



Research article

Combination of coumarin and doxorubicin induces drug-resistant acute myeloid leukemia cell death

Nouf S. Al-Abbas^{a,*}, Nehad A. Shaer^b^a Biology Department, Juumum College University, Umm Alqura University, Saudi Arabia^b Chemistry Department, Umm Al-Qura University, Saudi Arabia

ARTICLE INFO

Keywords:

Anticancer drugs
Apoptosis
Natural medicine
FACS assay
Cell viability

ABSTRACT

Background: Chemotherapy remains to be the method of choice used by clinicians to treat acute myeloid leukemia (AML) patients. However, the most common problem usually faced in the course of treatment is multidrug resistance (MDR). Nowadays, combination therapy involving natural products as adjuvant therapy to chemotherapy and radiotherapy has been used for many of health problems. Coumarin is a natural compound with known chemotherapeutic activity, as well as other pharmacological properties. We focused on the combination of coumarin and doxorubicin in overcoming of drug-resistance in acute myeloid leukemia.

Methods: Cell viability, Apoptotic and necrotic cell death with FACS, oxidative stress detection, and protein expression analysis were used in this study.

Results: Coumarin as a single drug exerts a significant cell death on Human acute myeloid leukemia (HL60); however, it does not show the same effect on drug-resistant acute myeloid leukemia (HL60/ADR). Comparing the effects of doxorubicin and coumarin as single drugs versus a combination of coumarin and doxorubicin showed a significant apoptotic cell death.

Conclusion: In AML patients, the development of multiple drug resistance (MDR) is the biggest challenge in treating AML patients. Combination therapy with coumarin may be a good choice to overcome the drug resistance in AML patients.

1. Introduction

Cancer is a major cause of death worldwide, according to World Health Organization (WHO) reports. More than 13 million cancer patients will die by 2030 [1,2]. It was reported that 20% of the people would suffer from some form of cancer before being 75 years old [3]. Despite the number of clinical trials carried out and advances made in diagnosis and treatment, some cancer patients do not respond normally to the established cancer treatments. However, chemotherapy remains to be the method of choices in treating cancer patients. Drug resistance is considered the main obstacle for many of the anticancer agents, which subsequently requires the administration of higher doses of the chemotherapeutic agents to overcome drug resistance [4, 5]. Acute myeloid leukemia (AML) or acute non-lymphocytic leukemia is a complicated disease associated with abnormal differentiation and high cellular proliferative rate of hematopoietic stem cells [6]. Clinical drug resistance is generally recognized as being conferred by one of the following aspects: cellular drug resistance, leukemia regrowth, and drug pharmacokinetics

[7, 8, 9, 10]. Previously published results treated certain cell lines with drugs that possess cytotoxic effect. These agents include actinomycin, colchicine, and Adriamycin. However, cell lines tested developed resistance to those agents [11, 12]. The mechanism by which cancerous cells develop multidrug resistance is because of less presence of the drug intracellularly after prolonged administration, which subsequently allows the cells to develop a better efflux system for these molecules [13]. ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp) and MRP1 mediate the active drug efflux system [14, 15].

Natural medicine is one of the most commonly used complementary and alternative therapies by cancer patients [16, 17]. Coumarin (1, 2-benzopyrone) is a natural drug found in many resources as a higher plant, like Umbelliferae and Rutaceae, some essential oils like cassia leaf oil, cinnamon bark oil, and lavender oil are also rich in microorganisms as well, like novobiocin and coumermycin from *Streptomyces* and aflatoxins from *Aspergillus* species but also synthetic drug [18, 19]. Coumarin has a wide spectrum of biological effects as anti-inflammatory, antioxidant, antinociceptive, anti-cancer, antiasthmatic, antidepressant,

* Corresponding author.

E-mail address: nsabbas@uqu.edu.sa (N.S. Al-Abbas).

antituberculosis, anti- Alzheimer, and antihyperlipidemic [20, 21, 22, 23, 24, 25]. The anticancer activities of coumarins are well established; coumarin acts as a kinase inhibitor, suppresses cancer cell proliferation via cell cycle arrest, and exerts an antimitotic activity and affecting the P-gp of the cancer cell. It also inhibits angiogenesis, heat shock protein 90 (Hsp90), telomerase, carbonic anhydrase, monocarboxylate transporters, aromatase, and sulfatase [26, 27, 28].

2. Material and methods

2.1. Cell lines

Both cell lines used (Acute human myeloid leukemia (AML) HL60 and HL60/ADR) were purchased from ATCC (American Tissue Culture Collection). The two cell lines were cultured in RPMI cell culture medium

and supplemented with 10% inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotic solution. Incubation conditions were fixed at 37 °C and 5% CO₂ in humid conditions for the following experiments. In all experiments, the control plates were given the complete growth culture media only and subsequently under normal culture conditions, cells are able to divide and increase in number.

