a favorable selectivity index compared to cisplatin.
Conclusion: Based on the study outcomes and the established pharmacokinetic and safety profiles of Tinidazole, it offers an attractive and safer alternative for treating cervical cancer.

Keywords: Tinidazole, cervical cancer, HeLa cell line, NHF cell line, selective toxicity.

Introduction

Cervical cancer poses a significant global health challenge, primarily resulting from prolonged infections with high-risk human papillomavirus (HPV) types, particularly HPV-16 and HPV-18, which account for around 70% of cases. **(1, 2).** The disease occurs when an HPV infection leads to the malignant transformation of cervical epithelial cells, typically advancing from precancerous lesions (cervical intraepithelial neoplasia) over the years or decades. **(3, 4).** Epidemiologically, cervical cancer ranks as the fourth most common cancer among women globally, with an estimated 604,000 new cases and 342,000 deaths in 2020. It disproportionately impacts low- and middle-income countries due to limited access to

screening and vaccination **(5, 6).** Key risk factors include engaging in early sexual activity, having multiple partners, experiencing immunosuppression (such as HIV infection), and smoking **(7, 8).** Treatment varies by disease stage, ranging from surgical interventions (e.g., conization or hysterectomy) for early-stage cancer to chemoradiotherapy for more advanced cases **(9, 10).** The introduction of HPV vaccination has significantly lowered the incidence in countries with strong immunization programs, while regular Pap smears and HPV testing continue to be essential for early detection **(11-13).**

Chemotherapy is essential in the treatment of cervical cancer, especially for advanced or metastatic cases. Cisplatin-based regimens, frequently combined with paclitaxel, serve as the foundation of systemic therapy **(14). .** In locally advanced cases, concurrent chemoradiation with cisplatin enhances survival compared to radiation alone **(15).** Recent advancements include the use of bevacizumab for recurrent disease, which has improved overall survival. **(16).**

Despite the vital role of chemotherapy in treating cervical cancer, it has several drawbacks, mainly due to its non-selective mode of action. Platinum-based agents, such as cisplatin, remain the standard treatment; however, they frequently cause severe systemic toxicity, including nephrotoxicity, neurotoxicity, myelosuppression, and gastrointestinal complications, which adversely affect patients' quality of life. **(17) .** Moreover, many advanced or recurrent cervical cancers develop resistance to chemotherapy, which limits long-term effectiveness **(18).** The hypoxic tumor microenvironment in cervical cancer decreases drug penetration and effectiveness, leading to treatment failure **(19).** Furthermore, chemotherapy alone provides limited survival benefits in metastatic or persistent cases, often requiring concurrent radiotherapy, which increases toxicity **(20).**

Despite their targeted mechanism, monoclonal antibodies (mAbs) used in cancer therapy encounter limitations, such as high production costs, risks of immunogenicity, and variable responses among patients**. (Scott et al., 2012**). Their large molecular size limits tumor penetration, and acquired resistance due to antigen loss or alterations in signaling pathways reduces efficacy**. (Weiner et al., 2010).** Additionally, mAbs frequently lead to infusion reactions and organ-specific toxicities (e.g., cardiotoxicity associated with trastuzumab). **(Narayan et al., 2021).** To address these challenges, researchers investigated alternative therapies that focus on repurposing non-cancer drugs. Several studies were conducted in this area context.

Metformin, a medication for diabetes, exhibits anti-tumor properties by regulating the mTOR and AMPK pathways. **(21).** Similarly, statins show potential chemopreventive properties by inhibiting the cholesterol pathway. **(22)**. These approaches provide affordable alternatives with proven safety profiles, although additional clinical validation is required to confirm effectiveness across various cancer types. **(23).**

