JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2023/32092

RESEARCH ARTICLE

# Growth Inhibitory Effect of Wedelolactone in Combination with Cisplatin on PA-1 Ovarian Cancer Cell Line

**Gloria Jemmi Christobel Robinson[1,2*], Shyam Sundar Jaganathan[2], Abirami M. Padmanaban[2] and Shila Samuel[2]**

[1]Department of Biochemistry, V.V. Vanniaperumal College for Women, Virudhunagar - 626001, Tamil Nadu, India; magie.jona@gmail.com

[2]VRR Institute of Biomedical Sciences (Affiliated to University of Madras), Chennai - 600017, Tamil Nadu, India

## Abstract

Drug resistance and poor therapeutic outcomes are the emerging problems pertaining to cisplatin treatment in ovarian cancer. The effectiveness of the conventional chemotherapeutic medication could be improved by combining with natural drugs. In the current study, Wedelolactone (WDL) a natural coumestan, in combination with Cisplatin (Cis) was determined to be a potent anti-cancer drug as evidenced by their capacity to bring about cytotoxicity by decreasing NF-κB expression in PA-1 ovarian cancer cells. "Cell viability assays" were carried out and the effective combination of wedelolactone with Cisplatin were confirmed by PCR and western blot analysis. The determined $IC_{50}$ (10μM) of WDL displayed advantageous anti-cancer effect in PA-1 cells compared to Cis treatment. Furthermore, the combination of wedelolactone (5μM) and cisplatin(3μM) also down regulated NF-κB expression which is a key player of various cancer promoting events such as drug resistance, apoptotic inhibition, inflammation and angiogenesis. WDL potentiates the sensitivity of PA-1 cells towards cisplatin by decreasing the ETS1 and P-gp expression which are involved in MDR mechanism. Overall, this study suggest that Wedelolactone can be used to sensitize ovarian tumors to standard cancer chemotherapeutics.

**Keywords:** Cisplatin, Drug Resistance, ETS, NF-κB, P-gp, Wedelolactone

## 1. Introduction

Cisplatin is a mainstream platinum "chemotherapeutic" medication that are being in usage about "ovarian cancer" for about 2 decades. The identified reasons for Cisplatin treatment failure include their ability to bring about forbidding side effects and augmenting chemoresistance[1]. Multidrug resistance is a considerable challenge concerning cancer treatment. "Chemoresistance" enhances the response of genes that intervene in the survival signaling cascade and MDR genes[2]. The established mechanisms responsible for "chemoresistance" includes amplified DNA repair, reduced drug uptake, turning on "pro survival pathways", aberrant apoptotic mechanisms or turning off the mechanisms essential for promoting cell death[3]. Moreover, the side effects of cisplatin especially organ toxicity viz nephro, cardio and hepatotoxicity are of extensive menace in ovarian cancer patients that reduces its clinical advantage[4-6]. On this notion, a reasonable, more determinative and safer approach to decline the detrimental effects of cisplatin without varying its anti-cancer potency is critically imperative. For this reason, the compounds that enhance the chemotherapeutic efficacy and turn off the survival signals in cancer cells could potentially take the role of "chemosensitizer". Being a multi-targeting compound, phytochemicals have been

*Author for correspondence

Article Received on: 01.12.2022 Revised on: 19.04.2023 Accepted on: 08.05.2023

evidenced to function as emphatic chemosensitizers in fusion with mainstream chemotherapeutic medications[7].

Research exploration in the last few years has brought to light about a myriad of phytochemicals bearing the capability to reverse resistance towards cisplatin with minimal side effects in "ovarian cancer" types. For instance, there is evidence stating that thymoquinone boosts cisplatin mediated cell toxicity in ovarian cancer cells. Similarly, berberine modulated cisplatin's sensitivity through PTEN–AKT signaling. Another study reports the enhancement of cytotoxic effect in ovarian tumor cells by Saikosaponin-d in combination with cisplatin through fine tuning mitochondrial fission. A naturally occurring flavonoid, Scutellarin improves the response of ovarian tumor cell lines to cisplatin by increasing its DNA binding ability[8-11]. Among the multitude of signaling mechanisms reported in ovarian cancer, there are strong reports confirming the involvement of NF-$\kappa$B mediating chemoresistance and tumor relapse[12-15].