2.2. Cell viability assay

To study the cell viability of HL60 and HL60/ADR cells in coumarin and doxorubicin treatment, both HL60 and HL60/ADR cells were cultured in RPMI provided with 10% FBS and 1% penicillin/streptomycin. The initial cell number (4×10^5 cell/mL) and treated with (100, 250, 500, and 1000 µg/mL coumarin) with or without 100 ng/mL doxorubicin and incubated for 24 h, positive and negative controls (100

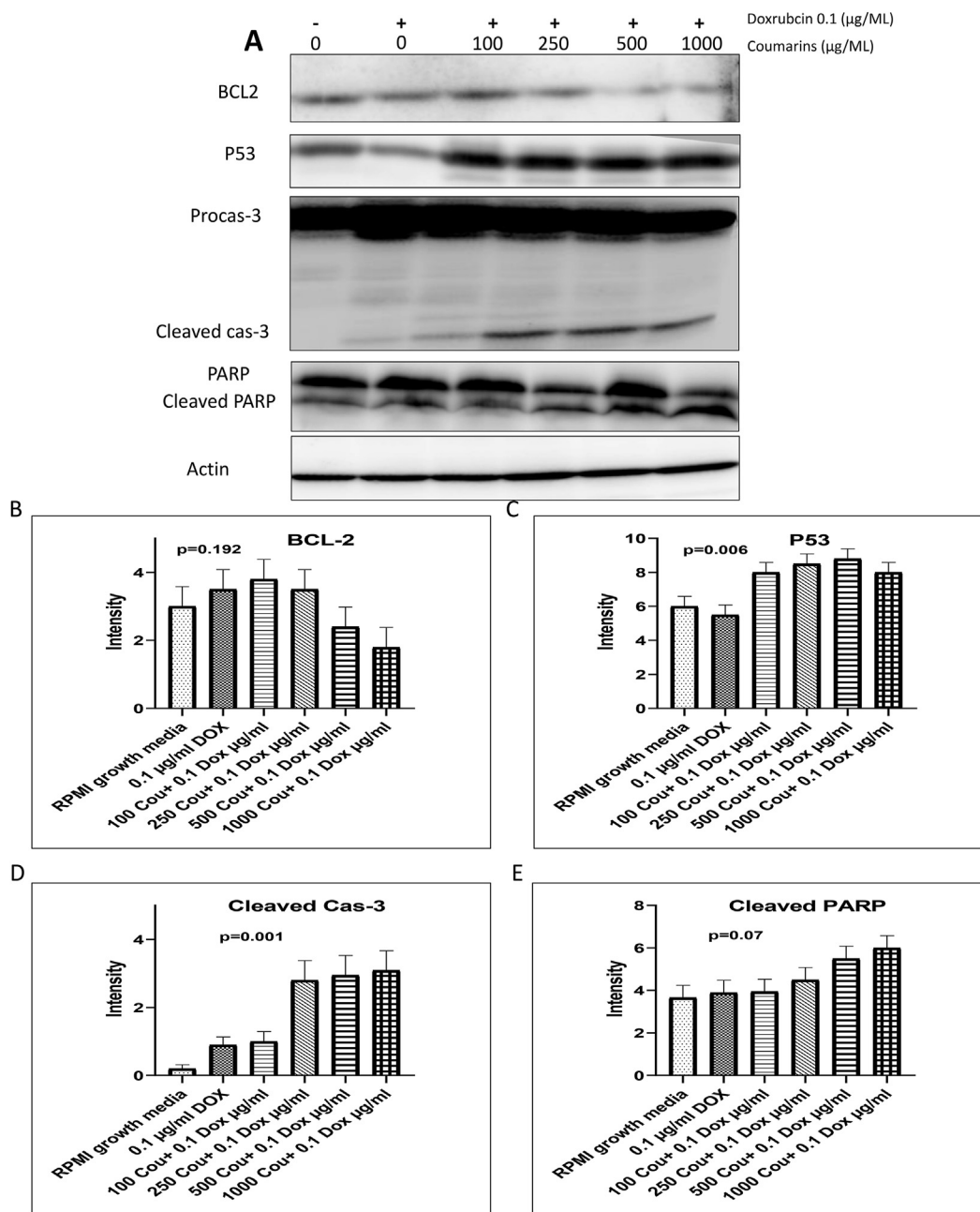


Figure 1. Western blotting of Cas-3, P53, PARP and Actin in HL60/ADR after co-treatment of 0.1 µg/ml of doxorubicin with (100, 250, 500, and 1000 µg/ml) coumarin) and (B-E) a quantification of western blot data using Image J software.