The class of antimicrobial agents known as 5-nitroimidazoles, which includes metronidazole, secnidazole, ornidazole, and tinidazole, is commonly utilized for treating anaerobic bacterial and protozoal infections. Recent research indicates their potential for repurposing in non-

infectious conditions, especially in cancer and inflammatory diseases. These agents produce cytotoxic effects by generating free radicals through nitroreductase, leading to DNA damage and triggering apoptosis in rapidly dividing cells. **(24)**. Metronidazole has demonstrated anti-tumor activity in preclinical models, potentially because of its hypoxia-selective toxicity, which positions it as a candidate for targeting hypoxic tumor microenvironments. **(25).** Tinidazole, which has a longer half-life, has been investigated for its potential role in reducing mucositis caused by chemotherapy. **(26).**

Despite limited studies on the anticancer impact of tinidazole, there are still restrictions on exploring its effects on cervical cancer and investigating its selective toxicity on cancer cells. This study was conducted to investigate the anticancer effects of tinidazole on cervical cancer and explore its selective toxicity.

1- Materials and methods:

2-1- medications:

Tinidazole and cisplatin, acquired as raw materials from the Samarra Pharmaceutical Factory in Iraq, were serially diluted using MEM media, resulting in concentrations from 0.1 to 1000 µg/ml.

2-2- Cytotoxicity Assay

Cytotoxicity was evaluated in HeLa cancer cells to assess the anticancer properties of Tinidazole and cisplatin. Furthermore, the cytotoxic effects of both drugs were tested on the NHF cell line, which serves as a model for normal healthy cells. The cytotoxicity and safety profiles of Tinidazole and cisplatin were assessed by measuring the viability of both cancerous and healthy cells across concentrations of 0.1 to 100 µg/ml for each treatment.

2-2-1—Cell Lines Used: The following cell lines were utilized.

1- **HeLa cell line:** it's derived from human cervical cancer cells. **(27, 28).**
2- **NHF cell line**: it's derived from normal human adipose tissue. **(29).**

2-2-2- Cell culture conditions:

The cell lines were cultured in MEM medium (US Biological, USA) and enriched with 10% (v/v) fetal bovine serum (FBS) (Capricorn-Scientific, Germany). To avoid bacterial contamination, the medium contained 100 IU/mL penicillin and 100 µg/mL streptomycin (Capricorn-Scientific, Germany). Cells were kept in a humidified incubator at 37°C, and all experiments were performed using cells in the exponential growth phase. **(30)**.

2-2-3- MTT cytotoxicity assay:

The MTT colorimetric assay tested cell viability by analyzing Tinidazole activity. In this approach, active cells convert the yellow MTT tetrazolium salt into purple formazan crystals, facilitated by mitochondrial dehydrogenase enzymes. The assay involves culturing cells in 96-well plates and using diverse concentrations of test compounds. After an appropriate incubation

period, MTT reagent is added to each well for further incubation. Only living cells with active metabolism can convert MTT into the insoluble purple formazan product. Once the crystals are dissolved, the absorbance of the solution is measured spectrophotometrically at a designated wavelength, providing a quantitative assessment of cell viability.

The number of viable cells directly influences the amount of formazan generated. A reduction in formazan production post-treatment with the test chemical indicates cytotoxicity, impacting absorbance. The dose-response curve is useful for determining the half-maximal inhibitory concentration (IC50). **(31)** .

Cells were inoculated into 96-well microplates at a density of 10,000 cells per well and incubated at 37°C for 24 hours to reach confluence. Along tow incubation periods, 24 and 72 hours, the cytotoxicity of the tested compounds was assessed using the MTT assay, with a concentration range of 0.1 to 1000 µg/ml and six replicates for each concentration. The untreated wells, totaling 20, served as negative controls.

After 24 and 72 hours of treatment, 28 µL of MTT solution (2 mg/mL) was applied into each well and incubated for 3 hours. Subsequently, the formazan crystals were dissolved with 100 µL of DMSO during a 15-minute incubation period. Absorbance readings at 570 nm were recorded using a microplate reader. The percentages of cytotoxicity were computed using the following formula.**(32)**

$$\textbf{Growth inhibition}\ \% = \frac{\textbf{optical density of control wells} - \textbf{optical density of treated wells}}{\textbf{optical density of control wells}} * \mathbf{100}\%$$

2-3- selective toxicity index:

The selective toxicity index score assessed the effectiveness of Tinidazole against cancer cells over 24- and 72-hour incubation periods. Following the determination of IC50 levels for Tinidazole and cisplatin, the selective cytotoxicity index was calculated using a mathematical equation based on cell growth curves for each HeLa and NHF cell line. **(33)**

$$\textbf{Selective toxicity Index}\ (SI) = \frac{\textbf{IC 50 of normal cell lines}}{\textbf{IC 50 of cancer cell lines}}$$

An SI score greater than 1.0 indicates that a drug is more effective at targeting cancer cells compared to normal cells. The estimation of the IC50 level was conducted using GraphPad Prism 9.5.0 (730) software. **(34)**

2-4- Ethical approval:

This research utilized only in vitro cell line models, excluding any involvement of human subjects or laboratory animals. All procedures followed institutional ethical guidelines for lab-based studies.

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2-5- Statistical Analysis:

Cytotoxicity results are presented as mean ± standard deviation (SD). We assessed intergroup variability using one-way ANOVA followed by post-hoc pairwise comparisons with LSD tests. For direct comparisons between groups, we utilized paired t-tests. All analyses were performed in SPSS (version 20), and statistical significance was set at $p < 0.05$. **(35).**

To improve data interpretation, we introduced a letter-coding system in our tables. Groups represented by the same letter indicate statistically similar means, whereas different letters signify significant differences ($p < 0.05$) between groups. This visual approach facilitates quick comparisons among multiple groups, minimizes the requirement for repetitive statistical annotations, and upholds rigor while enhancing readability.

3- Results:

3-1- Cytotoxic study:

3-2-1- Tinidazole Cytotoxicity:

The cytotoxic effects of Tinidazole on the viability of cervical cancer cell lines demonstrate its capacity to reduce cellular viability, particularly with extended incubation periods. A decrease in the IC50 value reinforces this effect, indicating that growth inhibition occurs in a time-dependent manner. Additionally, higher concentrations of Tinidazole yield a more significant inhibitory effect, signifying a concentration-dependent mode of inhibition.

Additionally, the inhibitory effect of metronidazole on the NHF cell line was both concentration- and time-dependent, with a greater emphasis on the concentration effect. Table (1)

Table 1: Effects of Tinidazole on the viability of HeLa and NHF cells at 24 and 72 hours

Concentration (µg/ml)	Cellular growth suppression (mean ± SD)					
	HeLa cell line			NHF cell line		
	24 hr.	72 hr.	*P*- value	24 hr.	72 hr.	*P*- value
0.1	**C** 1.00 ± 1.000	**C** 22.00 ± 2.000	**0.0001***	**B** 0.00 ± 0.000	**C** 0.00 ± 0.000	**N. S**
1	**BC** 2.00 ± 1.000	**C** 30.00 ± 3.000	**0.0001***	**B** 0.00 ± 0.000	**BC** 2.00 ± 2.000	**0.158**
10	**AC** 8.00 ± 3.000	**C** 32.00 ± 6.000	**0.003***	**AB** 3.00 ± 2.000	**AB** 9.00 ± 2.000	**0.021***
100	**A** 10.00 ± 3.000	**B** 53.00 ± 3.000	**0.0001***	**AB** 6.00 ± 1.000	**A** 13.00 ± 3.000	**0.019***
1000	**AB** 13.00 ± 3.000	**A** 69.00 ± 3.000	**0.0001***	**A** 9.00 ± 4.000	**A** 15.00 ± 2.000	**0.081***
LSD value	**8.76**	**13.32**	-	**7.56**	**7.40**	-
IC 50	**5244 µg/ml**	**82 µg/ml**	-	**6491 µg/ml**	**4213 µg/ml**	-
*: significant at (P<0.05)						

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3-2-2- Cisplatin cytotoxicity:

To serve as a positive control for comparison, cisplatin was selected. Its cytotoxic effects showed its capacity to hinder the growth of both cell lines (HeLa and NHF) in a manner that depended on both concentration and duration. The IC50 value decreased at 72 hours relative to 24 hours of incubation, indicating a time-dependent impact inhibition. table (2)

Table 2: Cisplatin's impact on the viability of HeLa and NHF cells at 24 and 72 hours.

