Original Article

# Evaluate the Effect of Artesunate and Cisplatin alone and in Combination in Viability and HIF-1α Gene on Prostate Cancer Cell Line and Compare it with the Viability on Normal HECK-293 Cell Line



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## ABSTRACT

**Objective:** To evaluate the effect on viability and hypoxia-inducible factor 1-alpha expression in prostate cancer 3 cell line and normal heck cell line of artesunate and cisplatin and comparing their effect alone and in combination

**Study Design:** Comparative study

**Place and Duration of Study:** This study was conducted at the Medicine College, Babylon University, Hillah, Iraq, from 1st October 2024 to 31st March 2025.

**Methodology:** Prostate cancer 3 cell lines and heck-293 cell line cultured in Roswell Park Memorial Institute supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells maintained in a humidified incubator at 37 °C. Roswell Park Memorial Institute colorimetric assay performed by seeding prostate cancer cell line in 96-well plate at density of (1x10^4) cells/well. Incubated for 24hour then treated with various concentrations of artesunate and cisplatin alone and in combination then after 24 hours incubation treated with 5mg/ml MTT solution and incubated for four hours then add Dimeythyl solution and measured the result by using microplate reader at 570 nm

**Results:** Artesunate showed significant inhibition at PC3 in one-way Anova $p<0.001$ the IC50 is 148.61 µg/ml and in normal heck cell line IC50 is 946.125 µg/ml the selectivity index is 6.4. artesunate also upregulates the HIF-1α gene of PC3 cell line in low concentration $p<0.001$but reduces this regulation in higher concentration. the IC50 of cisplatin in heck cell line is 501.62 µg/ml and in PC3 cell line is 606.9 µg/ml. cisplatin shows up significant up regulation on HIF-1 gene in all concentration. Combination index shows synergy in low doses $CI < 1$

**Conclusion:** Artesunate exhibit significant anticancer effect on PC3 cell line and moderate safety profile comparing with cisplatin which is lower potency and safety. artesunate reducing the up regulation of survival gene HIF-1α opposite to cisplatin which up regulates HIF-1α gene in all concentrations


**Key Words:** Artesunate, Cisplatin, Prostate cancer, Synergy.

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## INTRODUCTION

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One of leading malignancy and worldwide spreading cancer is prostate cancer[1]. Which diagnosed in male globally and it considered one of leading cause of mortality in the male because of cancer which considered a public health problem[2]. Prostate cancer is considered a solid malignancy which is developed under hypoxic conditions microenvironment this hypoxic condition considered critically associated with tumor progression[3]. The expression of HIF-1α in Normoxic conditions is considered on of the important factor in resistance of cancer chemotherapeutic[4]. The hypoxia inducible factors specially (HIF-1α) is stabilized by hypoxic microenvironment of the tumor this factor contributes to regulating and control the

genes which responsible for cellular survival and adaptation such as angiogenesis and metastasis[5]. The aggressive characteristics of prostate cancer tumor like metastasis, angiogenesis and the affection of clinical outcome of patient is related with elevation the expression of HIF-1α[6].

One of the principal transcriptional factor which make the prostate cancer cells adapt to hypoxic microenvironment by angiogenesis is HIF-1α and its considerable target to enhance anticancer therapy[7]. Cisplatin is a chemotherapeutic drug which containing platinum its mechanism of action prevents gene transcription by making crosslinks between DNA double strands furthermore its prevent DNA replication and initiate programed cell Lethality[8]. Cisplatin resistance is one of the problem which affect the clinical outcomes HIF-1α is considered a important factor in the cisplatin resistance under hypoxic microenvironment and that leads to increases the survival of cancer cell[9]. Across many type of tumor type a potent anticancer effects have shown by artesunate which is semi-synthetic derivative of artemisinin that can give a synergistic effect when combined with cisplatin to improve anticancer activity[10]. The combination of chemotherapeutics such as cisplatin with artesunate can give a synergistic antitumor by increasing the effectiveness and induction of apoptosis. so the assessment of HIF-1α can give an important information about the survival status of cancer while using the anticancer medication alone and in combination and its present as considerable target[11].