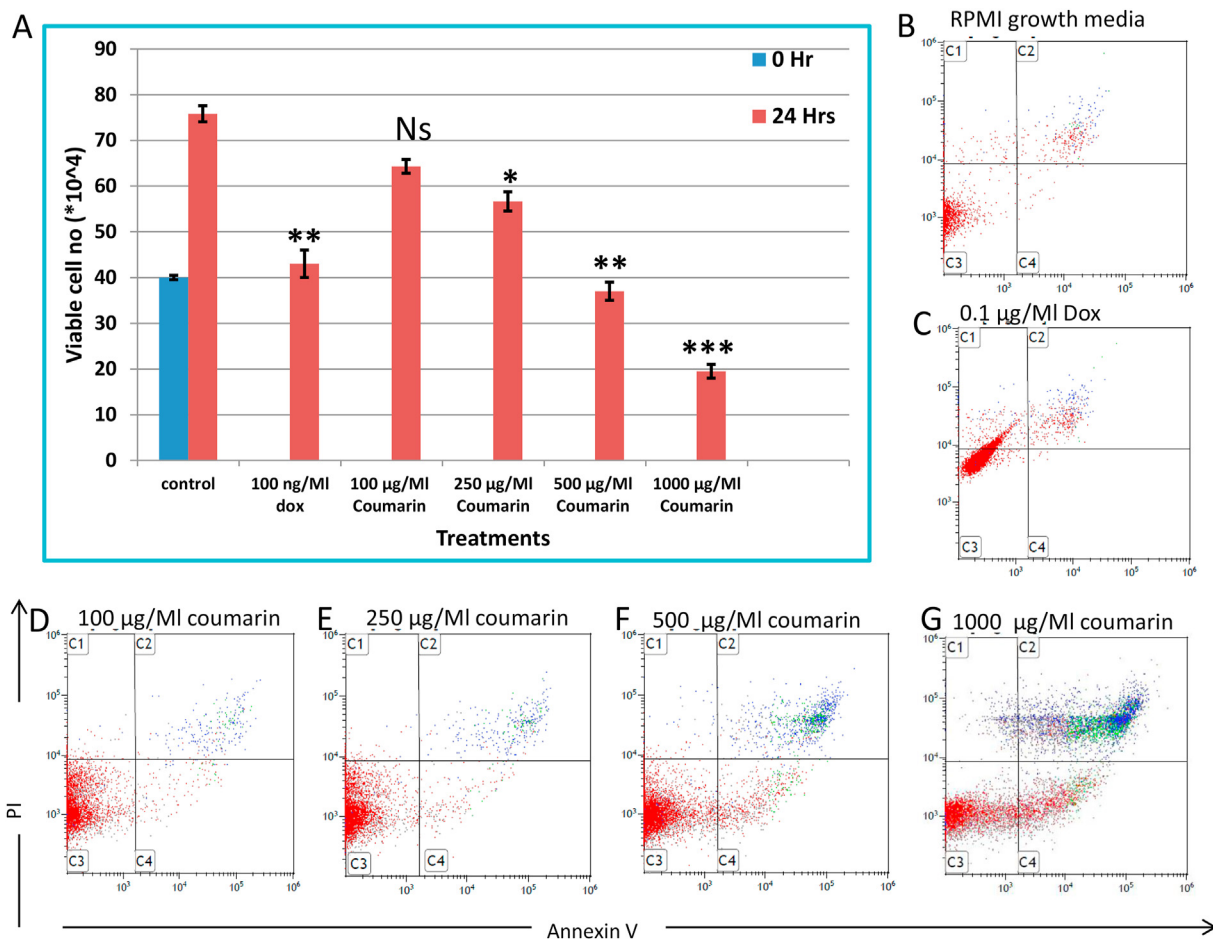


Figure 2. (A) cytotoxic effect of coumarin and doxorubicin treatment on HL60 cells using trypan blue exclusion dye. Apoptotic and necrotic cell death were assessed in HL60 using Annexin V and PI staining and analyzed using flow cytometer in (B) negative control, (C) treated with 0.1 µg/ml of doxorubicin as a positive control, and (D-G) treated with (100, 250, 500, and 1000 µg/ml) coumarin.

ng/mL doxorubicin and normal growth media), respectively were involved. Viable cells were detected by trypan blue dye exclusion method.

2.3. Apoptosis and necrosis using FACS analysis

The apoptotic and necrotic cell death by coumarin and doxorubicin were measured using Annexin V and 7AAD. Briefly, each cell line (HL60 or HL60/ADR) was cultured in 10 cell culture dishes with an initial seeding count of 4×10^5 cell/mL in RPMI growth media. Cells were treated with (100, 250, 500 and 1000 µg/mL coumarin) with or without 100 ng/mL doxorubicin and incubated for 24 h, positive and negative control (100 ng/mL doxorubicin and normal growth media) were involved. Then, the cells washed once with PBS, suspended in 100 µL 1X Annexin V binding buffer, add 5 µL FITC Annexin V. Cells were allowed to react at room temperature for 15 min. After the allowed time, 5 µL of 7AAD were added to each tube. Finally, 400 µL of 1X Annexin V binding buffer were added to each tube and analyzed by flow cytometer.

2.4. ROS detection

HL60/ADR cells were cultured in RPMI growth medium containing (100, 250, 500, and 1000 µg/mL coumarin) with or without 100 ng/mL doxorubicin and incubated for 24 h. Positive and negative controls (100 ng/mL doxorubicin and normal growth media) were involved and ROS inducer (pyocyanin) as a positive control was involved at a concentration of 500 µM for 30 min. After treatment,

cells were centrifuged and suspended in 200 µL of ROS detection Mix (ROS-ID® Hypoxia/Oxidative Stress Detection Kit, ENZ-51042-K500) for 30 min at normal cell culture conditions. After washing twice with PBS, cells were attached to the glass slides using the cytopsin, and Images were obtained with confocal microscopy using 490/525 nm filter and µM scale bar.