Concentration (µg/ml)	Cellular growth suppression (mean ± SD)					
	HeLa cell line			NHF cell line		
	24 hr.	72 hr.	*P*- value	24 hr.	72 hr.	*P*- value
0.1	**C** 0.00 ± 0.000	**D** 2.00 ± 1.000	**0.026***	**D** 4.00 ± 2.000	**E** 10.00 ± 1.000	**0.010***
1	**C** 0.00 ± 0.000	**C** 10.00 ± 3.000	**0.004***	**CD** 14.00 ± 4.000	**D** 21.00 ± 1.000	**0.042***
10	**C** 7.00 ± 2.000	**C** 17.00 ± 1.000	**0.001***	**BC** 20.00 ± 2.000	**C** 33.00 ± 3.000	**0.003***
100	**B** 27.00 ± 5.000	**B** 40.00 ± 3.000	**0.018***	**B** 31.00 ± 1.000	**B** 53.00 ± 3.000	**0.0001***
1000	**A** 39.00 ± 2.000	**A** 51.00 ± 1.000	**0.001***	**A** 50.00 ± 5.000	**A** 68.00 ± 2.000	**0.004***
LSD value	**9.34**	**7.46**	-	**11.5**	**7.98**	-
IC 50	**1278 µg/ml**	**917 µg/ml**	-	**967 µg/ml**	**87 µg/ml**	-
*: significant at (P<0.05)						

To illustrate Tinidazole's anticancer efficacy, its cytotoxicity was compared with that of standard chemotherapy (cisplatin) during each incubation period. The results demonstrated that Tinidazole's cytotoxicity was significantly greater than that of cisplatin, particularly during the 72-hour incubation period: Table 5, Figure 1, 2.

A further comparison examined safety, which included assessing the effects of Tinidazole and cisplatin on both HeLa and NHF cells. This comparison revealed that Tinidazole exhibited significantly higher cytotoxicity on cancer cells than on the NHF cell line. In contrast, the impact of cisplatin was greater on the NHF cell line compared to the HeLa cell line. Table (3,4)

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Table (3): comparing the effects of Tinidazole on the growth of HeLa and NHF cell lines at 24 and 72 hours

Concentration (µg/ml)	Cellular growth suppression (mean ± SD)					
	24 hr.			72 hr.		
	HeLa cell line	NHF cell line	*P*- value	HeLa cell line	NHF cell line	*P*- value
0.1	**C** 1.00 ± 1.000	**B** 0.00 ± 0.000	**0.158**	**C** 22.00 ± 2.000	**C** 0.00 ± 0.000	**0.0001***
1	**BC** 2.00 ± 1.000	**B** 0.00 ± 0.000	**0.026***	**C** 30.00 ± 3.000	**BC** 2.00 ± 2.000	**0.0001***
10	**AC** 8.00 ± 3.000	**AB** 3.00 ± 2.000	**0.074**	**C** 32.00 ± 6.000	**AB** 9.00 ± 2.000	**0.003***
100	**A** 10.00 ± 3.000	**AB** 6.00 ± 1.000	**0.094**	**B** 53.00 ± 3.000	**A** 13.00 ± 3.000	**0.0001***
1000	**AB** 13.00 ± 3.000	**A** 9.00 ± 4.000	**0.238**	**A** 68.00 ± 3.000	**A** 15.00 ± 2.000	**0.0001***
LSD value	**8.76**	**7.56**	-	**13.32**	**7.40**	-
IC 50	**5244 µg/ml**	**6491 µg/ml**	-	**82 µg/ml**	**4213 µg/ml**	-
*: significant at (P<0.05)						

Table (4): Comparing the effects of cisplatin on the growth of HeLa and NHF cell lines after 24 and 72 hours.