# METHODOLOGY

This comparative study was conducted at Medicine College, Babylon University, Hillah, Iraq from 1st October 2024 to 31st March 2025 letter No. 3434/QM/Approval/JSDJNEHU dated September 7, 2024. MTT assay of artesunate in concentration of (1000, 500, 250, 125, 62.5, 31.25) µg/ml. and cisplatin in concentrations of (500, 250, 125, 62.5, 31.25, 15.6) µg/ml on normal heck cell and artesunate in concentrations of (200, 100, 50, 25, 12.5, 6.25) µg/ml and cisplatin in concentration of (500, 250, 125, 62.5, 31.25, 15.6) µg/ml on pc3 cell then a combination of cisplatin in fixed concentration 31.25 µg/ml and Various concentrations of artesunate (200, 100, 50, 25, 12.5, 6.25) µg/ml.

The effect of artesunate in gene HIF-1α on pc3 cell line at concentrations of (6.25, 50, 200) µg/ml, the effect of cisplatin on the same gene in pc3 cell line at concentrations of (31.25, 125, 500) µg/ml and finally in combination of fixed concentrations of Cisplatin 31.25 µg/ml. plus artesunate (6.25, 50, 200) µg/ml

**Cell line and cell culture:** Prostate cancer (PC3) cell line obtain from LONZA Biologics (slough UK) and normal human cell HECK-293 obtained from (Pasteur Institute of Iran). PC3 and HEK displaced from liquid nitrogen in -196C and culture in culture media RPMI with 10% FBS and 1% penicillin-streptomycin in humidified incubator (5% co2 and 37C), and renew the culture media every 24hr after that add 3ml of trypsin to the flask and leave it for 5 min then shake the flask gently until all cells detached from the flask add 10 ml of culture media to the flask and mixed well with pipet and then take 250 µl and seeded it in each well.

**HIF-1A expression:** Total RNA isolation from PC3 cells using the FavorPrep™ Blood/Cultured Cell Total RNA Kit according to the instructions of manufacturer. Briefly, cells were harvested and lysed using FARB buffer containing β-mercaptoethanol, followed by mechanical disruption with acid-washed glass beads. The lysate was centrifuged and mixed with 70% RNase-free ethanol, then applied to a FARB Mini Column. The column was washed with the provided buffers and treated with DNase I to remove genomic DNA contamination.

# RESULTS

In the MTT viability assay, artesunate significantly inhibited normal HEK-293 cells ($P<0.001$, one-way ANOVA). At 1000 ($P<0.001$), 500 µg/ml, and 250 µg/ml ($P<0.01$) by t-test, the IC50 is 946.125 µg/ml (Table 1). Cisplatin shows significant cytotoxicity in one-way ANOVA ($p<0.001$), at 500 µg/ml ($p<0.001$) and 250 µg/ml ($p<0.001$) by t-test. The inhibitory concentration 50% of cisplatin in normal HEK-293 cells was 501.62 µg/ml (Table 2). In the PC3 cell line, artesunate also shows a significant anticancer effect ($p<0.001$ in one-way ANOVA), at concentrations of 200 µg/ml, 100 µg/ml ($p<0.001$), and 50 µg/ml ($p<0.05$) by t-test (Table 3). The IC50 of artesunate on the PC3 cell line is 148.61 µg/ml. Cisplatin has significant anticancer activity on the PC3 cell line, with one-way ANOVA $p<0.001$. At concentrations of (500, 250, 125, 62.5) µg/ml, $p<0.001$, and at 31.25 µg/ml $p<0.01$ by t-test and the 50% inhibition concentration is 606.9 µg/ml (Table 4).

For the combination of fixed concentrations of cisplatin 31.25µg/ml and different concentrations of artesunate, the one-way ANOVA revealed a significant effect of the combination on the PC3 cell line ($p<0.0001$). At 31.25µg/ml cisplatin and (200, 100, 50, 25, 6.25)µg/ml of artesunate ($p<0.0001$) by t-Test (Table 5) and the inhibitory concentration 50% of the combination is 243.93 µg/ml (Fig. 1). Combination index shows synergistic effect in concentration of artesunate (6.25, 12.5, 25, 50) µg/ml plus 31.25 µg/ml cisplatin and in 100, 200 µg/ml shows antagonism (Table 6). The selectivity index of artesunate is 6.4

GAPDH is the housekeeping gene used as a negative control in HIF-1α expression evaluation. Cisplatin elevated HIF-1α gene expression at three concentrations (31.25, 125, 500) µg/ml, significantly ($p<0.0001$) in one-way ANOVA (Fig. 2)

**Table No. 1: Effect of artesunate on viability of HEK-297**

| Concentration of Artesunate µg/ml | Viability | Inhibition | P value |
|---|---|---|---|
| 31.25 | 90.49429658 | 9.505703422 | P>0.05 |
| 62.5 | 91.12801014 | 8.871989861 | P>0.05 |
| 125 | 92.90240811 | 7.097591888 | P>0.05 |
| 250 | 77.94676806 | 22.05323194 | P<0.01 |
| 500 | 68.56780735 | 31.43219265 | P<0.01 |
| 1000 | 48.79594423 | 51.20405577 | P<0.001 |