2.5. Western blot analysis

The effect of combining coumarin and doxorubicin on the apoptotic cell death was investigated in HL60/ADR cells using Western blot analysis (Figure 1 A). The cells were treated with (100, 250, 500, and 1000 µg/mL) concentrations of coumarin combined with doxorubicin at 100 ng/mL for 24 h. After treatment, cells were harvested in a proper amount of RIPA buffer, collected in Eppendorf tubes, and left for 10 min on ice with regular vortexing. Then the tubes were centrifuged at 12000 rpm for 20 min at 4 °C. The supernatants were transferred to another Eppendorf and kept at -80 °C to be used for protein electrophoresis using 30 µg protein from each sample. Protein samples were loaded in SDS-PAGE and the proteins were transferred to nitrocellulose membranes. Membranes were processed for blocking and incubated overnight in 4 °C with anti *Bcl-2*, *p53*, Caspase-3, PARP, and actin (cell signal 1:1000) primary antibody and then incubated with the proper secondary antibody for 1 h at 25 °C. Finally, the proteins were visualized by enhanced chemiluminescence luminal (ECL) solution. The membranes were then scanned on C-Digits scanner using Image Studio Digits version 3.1.

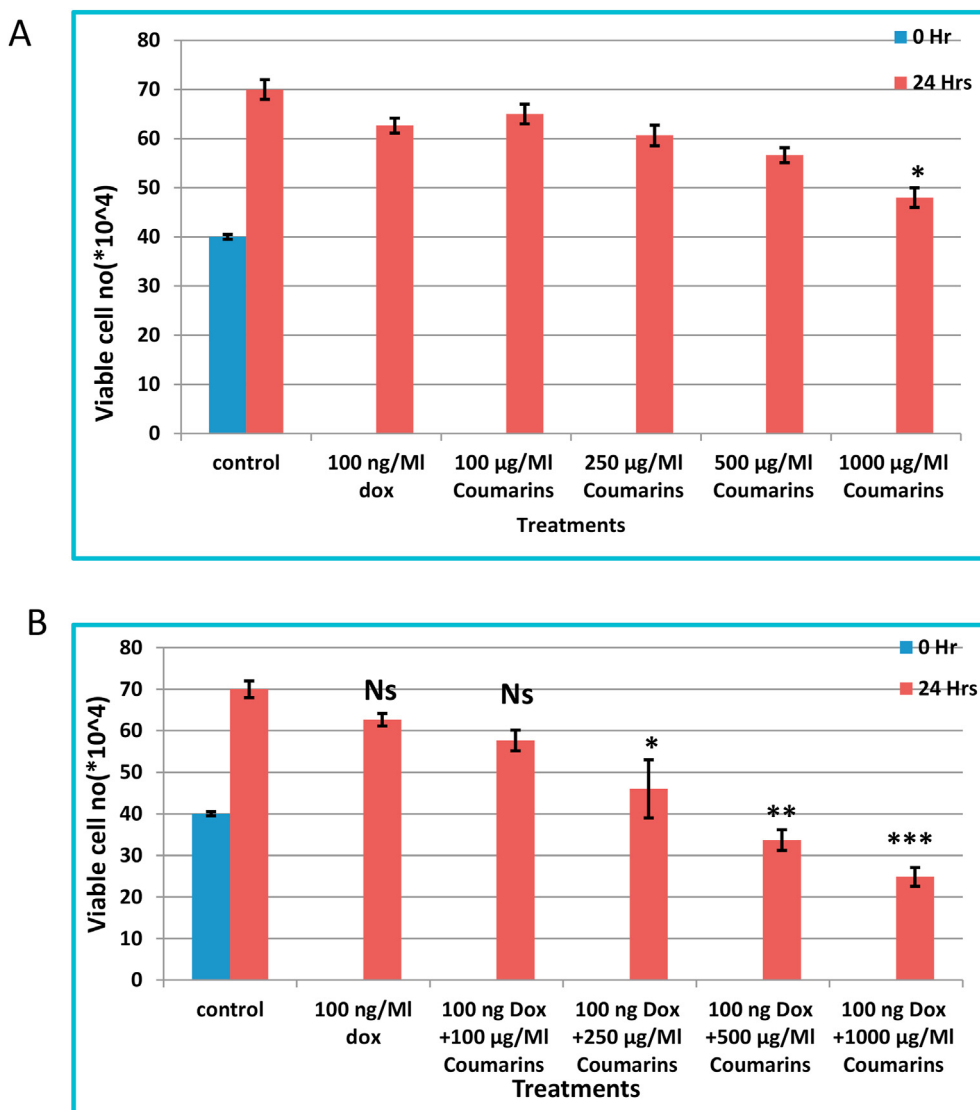


Figure 3. Cell viability was assessed in HL60/ADR cells using trypan blue exclusion dye; (A) treated with different coumarin concentrations and (B) co-treated with 100 ng/ml doxorubicin with different coumarin concentrations.

2.6. Statistical analysis

The quantitative data were expressed as averages \pm SD. Data were then plotted on graphs and analyzed statistically. Statistical differences were investigated using One-way analysis of variance (ANOVA) followed by Student's t-test.

3. Results

3.1. Coumarin induces HL60 cell death

Cell viability was investigated in HL60 under different concentrations of coumarin (0, 100, 250, 500, and 1000 μ g/mL), using trypan blue assay after 24 h. Quantitatively, it was shown that HL60 cells exert significant cell death with (250, 500, and 1000 μ g/mL) with $P < 0.05$, 0.01, and 0.001, respectively; comparing with 100 ng/mL ($P < 0.01$) as a positive control as shown in Figure 2A. Analyzing cell viability, FACS results confirm HL60 cell death under the same experimental conditions using Annexin V and 7AAD as in Figure 2 B–G.