Being a critical mediator of major dysregulated events involved in tumor occurrence and progression, NF$\kappa$B brings about drug resistance by apoptosis inhibition and mediating MDR proteins expression[16]. The ABC transporters that act as chemotherapy "drug efflux" pump and the major contributors of "chemotherapy resistant tumors" generally includes: P-glycoprotein or MDR1, multidrug resistance-associated protein 1 and breast cancer resistance protein[17]. In ovarian cancer the foremost mechanism involved is the excessive P-glycoprotein expression, a product of ABCB1 or MDR1 gene. Suppression of P-gp to counteract MDR was noted in a scientific finding[18]. Amplified P-gp expression towards cisplatin treatment and "reduced apoptosis" due to its overexpression have been studied in HeLa cells[19]. A research finding has declared that elevated expression of MDR transporters by the stimulation of NF-κB in colon cancer cells[20]. Another key factor involved in MDR mechanism is ETS1 a transcription factor of IKKA and MDR1 which codes for P-gp[21,22]. Inordinate expression of ETS1 in ovarian cancer has been recorded[23]. Apart from MDR, apoptosis circumvention also aids in cancer advancement and resistance to cisplatin. Therefore, ripping off NF-κB mediated signaling would be an ultimate remedial strategy in ovarian cancer treatment[24,25]. Phytochemicals investigated as a significant regulator of ETS1 and P-gp could function as reassuring drug not only to reverse MDR but also to resensitize ovarian cancer cells towards cisplatin treatment. Pertaining to these findings, Wedelolactone a well-known inhibitor of IKKAα, together with cisplatin would modulate the PA-1 ovarian cancer cell proliferation and survival in combination with chemotherauptic agents. Wdl was of the interest of our study since several studies have previously proved it as an anti breast cancer agent and it has been known to target several intracellular molecules. Although there were no studies in ovarian cancer and as an adjuvant to cisplatin, we studied the association of wdl with cisplatin in reducing the dose and enhancing the cytotoxicity. Thus, our study aims to mount whether Wedelolactone, a natural coumestan could enhance the chemosensitivity of Cisplatin by down regulating NF$\kappa$B in PA-1 cells (ovarian cancer cell line) and to unveil partially the underlying chemosensitization mechanism.

## 2. Materials and Methods

### 2.1 Chemicals and Maintenance and Treatment of PA-1 Cell Line

Wedelolactone (purity ≥ 95%), Cis-dichlorodiammine platinum (II) were procured from Sigma Aldrich Co. Ltd. USA. Antibodies used were purchased from Santa Cruz Biotech., USA. PA-1 cells obtained from the National centre for cell science (Pune, India) were maintained and grown at 37 °C with 5% $CO_2$ in a humidified incubator. They were cultured as a monolayer in T25 flask to attain 70% confluence using Dulbecco's Modified Eagle's Medium, 10% fetal bovine serum and penicillin- 50 IU/mL, streptomycin -3.5µg/mL and gentamycin -2.5µg/mL. 2.5 x $10^5$/ ml per 5mL of PA-1 cells were seeded before treatment. On the drug treatment day, after washing with 2 ml of sterile PBS twice, culture medium containing the appropriate drugs (Cis, WDL) was added to the wells.

### 2.2 Cell Viability Assay

$5 \times 10^3$ cells per well in 200µL DMEM and 10% FBS Cells were seeded in 96 well plates and left overnight. Cells that were not attached were removed by mild washing after 24 hrs. Cells were then treated with varying concentrations of Wedelolactone and cisplatin. Negative control of serum free medium + DMSO was assessed. After 48 hrs of treatment, the plates were incubated with 10µL MTT solution for 3 h at 37 °C. Then the absorbance was read at 590 nm using a microplate reader (Bio-Rad, USA). Cell viability was expressed as a percentage of "untreated cells", (negative control- 100%). IC50 - concentration at which

**Table 1.** List of primers

| TARGET | FORWARD | REVERSE |
|---|---|---|
| NFkB | GAAGGAATCGTACCGGGAACA | CTCAGAGGGCCTTGTGACAGTAA |
| IKKα | GCCATGTACCCATACGATGTTCC | GCTCCAATAATCAACAGTGGCTG |
| IkBα | TGAATTCAGTCCATGGCTTGCAGGC | GGATCTCCTGCAGCTCCTTGACCAT |
| GAPDH | CCACCCATGGCAAATTCCATGGCA | TCTAGACGGCAGGTCAGGTCCTCC |

cell growth was inhibited by 50 per cent was determined from the dose response curves.

### 2.3 Trypan Blue Assay

Around $5\times10^3$ PA-1 cells per well were incubated at 37 ºC under 95% air and 5% $CO_2$ well in 96-well plates for 24 hrs. Once the confluency reached 75-80 %, they were treated with varying concentrations of Wedelolactone and cisplatin in individual or in combination for 48hrs. After incubation, viability of cells was measured using the "Trypan blue dye exclusion method". After incubation the cells were harvested and washed with PBS. Equal volumes of 10µL of cell suspension in PBS and 0.4% Trypan blue dye in equal volumes were mixed and incubated for 3 minutes at RT and loaded onto the counting slides. Cell viability and total cell were counted using the Bio Rad "TC20 automated" cell counter.

### 2.4 Semi-Quantitative Reverse Transcription PCR Analysis

Total RNA was extracted from the control and treated cells using routine Trizol method following manufacturer's protocol, and converted to cDNA using the iSCRIPT cDNA conversion kit (Bio-rad). Pre-designed primers were used for the target gene IKBα, IKKα, NFκB and GAPDH (Table 1) were procured and gene expression analysis were performed. All reactions were performed in triplicates.