**Table No. 2: Effect of cisplatin on viability of HEK-297**

| Concentration of Cisplatin µg/ml | Viability | Inhibition | P value |
|---|---|---|---|
| 15.6 | 94.67680608 | 5.323193916 | P>0.05 |
| 31.25 | 91.63498099 | 8.365019011 | P>0.05 |
| 62.5 | 99.23954373 | 0.760456274 | P>0.05 |
| 125 | 90.87452471 | 9.125475285 | P>0.05 |
| 250 | 73.63751584 | 26.36248416 | P<0.01 |
| 500 | 49.42965779 | 50.57034221 | P<0.001 |

**Table No. 3: Effect of artesunate on viability of PC3**

| Concentration of Artesunate µg/ml | Viability | Inhibition | P value |
|---|---|---|---|
| 6.25 | 93.90846923 | 6.09 | P>0.05 |
| 12.5 | 83.36664913 | 16.63335087 | P>0.05 |
| 25 | 75.47606523 | 24.52393477 | P>0.05 |
| 50 | 70.36296686 | 29.63703314 | P<0.05 |
| 100 | 52.75118359 | 47.24881641 | P<0.001 |
| 200 | 43.02998422 | 56.97001578 | P<0.001 |

**Table No. 4: Effect of cisplatin on viability of PC3**

| Concentration of cisplatin µg/ml | Viability | Inhibition | P value |
|---|---|---|---|
| 15.6 | 109.6685955 | -9.668595476 | P>0.05 |
| 31.25 | 78.21146765 | 21.78853235 | P<0.01 |
| 62.5 | 69.56338769 | 30.43661231 | P<0.001 |
| 125 | 59.92635455 | 40.07364545 | P<0.001 |
| 250 | 58.74802735 | 41.25197265 | P<0.001 |
| 500 | 57.06470279 | 42.93529721 | P<0.001 |

**Table No. 5: Effect of combination of fixed 31.25 µg/ml cisplatin plus different concentrations of artesunate**

| Different concentrations of artesunate µg/ml plus 31.25 µg/ml cisplatin | Viability | Inhibition | P value |
|---|---|---|---|
| 6.25 | 51.5704315 | 48.42956853 | P<0.001 |
| 12.5 | 61.3420051 | 38.65799492 | P<0.01 |
| 25 | 59.9143401 | 40.0856599 | P<0.001 |
| 50 | 60.3743655 | 39.62563452 | P<0.001 |
| 100 | 63.3248731 | 36.6751269 | P<0.001 |
| 200 | 47.5412437 | 52.45875635 | P<0.001 |

**Table No. 6: Combination index value of interaction between artesunate and cisplatin in PC3 cell line**

| Artesunate concentration (µg/ml) | 31.25 µg/ml cisplatin | Inhibition | Combination index | Result |
|---|---|---|---|---|
| 6.25 | 31.25 | 48.42956853 | 0.1 | Synergistic |
| 12.5 | 31.25 | 38.65799 | 0.234 | Synergistic |
| 25 | 31.25 | 40.0856599 | 0.325 | Synergistic |
| 50 | 31.25 | 39.62563 | 0.58 | Synergistic |
| 100 | 31.25 | 36.67513 | 1.21 | Antagonism |
| 200 | 31.25 | 52.45876 | 1.32 | Antagonism |

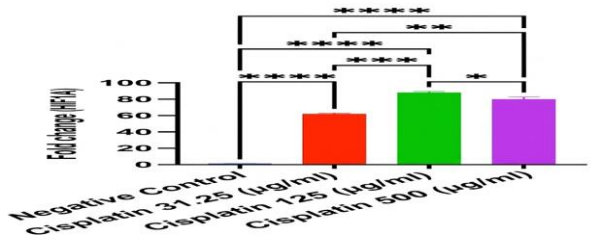



**Figure No. 1: Cisplatin in Different Concentrations Effect on Regulation of HIF-1α on PC3 cells (*=p<0.05), (**=p<0.01),(***=p<0.001)**