3.2. The combination of coumarin and doxorubicin show marked cell death in drug-resistant acute myeloid leukemia

Cell death was investigated in HL60/ADR cells under different concentrations of coumarin (0, 100, 250, 500, and 1000 μ g/mL) with or without doxorubicin (100 ng/mL) using trypan blue exclusion method after 24 h of treatment. Quantitatively, coumarin as a single drug show significant reduction in cell viability at 1000 μ g/mL ($P < 0.05$) as shown in Figure 3A, while it was shown that HL60/ADR cells exert significant cell death with (250, 500, and 1000 μ g/mL) with $P < 0.05$, 0.01, and 0.001, respectively, comparing with 100 ng/mL ($P < 0.01$) as a positive control as shown in Figure 3B.

3.3. Apoptotic cell death in drug-resistant acute myeloid leukemia was observed with a combination of coumarin and doxorubicin

Consistent with cell viability results, flow cytometry analysis with Annexin V and 7AAD of HL60/ADR treated with coumarin (0, 100, 250, 500, and 1000 μ g/mL) with or without doxorubicin (100 ng/mL) reveal that coumarin as a single drug show high apoptotic cell only at 1000 μ g/

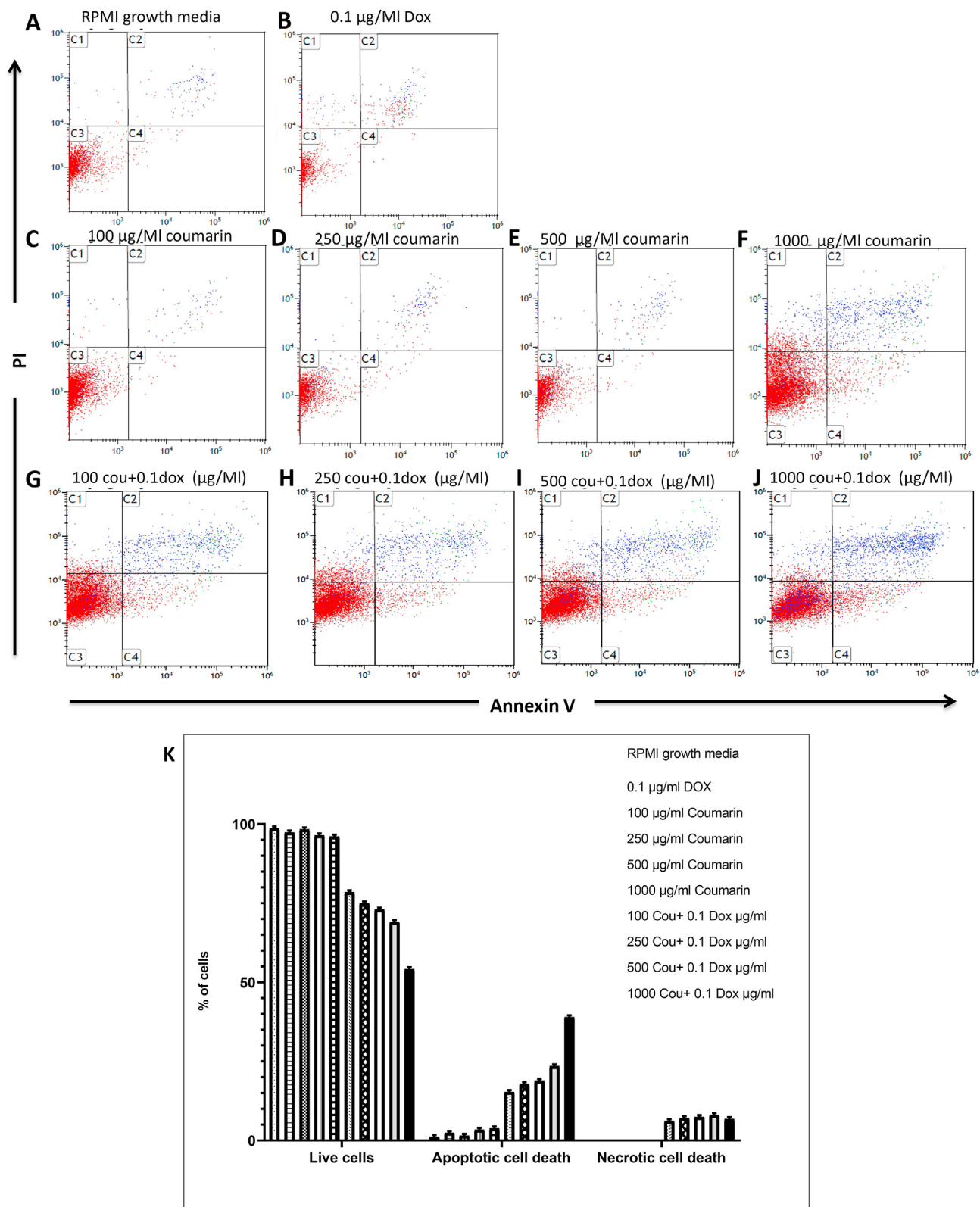


Figure 4. Apoptotic and necrotic cell death were assessed in HL60/ADR cells using Annexin V and PI staining and analyzed using flow cytometer in (B) (negative control), (C-F) HL60/ADR cells treated with (100, 250, 500, and 1000 µg/ml) coumarin , and (G-J) combination of 0.1 µg/ml of doxorubicin with (100, 250, 500, and 1000 µg/ml) coumarin and (K) quantification of Flowcytometry results.