### 2.5 Protein Preparation and Western Blot Analysis

Treated PA-1 ovarian cancer cells were lysed with RIPA buffer, containing the protease inhibitor cocktail and sodium orthovanadate for 30 min at 4˚C. Cell lysates were centrifuged at 4 °C for 10 min at 12,000 rpm and then protein concentrations were determined. Western blotting was carried out by separating equal concentration of proteins using SDS-PAGE and blotting onto a nitrocellulose membrane. Then the membranes were blocked in 4% BSA and antibodies for ETS1, P-gp, NFκB p65, p-NFκBp65, IKBα, p-IKBα, IKKα, p-IKKα, internal control (β-actin). Followed by blocking was the detection of specific proteins during which the membranes were incubated in a chemiluminescent substrate solution. ImageJ program was used for densitometry analysis.

### 2.6 Statistics Analysis

Data were represented as Mean ± Standard Error of the Mean (SEM). Statistical Significance was analysed using Anova and Dunnett's multiple comparison test between the groups, using Graph pad Prism7.0 software package. $P<0.05$ was considered to be statistically significant. Experiments were carried out in triplicates.

## 3. Results and Discussion

Considerable drawbacks involved in "chemotherapy treatment" are the serious toxicity and drug resistance. Numerous studies have affirmed that natural compounds with chemo preventive property could improve the ovarian cancer response rate in combination with chemotherapeutic agents both *in vitro* and *in vivo*[26-29]. Though Wedelolactone presents limited bioavailability, low doses of physiologically practicable concentrations are appropriate for its chemotherapeutic effect. We ascertained that Wedelolactone inhibited the PA-1 ovarian cancer cell proliferation, improved the cytotoxic effect of Cisplatin, reduced ETS1 and P-gp expression and also reduced cisplatin- induced hyper activation of NF κB. Similar finding was reported that curcumin enhanced the effects of paclitaxel and reversed MDR through NF-κB inhibition in SKOV3 cells[30].

Cisplatin establishes both interstand DNA and protein- DNA crosslinks to bring about growth inhibition and cell death[31]. Hindrance of cell proliferation is achieved mainly through NF-κB signaling by Cisplatin. Continuous cisplatin therapy results in accumulation of the drug with least response and NF-κB over expression which forms basis for chemoresistance[32]. Many findings