Concentration (µg/ml)	Cellular growth suppression (mean ± SD)					
	24 hr.			72 hr.		
	HeLa cell line	NHF cell line	*P*- value	HeLa cell line	NHF cell line	*P*- value
0.1	**C** 0.00 ± 0.000	**D** 4.00 ± 2.000	**0.026***	**D** 2.00 ± 1.000	**E** 10.00 ± 1.000	**0.001***
1	**C** 0.00 ± 0.000	**CD** 14.00 ± 4.000	**0.004***	**C** 10.00 ± 3.000	**D** 21.00 ± 1.000	**0.004***
10	**C** 7.00 ± 2.000	**BC** 20.00 ± 2.000	**0.001***	**C** 17.00 ± 1.000	**C** 33.00 ± 3.000	**0.001***
100	**B** 27.00 ± 5.000	**B** 31.00 ± 1.000	**0.246**	**B** 40.00 ± 3.000	**B** 53.00 ± 3.000	**0.006***
1000	**A** 39.00 ± 2.000	**A** 50.00 ± 5.000	**0.024***	**A** 51.00 ± 1.000	**A** 68.00 ± 2.000	**0.0001***
LSD value	**9.34**	**11.5**	-	**7.46**	**7.98**	-
IC 50	**1,278 µg/ml**	**967 µg/ml**	-	**917 µg/ml**	**87 µg/ml**	-
*: significant at (P<0.05)						

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Table (5): A comparison of 24 and 72 hours. Hela cell line growth inhibition by Tinidazole and cisplatin.

Concentration (µg/ml)	Cellular growth suppression (mean ± SD)					
	24 hr.			72 hr.		
	Tinidazole	Cisplatin	*P*- value	Tinidazole	Cisplatin	*P*- value
0.1	**C** 1.00 ± 1.000	**C** 0.00 ± 0.000	**0.158**	**C** 22.00 ± 2.000	**D** 2.00 ± 1.000	**0.0001***
1	**BC** 2.00 ± 1.000	**C** 0.00 ± 0.000	**0.026***	**C** 30.00 ± 3.000	**C** 10.00 ± 3.000	**0.001***
10	**AC** 8.00 ± 3.000	**C** 7.00 ± 2.000	**0.656**	**C** 32.00 ± 6.000	**C** 17.00 ± 1.000	**0.013***
100	**A** 10.00 ± 3.000	**B** 27.00 ± 5.000	**0.007***	**B** 53.00 ± 3.000	**B** 40.00 ± 3.000	**0.006***
1000	**AB** 13.00 ± 3.000	**A** 39.00 ± 2.000	**0.0001***	**A** 68.00 ± 3.000	**A** 51.00 ± 1.000	**0.001***
LSD value	**8.76**	**9.34**	-	**13.32**	**7.46**	-
IC 50	**5244 µg/ml**	**1278 µg/ml**	-	**82 µg/ml**	**917 µg/ml**	-
*: significant at (P<0.05)						