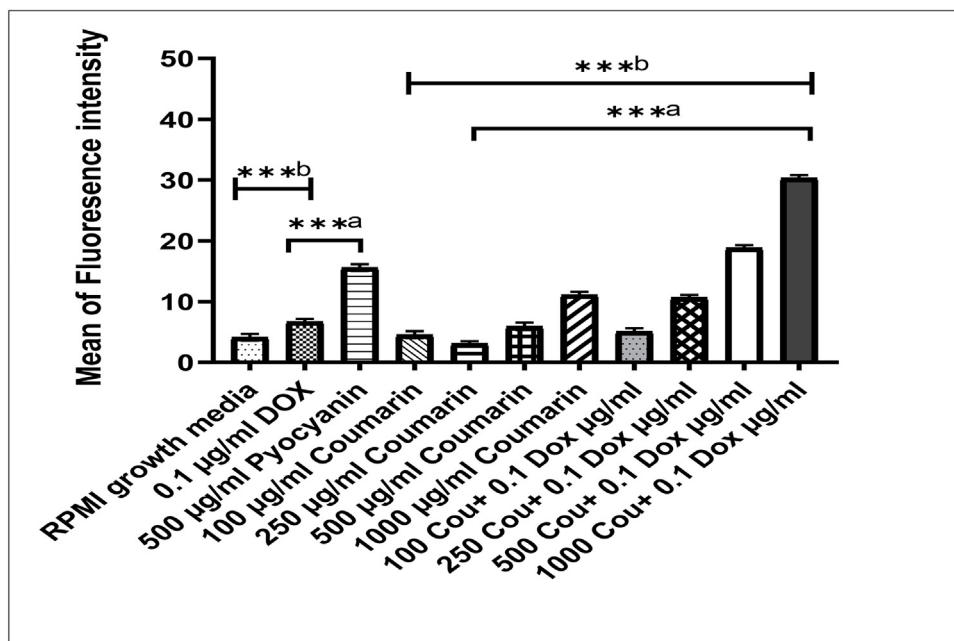
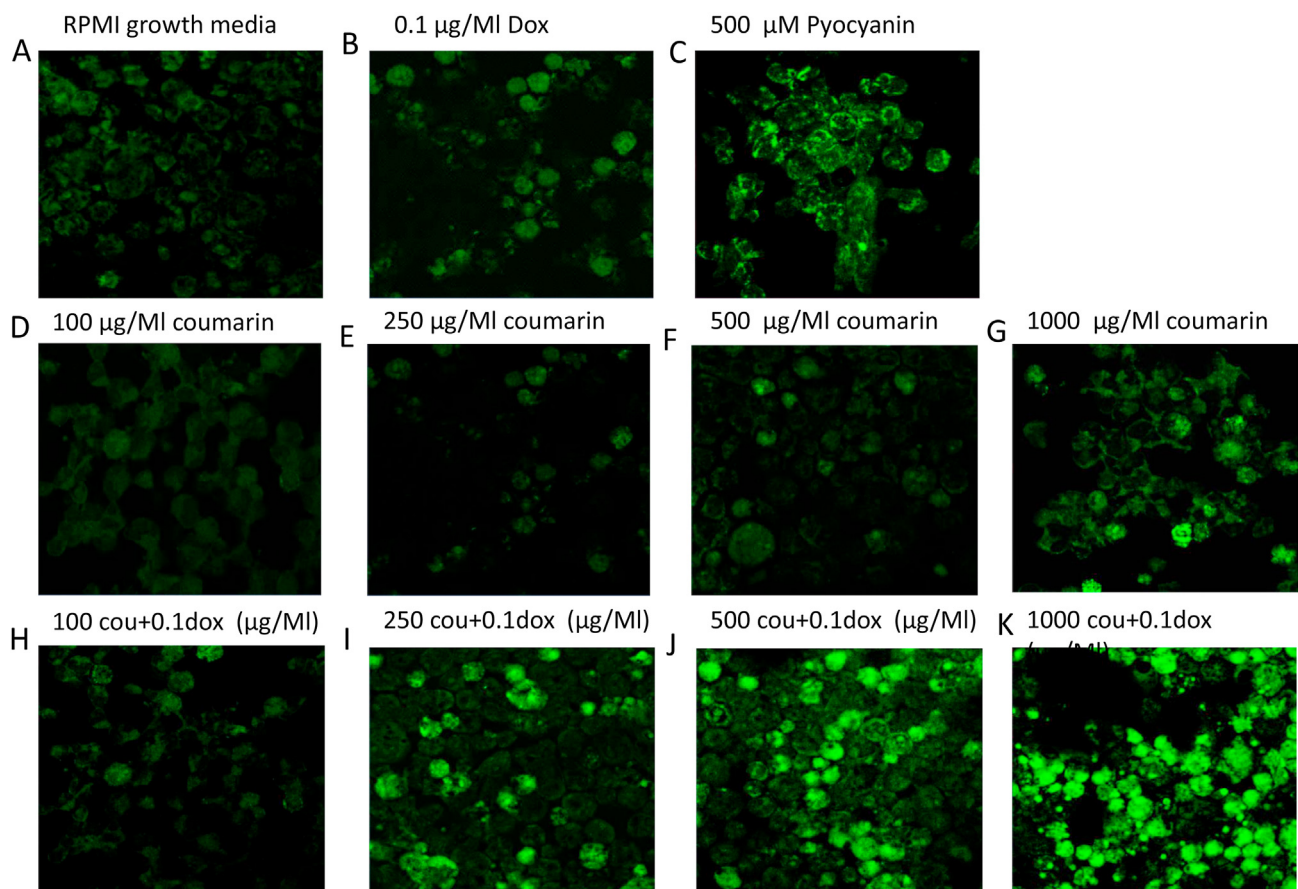