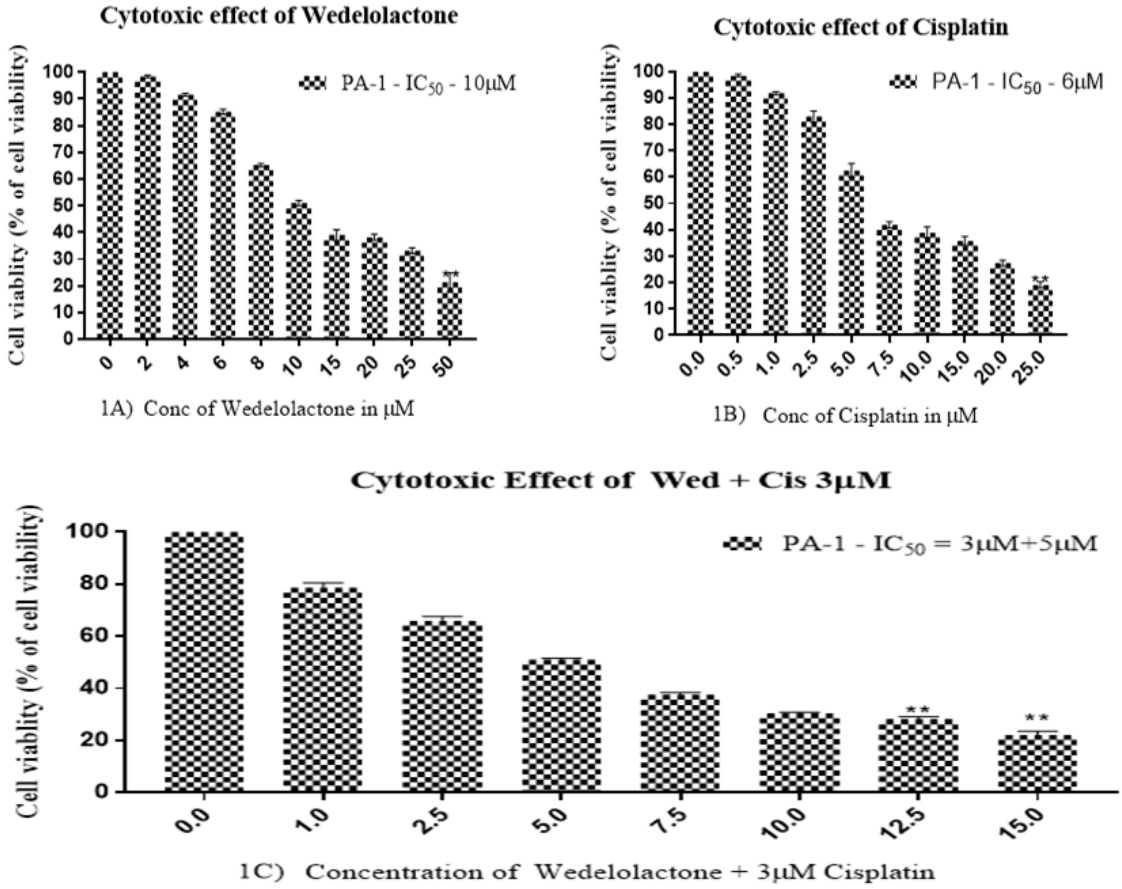


**Figure 1.** Effect of Wedelolactone and Cisplatin combination treatment on cell viability of PA-1 ovarian cancer cells **(1A)**. Depicts cell viability of wedelolactone determined by MTT assay **(1B).** Depicts cell viability of cisplatin determined by MTT assay **(1C).** Depicts the combined effect of wedelolactone and cisplatin. Data are shown as mean ± SD of three independent experiments.

have imparted the link between NF-κB and drug resistance towards "chemotherapeutic agents" in many other tumor cells[33,34]. An investigation outlined that curcumin improved the cisplatin sensitivity in KCP 4 cells by down regulating NF-κB[35]. Thus in this extensive research, Wedelolactone was found to improve the cisplatin sensitivity by downregulating NF-κB expression. The combination of WDL and Cisplatin (5 and 3 µM) exerted the anti-proliferative effect on PA-1 cells through NF-κB inhibition.

Cytotoxicity of Wedelolactone and Cisplatin against the PA-1 human cancer cell line was evaluated by MTT reduction assay. "$IC_{50}$" values for Wedelolactone and Cisplatin were determined from the concentration Vs cell survival % curves. (Figure 1) depicts the absorbance determined by "MTT" assay in cells treated with varying concentrations of Wedelolactone (10µM) and Cis (6µM). Examining cell membrane strength is the most common means to evaluate cell viability and cytotoxicity. Compounds that have cytotoxic effects often compromise cell membrane strength. Tryphan blue is normally let off from the inside membrane of healthy cells; however, if the cell membrane lose its intactness they freely cross the membrane to stain the intracellular components. The treated cells revealed enormous reduction on cell proliferation after 48 hrs treatment (Figure 2).

NF-κB being a key player of cell proliferation, determination of the expression of NF-κB and its regulators form a reasonable basis for exploring drug resistance mechanism. PA-1 cells were treated with combination of 5µM WDL and 3µM of cisplatin for 48hrs and analyzed for NF-κB, IKKα, IkBα mRNA and protein expression. Observations were then compared with control cells and individually treated cells (WDL

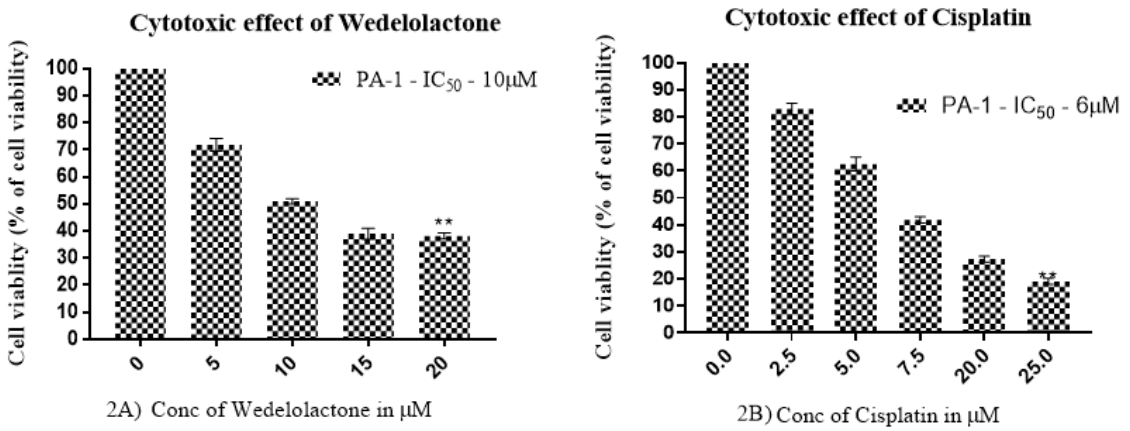


**Figure 2.** Effect of Wedelolactone and Cisplatin treatment on cell viability of PA-1 ovarian cancer cells **(2A).** Depicts cell viability of wedelolactone determined Trypan blue dye exclusion assay **(2B).** Depicts cell viability of cisplatin by Trypan blue dye exclusion assay. Data are shown as mean ± SD of three independent experiments.