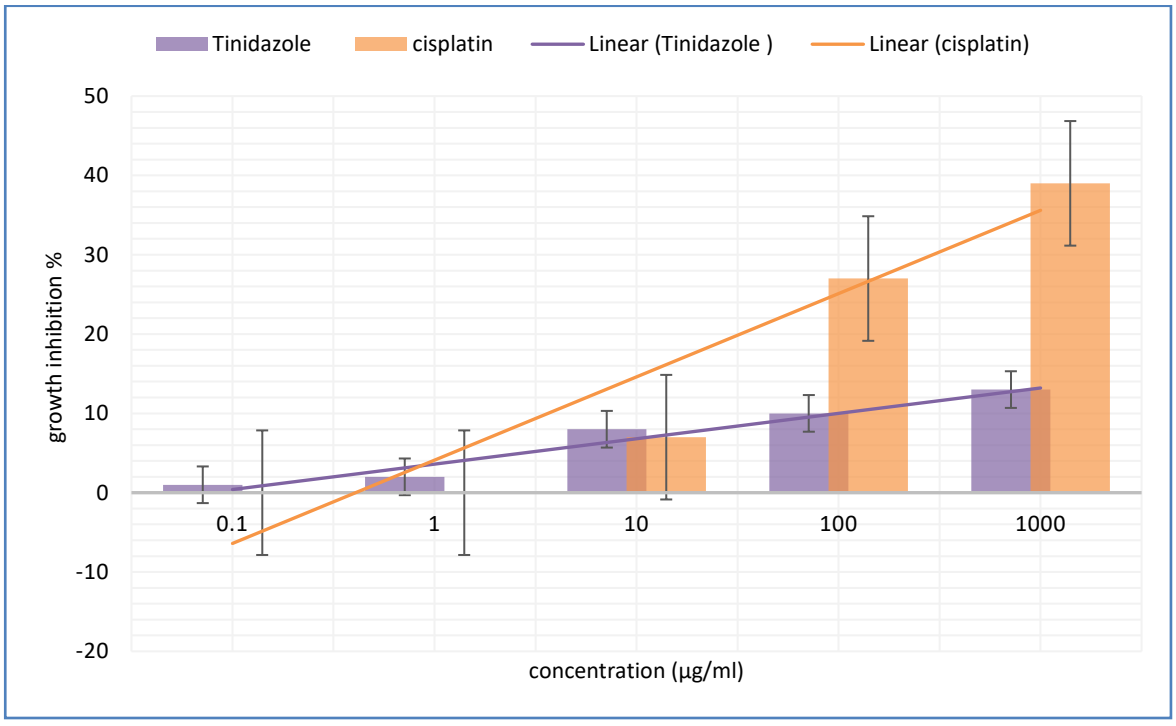

Figure 1: Comparison of growth inhibition in the HeLa cell line between Tinidazole and cisplatin after 24 hours of incubation.

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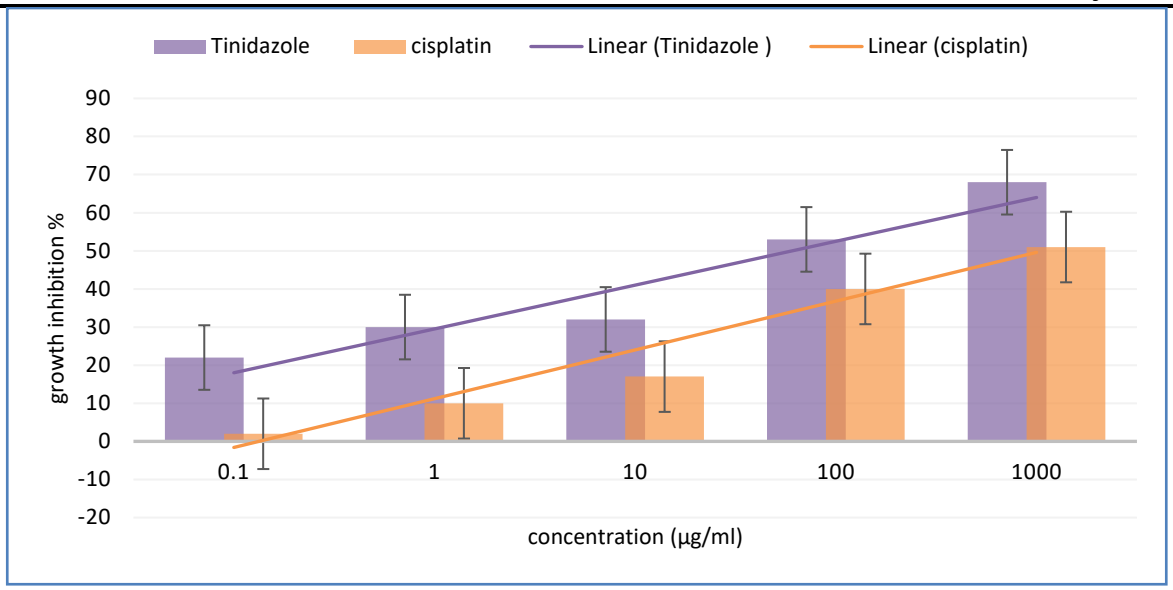

Figure (2): Comparison of growth inhibition in the HeLa cell line between Tinidazole and cisplatin after 72 hours of incubation

3-3- Selective toxicity index study:

At 24 hours, the SI score for Tinidazole was 1.23, rising to 51.3 at 72 hours, which suggests that Tinidazole has a favorable selective toxicity against cancer cells compared to healthy cells. As the incubation duration increased, the selective toxicity also became more pronounced. In contrast, cisplatin had an SI score of 0.75 at 24 hours and 0.094 at 72 hours, indicating a lower selective toxicity toward cancer cells compared to healthy cells. See Figure 3.