Figure 5. Confocal imaging of ROS in HL60/ADR after treatment with; (A) negative control, (B) 0.1 µg/ml of doxorubicin, (C) 500 µM Pyocyanin, (D-G) treated with (100, 250, 500, and 1000 µg/ml) coumarin, and (H-K) combination of 0.1 µg/ml of doxorubicin with (100, 250, 500, and 1000 µg/ml) coumarin and (L) quantification of the intensity of fluorescence using imge J software.

mL ($P < 0.001$), while a similar trend of markedly apoptotic cell death with (100, 250, 500, and 1000 $\mu\text{g/mL}$) comparing with 100 ng/mL as a positive control and RPMI normal growth media as shown in Figure 4(A–J).

3.4. ROS generation considerably increased with the combination of doxorubicin (100 ng/mL) with the increasing concentration of coumarin

Increasing the level of oxidative stress was markedly observed with the combination of coumarin and doxorubicin through an increase of the ROS signal. The ROS signal was dramatically increased with coumarin concentration (100, 250, 500, 1000 $\mu\text{g/mL}$) with doxorubicin (100 ng/mL) versus treatment of coumarin alone. The ROS signal of the combination treatment was also high comparing with the positive ROS inducer (500 μM pyocyanin) as shown in Figure 5 (A–K).

3.5. Coumarin and doxorubicin repressed *Bcl-2* and increased *p53*, cleaved *casp-3*, and *PARP* protein expressions

Western blot observations demonstrated that there is a significant decrease in the expression of apoptotic inhibitor protein *Bcl-2* with the combination of coumarin (500 and 1000 $\mu\text{g/mL}$) with 100 ng/mL doxorubicin. On the contrary, the combinations of coumarin (100, 250, 500 and 1000 $\mu\text{g/mL}$) with 100 ng/mL doxorubicin produced a significant increment of apoptotic-induced protein *p53*. Cleaved caspase-3 and cleaved PARP were markedly observed with the combination of coumarin (250, 500 and 1000 $\mu\text{g/mL}$) with 100 ng/mL doxorubicin.

4. Discussion

Acute myeloid leukemia (AML) as a clonal disorder is characterized by the increase of an immature, abnormally differentiated myeloid cell population in the bone marrow and peripheral blood. AML is the most common acute leukemia in adults aged above 45 years [29]. About one-third of the AML patients do not attain a complete remission with conventional chemotherapy treatment developed drug-resistant AML, which is considered a highly heterogeneous disease showing aggressive behavior toward all of the normal strategies of treatment therapy [29, 30]. Natural compounds and their derivatives as enhancer drugs play an important role in cancer treatment. Natural compounds and/or their derivatives mainly contribute with 60% of therapeutic drugs compositions used in cancer treatment [31]. In addition to the high cytotoxic effect of the chemotherapeutic agents towards the normal rapidly proliferating cells, drug resistance is the main obstacle in blood cancer treatment. In order to mitigate the side effects and drug resistance, modified therapeutic regimens such as combination therapy have been introduced [32, 33, 34]. Therefore, our research focused on the combination of coumarin as adjuvant natural therapy with doxorubicin to study the enhancement role of coumarin in overcoming doxorubicin resistance in AML patients. Coumarin and its derivatives have been reported by many studies to show cell cycle arrest, inhibit cell proliferation, and induce apoptotic cell death in chronic myeloid leukemia and histiocytic lymphoma [35, 36]. Inconsistent with the previous studies, our cell viability results show the high cytotoxic effect of coumarin on HL60 cells at (250, 500, 1000 $\mu\text{g/mL}$) with $P < 0.05$, 0.01, 0.001, respectively; in comparing with doxorubicin (100 ng/mL), with $P < 0.01$. In parallel with cytotoxicity results, FACS analysis with Annexin V and 7AAD show that coumarin at 500, 1000 $\mu\text{g/mL}$ exert marked apoptotic cell death.