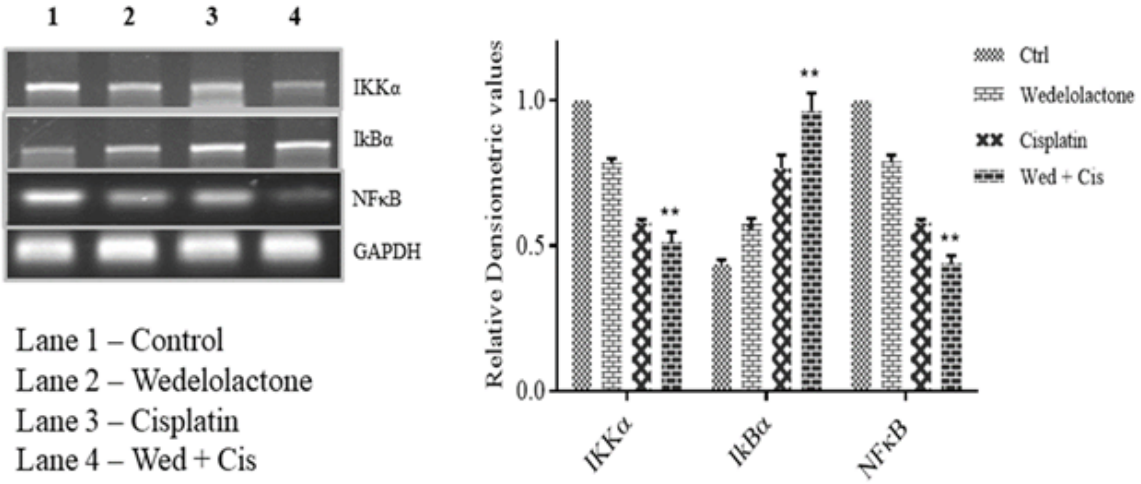


**Figure 3.** Effect of WDL+Cis combination on the mRNA expression of NFkB circuit of PA-1 cells. Densitometry images shown were the folds of induction relative to the untreated cells. Values are expressed as means ± SD. **p<0.01, compared with control.

and Cis alone). Figure 3 presents the PCR image of NF-κB, IKKα, IkBα of 4 groups and its respective densitometric data. A notable decrease in NF-κB, IKKα mRNA expression was noted in combination treatment group compared to Cisplatin and WDL alone (Figure 3). Similar results were observed in the protein expression by immunoblot (Figure 4). Increase in IkBα mRNA and protein expression was simultaneously observed in combined treatment of WDL+Cis which reveals the concept of NF-κB downregulation by inhibiting IKKα (Figure 3 and Figure 4). More significance was observed in WDL+Cis group compared to untreated. The given results were represented as mean ± SEM of triplicates.

In an endeavour to explore the drug influx into PA-1 cells, the expression of ETS1 and P-gp involved in drug resistance was assessed by western blot. In Wedelolactone-Cisplatin combination treatment decreased ETS1 and P-gp expression was observed revealing its chemosensitisation effect. Moreover, ETS1 and P-gp expression was observed more in Cisplatin treated PA-1cells than untreated cells. The densitometry data (Figure 4) clearly confirmed the