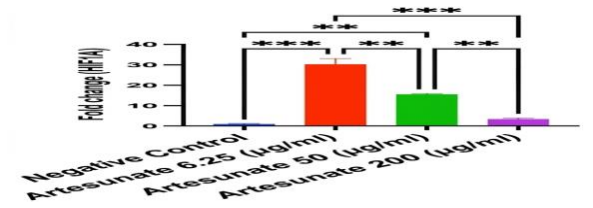



**Figure No. 2: Artesunate in Different Concentrations Effect on Regulation of HIF-1α on PC3 cells (*=p<0.05), (**=p<0.01),(***=p<0.001).**

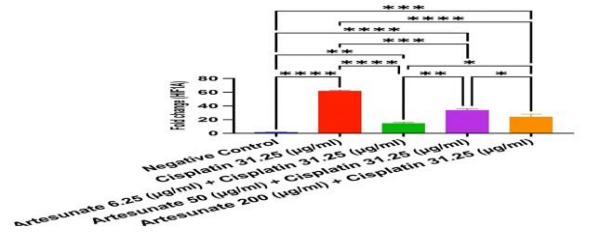



**Figure No. 3: Effect of Combination of Different Concentrations of Artesunate with Fixed Concentration of Cisplatin on HIF-1α Gene Expression in PC3 Cell Line (*=p<0.05), (**=p<0.01),(***=p<0.001)**

Artesunate elevated HIF-1α gene expression at three concentrations (6.25, 50, 200) µg/ml, significantly ($p<0.0001$) in one-way ANOVA and in Tukey's multiple comparisons ($p<0.0001$) in 6.25µg/ml, ($p<0.001$) at 50 µg/ml, and not significant in concentration of 200µg/ml (Fig. 3).

The combination of fixed concentrations of Cisplatin (31.25) µg/ml and three concentration of artesunate (31.25, 125, 500) µg/ml affecting the HIF-1α expression elevated its regulation significantly $p<0.0001$ in one-way Anova and in Tukey's multiple comparisons ($p<0.01$) in concentration (31.25 µg/ml cisplatin plus 6.25 µg/ml artesunate), in concentration of (31.25µg/ml cisplatin plus 50µg/ml artesunate) $p<0.0001$and in concentration of (31.25 µg/ml cisplatin plus 200 µg/ml artesunate) $p<0.001$.

# DISCUSSION

Artesunate shows dose dependent inhibition on pc3 cell line were IC50 148.61µg/ml which is much less effect compared with its effect on normal heck cell which IC50 946.125 µg/ml. The calculated selectivity index (SI) was 6.4 which indicates its toxic to pc3 more than 6-fold than its toxicity on normal heck cell and wide therapeutic window. The selectivity of artesunate to pc3 cell line is because of high iron concentration in pc3 in comparison to normal cell[12], its main cause of cell death because of ferroptosis due to cleavage of endoperoxides bridge in presence of iron and produces reactive oxygen species which kills the cell.[13] Artesunate up regulate HIF-1α expression significantly in low concentration because of ORS (Reactive Oxygen Species) activity[14] and that regulation start to decrease with increasing concentration because the effect of artesunate that decrease the production of HIF-1[15].

The cisplatin IC50 on normal heck cell is 501.62 µg/ml which is lower than its IC50 606.9 µg/ml on pc3 cell which indicate highly toxic effect of cisplatin on normal cell and Probable resistance of pc3 cell to cisplatin[16]. This toxicity of cisplatin came from nonselective interaction to DNA between normal cell and pc3 cell[17]. Cisplatin also elevate the expression of HIF-1α due to its role in increasing the oxidative stress which leads to cell death in addition to DNA adduct process[18].

In combination there is a significant inhibition effect due to synergistic (CI<1) effect of fixed concentration of cisplatin 31.25 µg/ml and the first four dose of artesunate as shown in Table No. 1 The combination upregulate expression of HIF-1α gene but in lesser extants as in cisplatin due to the effect of artesunate of HIF-1α as previously explain.

# CONCLUSION

Artesunate exhibits anticancer activity with moderate selectivity furthermore its decrease expression of the survival gene HIF-1α which is advantage over cisplatin in case of potency, safety and HIF-1α gene upregulation. The combination shows synergistic effect in low doses which encourage further study to use the combination as anticancer therapy.

**Author's Contribution:**

| | |
|---|---|
| Concept & Design or acquisition of analysis or interpretation of data: | Ali Abdulmuttaleb Saleh, Majid Kadhoom Abass |
| Drafting or Revising Critically: | Ali Abdulmuttaleb Saleh, Rana A. Ghaleb |
| Final Approval of version: | All the above authors |
| Agreement to accountable for all aspects of work: | All the above authors |

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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