Table 6: Comparison of Tinidazole and cisplatin SI over 24 and 72 hours.

Incubation periods	Selective toxicity score	
	Tinidazole	Cisplatin
24 hours	1.23	0.75
72 hours	51.3	0.094
(An SI greater than 1.0 indicates that a drug is more effective against tumor cells compared to its toxicity towards normal cells)		

3-4- Morphological Observations of the study cell line:

Figure 3 shows the morphological changes observed in HeLa and NHF cell lines after 72 hours of exposure to 1000 µg/ml of Tinidazole and cisplatin.

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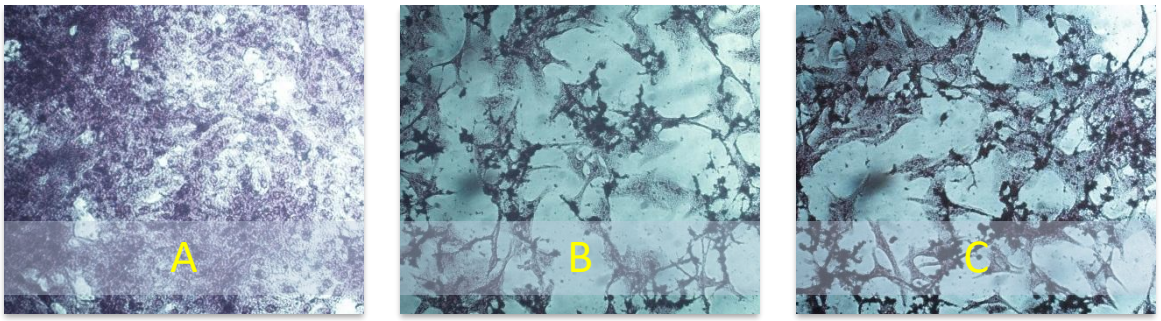

Figure (3): Morphological features of the HeLa cell line (400x). (**A**) Untreated HeLa cell cancer line. (**B**) HeLa cells were subjected to 1000 µg/ml Tinidazole for 72 hours. (**C**) HeLa cells treated with 1000 µg/ml cisplatin for 72 hours.

Discussion

Reassessing existing non-cancer medications offers an opportunity for alternative therapies. This study investigates the anticancer effects of Tinidazole. The limited research on the anticancer effects of Tinidazole significantly influenced our decision to explore this medication. Moreover, several studies guided our choice of Tinidazole due to its related compound's potential anticancer effects. Moreover, another factor contributing to our choice includes its safety, supported by the well-established pharmacokinetics and safety profile of Tinidazole.

Our study MTT assay findings exhibited that Tinidazole inhibited cervical cancer growth and exhibited reduced cytotoxicity towards normal healthy cell lines. Tinidazole showed improved anticancer effectiveness compared to the cytotoxicity of cisplatin. In contrast to cisplatin, Tinidazole demonstrates enhanced safety due to its favorable selectivity index score, which signifies its selective toxicity towards cancer cells while sparing healthy cells.