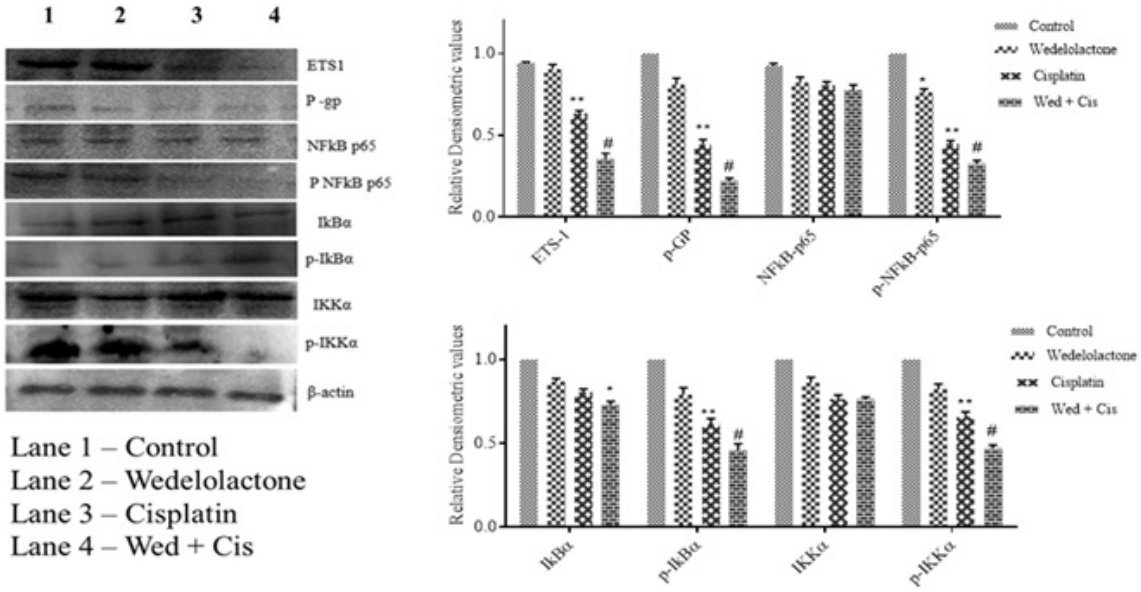


**Figure 4.** PA-1 cells treated with WDL+Cis combination exhibited decreased p-NFkB p-IKKα, Ets1, P-gp protein expression and increased expression of IkBα compared to individual treatment and untreated cells. Values are expressed as means ± SD. *p<0.05, **p<0.01, #p<0.001compared with control.

combination effect was significant on reducing ETS1 and P-gp expression than the individual Wedelolactone and Cisplatin treatment. The results therefore suggested that combination treatment could prevent the development of cisplatin resistance in ovarian cancers.

The drug resistant membrane protein, P-gp was found to be expressed to a higher extent in control and cisplatin treated PA-1 cells. This in turn is correlated with the increased expression of ETS1 which acts as a transcription factor for P-gp[36]. Accumulating evidences have revealed ETS1 over expression in drug resistant cancer cells[37,38]. Additionally, this might also contribute to the molecular basis of PA-1 drug resistance. ETS1 being a transcription factor of IKKAα could be conducive to NF-κB hyper activation[38]. The current research results indicated an ample reduction in ETS1 and P-gp protein expression in WDL- Cisplatin treated PA-1 cells highlighting its combination interaction. Expression of ETS1 and P-gp in cisplatin treated cells suggest the resistance mechanism towards "chemotherapeutic drug". Thus WDL improves the drug uptake efficacy by altering P-gp and ETS1 expression.

## 4. Conclusion

In conclusion, our study shows that Wedelolactone, a natural coumestan, significantly enhances the chemosensitisation of cisplatin by altering the MDR proteins expression in PA-1 ovarian cancer cell lines via NF-κB signalling. These futuristic results ought to be evaluated further by implementing the *in vivo* studies for strengthening the efficacy and diminishing the toxicity without affecting the normal cells. Thus, "combination therapy" with the drug of natural origin might quite possibly offer a greater therapeutic effect for the treatment of "ovarian cancer".

## 5. Acknowledgement

We gratefully acknowledge Dr.P.Rasappan, Director-Research of VRR Institute of Biomedical Science for the financial support.

## 6. References

1. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. European Journal of Pharmacology. 2014; 740:364-78. https://doi.org/10.1016/j.ejphar.2014.07.025
2. Januchowski R, Sterzyńska K, Zaorska K, Sosińska P, Klejewski A, Brązert M, Nowicki M, Zabel M. Analysis of MDR genes expression and cross-resistance in eight drug resistant ovarian cancer cell lines. Journal of Ovarian Research. 2016; 9(1):1-1. https://doi.org/10.1186/s13048-016-0278-z

3. Chen SH, Chang JY. New insights into mechanisms of cisplatin resistance: from tumor cell to microenvironment. International journal of molecular sciences. 2019; 20(17):4136. https://doi.org/10.3390/ijms20174136
4. Dasari S, Njiki S, Mbemi A, Yedjou CG, Tchounwou PB. Pharmacological effects of cisplatin combination with natural products in cancer chemotherapy. International Journal of Molecular Sciences. 2022; 23(3):1532. https://doi.org/10.3390/ijms23031532
5. Kınal ME, Tatlıpınar A, Uzun S, Keskin S, Tekdemir E, Özbeyli D, Akakın D. Investigation of astaxanthin effect on cisplatin ototoxicity in rats by using otoacoustic emission, total antioxidant capacity, and histopathological methods. Ear, Nose and Throat Journal. 2021; 100(4):NP198-205. https://doi.org/10.1177/0145561319866826
6. Dieckmann KP, Struss WJ, Budde U. Evidence for acute vascular toxicity of cisplatin-based chemotherapy in patients with germ cell tumour. Anticancer Research. 2011; 31(12):4501-5.
7. Vinod BS, Maliekal TT, Anto RJ. Phytochemicals as chemosensitizers: from molecular mechanism to clinical significance. Antioxidants and Redox Signaling. 2013; 18(11):1307-48. https://doi.org/10.1089/ars.2012.4573
8. Wilson AJ, Saskowski J, Barham W, Yull F, Khabele D. Thymoquinone enhances cisplatin-response through direct tumor effects in a syngeneic mouse model of ovarian cancer. Journal of Ovarian Research. 2015; 8(1):1-0. https://doi.org/10.1186/s13048-015-0177-8
9. Tsuyoshi H, Wong VK, Han Y, Orisaka M, Yoshida Y, Tsang BK. Saikosaponin-d, a calcium mobilizing agent, sensitizes chemoresistant ovarian cancer cells to cisplatin-induced apoptosis by facilitating mitochondrial fission and G2/M arrest. Oncotarget. 2017; 8(59):99825. https://doi.org/10.18632/oncotarget.21076
10. Chen Q, Qin R, Fang Y, Li H. Berberine sensitizes human ovarian cancer cells to cisplatin through miR-93/PTEN/Akt signaling pathway. Cellular physiology and biochemistry. 2015; 36(3):956-65. https://doi.org/10.1159/000430270
11. Xie Z, Guo Z, Lei J, Yu J. Scutellarin synergistically enhances cisplatin effect against ovarian cancer cells through enhancing the ability of cisplatin binding to DNA. European Journal of Pharmacology. 2019; 844:9-16. https://doi.org/10.1016/j.ejphar.2018.11.040
12. Shen H, Liao B, Wan Z, Zhao Y, You Z, Liu J, Lan J, He S. PTOV1 promotes cisplatin-induced chemotherapy resistance by activating the nuclear factor kappa B pathway in ovarian cancer. Molecular Therapy-Oncolytics. 2021; 20:499-507. https://doi.org/10.1016/j.omto.2021.02.008
13. Zhang L, Chinnathambi A, Alharbi SA, Veeraraghavan VP, Mohan SK, Zhang G. Punicalagin promotes the apoptosis in human cervical cancer (ME-180) cells through mitochondrial pathway and by inhibiting the NF-kB signaling pathway. Saudi Journal of Biological Sciences. 2020; 27(4):1100-6. https://doi.org/10.1016/j.sjbs.2020.02.015
14. Liu YK, Jia YJ, Liu SH, Ma J. FSTL1 increases cisplatin sensitivity in epithelial ovarian cancer cells by inhibition of NF-κB pathway. Cancer Chemotherapy and Pharmacology. 2021; 87(3):405-14. https://doi.org/10.1007/s00280-020-04215-9
15. Motohara T, Yoshida GJ, Katabuchi H. The hallmarks of ovarian cancer stem cells and niches: Exploring their harmonious interplay in therapy resistance. In Seminars in Cancer Biology. Academic Press. 2021; 77:182-193. https://doi.org/10.1016/j.semcancer.2021.03.038
16. Neophytou CM, Trougakos IP, Erin N, Papageorgis P. Apoptosis deregulation and the development of cancer multi-drug resistance. Cancers. 2021; 13(17):4363. https://doi.org/10.3390/cancers13174363
17. Wind NS, Holen I. Multidrug resistance in breast cancer: from *in vitro* models to clinical studies. International Journal of Breast Cancer. 2011. https://doi.org/10.4061/2011/967419
18. Vaidyanathan A, Sawers L, Gannon AL, Chakravarty P, Scott AL, Bray SE, Ferguson MJ, Smith G. ABCB1 (MDR1) induction defines a common resistance mechanism in paclitaxel-and olaparib-resistant ovarian cancer cells. British Journal of Cancer. 2016; 115(4):431-41. https://doi.org/10.1038/bjc.2016.203
19. Takara K, Obata Y, Yoshikawa E, Kitada N, Sakaeda T, Ohnishi N, Yokoyama T. Molecular changes to HeLa cells on continuous exposure to cisplatin or paclitaxel. Cancer Chemotherapy and Pharmacology. 2006; 58(6):785-93. https://doi.org/10.1007/s00280-006-0226-5
20. Bentires-Alj M, Barbu V, Fillet M, Chariot A, Relic B, Jacobs N, Gielen J, Merville MP, Bours V. NF-κB transcription factor induces drug resistance through MDR1 expression in cancer cells. Oncogene. 2003; 22(1):90-7. https://doi.org/10.1038/sj.onc.1206056
21. Zhang Y, Wu J, Ye M, Wang B, Sheng J, Shi B, Chen H. ETS1 is associated with cisplatin resistance through IKKα/NF-κB pathway in cell line MDA-MB-231. Cancer Cell International. 2018; 18(1):1-2. https://doi.org/10.1186/s12935-018-0581-4
22. Gu L, Zhu N, Findley HW, Woods WG, Zhou M. Identification and characterization of the