The results of our study are consistent with numerous earlier studies into the anticancer properties of Tinidazole-related compounds (5-Nitroimidazole Antimicrobial Agent) as Metronidazole, Secnidazole, and Ornidazole. A recent study indicates that metronidazole might inhibit the growth of CHO (Chinese hamster ovary) cells, HeLa cells (from cervical cancer), and human marrow cells. Nevertheless, this effect appears to depend on the concentration of the drug and the degree of hypoxia conditions. **(36-38),** and showed cytotoxic effects on the MDA-MB-231 breast cancer cell line. Cytotoxicity was noted at elevated concentrations, up to 250 µg/ml, after 72 hours of incubation. **(31, 39, 40) .** Another recent study has examined the anticancer potential of metronidazole, particularly its selective cytotoxic effects in low-oxygen environments. Tumor microenvironments frequently exhibit hypoxia, leading to the enzymatic reduction of the nitro group in metronidazole, which produces reactive intermediates that harm DNA and initiate cancer cell death. **(41).** Preclinical studies show that combining metronidazole with radiotherapy enhances tumor sensitivity in hypoxic regions, improving therapeutic

outcomes. **(42).** Furthermore, in vitro studies involving colorectal and glioblastoma cell lines demonstrate that metronidazole derivatives exhibit selective antiproliferative effects, highlighting their potential for repurposing. **(43).** Recent study evidence indicates that metronidazole's anticancer mechanism, reliant on nitroreductase activity in hypoxic circumstances, may specifically target cancer cells, especially inside hypoxic tumor microenvironments. **(44)**. Hypoxia, a characteristic feature of solid tumors, activates the nitro group of metronidazole, generating cytotoxic free radicals that lead to DNA damage and trigger apoptosis. **(45, 46).** This targeted cytotoxicity protects healthy normoxic tissues, positioning metronidazole as a promising option for tumor-specific therapy.

Furthermore, regarding Ornidazole (another 5-Nitroimidazole antimicrobial agent), it has exhibited the ability to suppress angiogenesis and migration in non-small cell lung cancer (NSCLC) by targeting the VEGFA/VEGFR2/NRP-1 axis and inhibiting the PI3K/AKT pathway. These effects reduce tumor vascularization and metastatic potential, indicating Ornidazole's promise for repurposing as an adjunctive therapy for NSCLC by disrupting critical oncogenic signaling pathways.**(47)**

The research faces limitations, including laboratory validation linked to molecular studies, which are influenced by financial constraints and obstacles.

Conclusion:

This study aimed to identify a safe and effective anticancer treatment for cervical cancer by repurposing Tinidazole. MTT assay results showed that Tinidazole significantly inhibits the proliferation of cervical cancer cells more effectively than the growth inhibition caused by chemotherapy (cisplatin). The inhibition method depended on the concentration and duration of incubation. Regarding the selectivity index score, Tinidazole's cytotoxicity exhibited selectivity, indicating specific targeting of cancer cells rather than healthy ones.

In light of these findings, Tinidazole presents an attractive alternative therapeutic option for cervical cancer, especially given its established pharmacokinetic properties and safety profile. Yet, clinical trials are needed to validate efficacy and optimize dosing regimens for non-antimicrobial applications.

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Gathering and organizing data: Doaa Hassan Abd alwahab
Data analysis/interpretation: Doaa Hassan Abd alwahab
Article composition: Doaa Hassan Abd alwahab
Critique the essay for significant ideas: Doaa Hassan Abd alwahab.
Statistical analysis expertise: Doaa Hassan Abd alwahab
Ultimate article endorsement and guarantee: Doaa Hassan Abd alwahab

Acknowledgements:
The research team extends its gratitude to the researchers and instructional staff at ICMGR/Mustansiriyah University in Baghdad, along with the Iraqi National Cancer Research Center/University of Baghdad, for their vital support throughout this study. We also wish to express our sincere appreciation to the quality control team at the Samarra Pharmaceutical Factory for supplying the drugs utilized in this research.

Funding and sponsorship:
Self-funded

Conflicts of interest :
The authors state that they have no conflicts of interest to disclose.

Declaration of Generative AI and AI-assisted technologies in the writing process:
The authors clarify that this work does not use generative AI or AI-assisted technologies.

Abbreviations:
(ICCMGR): The Iraqi Centre for Cancer and Medical Genetics Research.
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide stain
MEM: Minimum Essential Medium
SAS: Statistical Analysis System
LSD: Least Significant Difference
NHF cell line: human-derived adipose tissue cell line
OD: optical density

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