IKKα promoter: positive and negative regulation by ETS-1 and p53, respectively. Journal of Biological Chemistry. 2004; 279(50):52141-9. https://doi.org/10.1074/jbc.M407915200
23. Takai NO, Miyazaki TA, Nishida MA, Nasu KA, Miyakawa IS. c-Ets1 is a promising marker in epithelial ovarian cancer. International Journal of Molecular Medicine. 2002; 9(3):287-92. https://doi.org/10.3892/ijmm.9.3.287
24. Alvero AB. Recent insights into the role of NF-kappaB in ovarian carcinogenesis. Genome Medicine. 2010; 2(8):1-3. https://doi.org/10.1186/gm177
25. Pundir M, Sharma A, Kumar J. Phytochemicals used as inhibitors in the treatment of ovarian cancer: A Mini-review. Materials Today: Proceedings. 2021. https://doi.org/10.1016/j.matpr.2021.09.505
26. Ghoneum A, Gonzalez D, Afify H, Shu J, Hegarty A, Adisa J, Kelly M, Lentz S, Salsbury F, Said N. Compound C Inhibits Ovarian Cancer Progression via PI3K-AKT-mTOR-NFκB Pathway. Cancers. 2022; 14(20):5099. https://doi.org/10.3390/cancers14205099
27. Ma L, Zhang M, Zhao R, Wang D, Ma Y, Ai L. Plant natural products: promising resources for cancer chemoprevention. Molecules. 2021; 26(4):933. https://doi.org/10.3390/molecules26040933
28. Siddiqui AJ, Jahan S, Singh R, Saxena J, Ashraf SA, Khan A, Choudhary RK, Balakrishnan S, Badraoui R, Bardakci F, Adnan M. Plants in anticancer drug discovery: from molecular mechanism to chemoprevention. BioMed Research International. 2022. https://doi.org/10.1155/2022/5425485
29. Ali Abdalla YO, Subramaniam B, Nyamathulla S, Shamsuddin N, Arshad NM, Mun KS, Awang K, Nagoor NH. Natural Products for Cancer Therapy: A Review of Their Mechanism of Actions and Toxicity in the Past Decade. Journal of Tropical Medicine. 2022. https://doi.org/10.1155/2022/5794350
30. Rocha CR, Silva MM, Quinet A, Cabral-Neto JB, Menck CF. DNA repair pathways and cisplatin resistance: an intimate relationship. Clinics. 2018; 73. https://doi.org/10.6061/clinics/2018/e478s
31. Pfister DG, Spencer S, Adelstein D, Adkins D, Anzai Y, Brizel DM, Bruce JY, Busse PM, Caudell JJ, Cmelak AJ, Colevas AD. Head and neck cancers, version 2.2020, NCCN clinical practice guidelines in oncology. Journal of the National Comprehensive Cancer Network. 2020; 873-98. https://doi.org/10.6004/jnccn.2020.0031
32. Mahapatra MK, Mandal CC. Natural Extracts Target NF-κB and Reactive Oxygen Species: Molecular Insights into Therapy Resistance and Toxicity. In Handbook of Oxidative Stress in Cancer: Therapeutic Aspects. Singapore: Springer Singapore. 2022; 1-28. https://doi.org/10.1007/978-981-16-1247-3_32-1
33. Barr MP, Gray SG, Hoffmann AC, Hilger RA, Thomale J, O'Flaherty JD, Fennell DA, Richard D, O'Leary JJ, O'Byrne KJ. Generation and characterization of cisplatin-resistant non-small cell lung cancer cell lines displaying a stem-like signature. PloS one. 2013; 8(1):e54193. https://doi.org/10.1371/journal.pone.0054193
34. Marinello PC, Panis C, Silva TN, Binato R, Abdelhay E, Rodrigues JA, Mencalha AL, Lopes NM, Luiz RC, Cecchini R, Cecchini AL. Metformin prevention of doxorubicin resistance in MCF-7 and MDA-MB-231 involves oxidative stress generation and modulation of cell adaptation genes. Scientific Reports. 2019; 9(1):1-1. https://doi.org/10.1038/s41598-019-42357-w
35. Oiso S, Ikeda R, Nakamura K, Takeda Y, Akiyama SI, Kariyazono H. Involvement of NF-κB activation in the cisplatin resistance of human epidermoid carcinoma KCP-4 cells. Oncology Reports. 2012; 28(1):27-32. https://doi.org/10.3892/or.2012.1801
36. Kato T, Fujita Y, Nakane K, Kojima T, Nozawa Y, Deguchi T, Ito M. ETS1 promotes chemoresistance and invasion of paclitaxel-resistant, hormone-refractory PC3 prostate cancer cells by up-regulating MDR1 and MMP9 expression. Biochemical and Biophysical Research Communications. 2012; 417(3):966-71. https://doi.org/10.1016/j.bbrc.2011.12.047
37. Vishnoi K, Ke R, Viswakarma N, Srivastava P, Kumar S, Das S, Singh SK, Principe DR, Rana A, Rana B. Ets1 mediates sorafenib resistance by regulating mitochondrial ROS pathway in hepatocellular carcinoma. Cell Death and Disease. 2022; 13(7):1-6. https://doi.org/10.1038/s41419-022-05022-1
38. Sakamoto K, Endo K, Sakamoto K, Kayamori K, Ehata S, Ichikawa J, Ando T, Nakamura R, Kimura Y, Yoshizawa K, Masuyama K. EHF suppresses cancer progression by inhibiting ETS1-mediated ZEB expression. Oncogenesis. 2021; 10(3):1-5. https://doi.org/10.1038/s41389-021-00313-2