Drug resistance is one of the main problems in AML treatment. In the AML patients, the occurrence of resistance to chemotherapeutic agents is called multidrug resistance (MDR). The HL60/ADR is known to be resistant to many chemotherapeutic agents used solely. Many research articles have used combination of chemotherapeutic agents to gain the benefit of synergistic effects of both agents used together [37, 38]. In a previous research [37], high doses of Adriamycin were used in combination to overcome the multiple drug resistance in HL60 cells. MDR is

caused by multidrug resistance protein1 (MDR1) gene product, known as P-glycoprotein (P-gp) which is an important ATP-dependent efflux pump of the cell membrane that pumps many drugs out of cells [39]. Treatment failure in AML patients and the generation of cellular resistance to a wide variety of anti-AML drugs is due to overexpression of P-gp [40]. DOX is one of the most potent intracellular ROS inducer anti-cancer drugs and causes acute cardiac arrhythmias and chronic cumulative cardiomyopathy as a side effect [41, 42, 43, 44]. Inconsistent to cell death results, intracellular oxidative stress increased by ROS generation, which increased with the combination of doxorubicin and coumarin and it was interestingly observed that the ROS induction was increased by an increase of coumarin concentration, while single treatment of coumarin exhibits no significant change in ROS generation compared with positive and negative controls. ROS are mediators of cell death and caspase-dependent apoptosis inducers [45]. Both DOX and coumarin have no significant cell death effect on adriamycin-resistant human acute myeloid leukemia (HL60/ADR) compared with HL60 cells as shown in cell viability and apoptotic cell death with flow cytometry results. Inconsistent with cell viability results, doxorubicin combination with coumarin showed an observed cell death in HL60/ADR cells and the rate of cell death markedly increased with the increase of coumarin concentration, which might be explained by the role of coumarin in inhibition of extracellular efflux of DOX.

Many studies reported the important role of *p53* tumor suppressor protein as apoptotic cell death regulator [46, 47]. It was showed that DOX induces *p53* activation and apoptosis [48]. In addition to cell viability, ROS and flow cytometry results, our Western blot results show that combination of DOX with different doses of coumarin has a positive effect on *p53* protein expression in HL60/ADR. It was demonstrated that DOX has a negative effect on the anti-apoptotic *Bcl-2* protein expression and affected oxidative stress by increasing hydrogen peroxide production in breast cancer cells [49]. Inconsistent with the published data, our results show a negative effect of combination between DOX and coumarin on *Bcl-2* protein expression. The caspase-3-like activity is increased through a protease cascade during apoptosis in the early stages [50]. It has been reported that DOX induces apoptosis through activation of caspase-3 [51]. In accordance with the previous results, the combination of DOX and coumarin induce apoptosis through the activation of caspase-3 and PARP proteins.

5. Conclusion

In summary, in this study, we demonstrate that single treatment with DOX or coumarin has a marked cytotoxic effect on human acute myeloid leukemia, but has no effect on drug-resistant acute myeloid leukemia. However, the combination of coumarin and doxorubicin shows a significant apoptotic cell death in drug-resistant acute myeloid leukemia.

Declarations

Author contribution statement

Nouf A-Abbas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nehad Al-Shaer: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- [1] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward, D. Forman, CA cancer, *J. Clin. Oncol.* 29 (2011) 69e90.
- [2] S.-K. Kim, *Handbook of Anticancer Drugs from Marine Origin*, Springer International Publishing, Switzerland, 2015.
- [3] J. Ferlay, H.R. Shin, F. Bray, D. Forman, C. Mathers, D.M. Parkin, GLOBOCAN 2008 v1.2. "Cancer Incidence and Mortality Worldwide: IARC Cancer Base No.10, International Agency for Research on Cancer, Lyon, France, 2010.
- [4] G.I. Solyanik, Multifactorial nature of tumor drug resistance, *Exp.Oncol.* 32 (2011) 181–185.
- [5] S. Vijayaraghavalu, C. Peeta, S. Lu, V. Labhasetwar, Epigenetic modulation of the biophysical properties of drug-resistant cell lipids to restore drug transport and endocytic functions, *Mol. Pharm.* 9 (2012) 2730–2742.
- [6] A. Ghoneum, S. Sharma, J. Gimzewski, Nano-hole induction by nanodiamond and nanoplatinum liquid, DPV576, reverses multidrug resistance in human myeloid leukemia (HL60/AR), *Int. J. Nanomed.* 8 (2013) 2567–2573.
- [7] E. Klumper, G.J. Ossenkoppele, R. Pieters, et al., In vitro resistance to cytosine arabinoside, not to daunorubicin, is associated with the risk of relapse in de novo acute myeloid leukaemia, *Br. J. Haematol.* 93 (1996) 903–910.
- [8] H.D. Preisler, Multidrug resistance is more than MDR1 activity, *Leuk. Res.* 19 (1995) 429–431.
- [9] B. Löwenberg, W.L. van Putten, I.P. Touw, R. Delwel, V. Santini, Autonomous proliferation of leukemic cells in vitro as a determinant of prognosis in adult acute myeloid leukemia, *N. Engl. J. Med.* 328 (1993) 614–619.
- [10] A. Raza, H.D. Preisler, R. Day, et al., Direct relationship between remission duration in acute myeloid leukemia and cell cycle kinetics: a leukemia intergroup study, *Blood* 76 (1990) 2191–2197.
- [11] J.L. Biedler, H. Riehm, Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross-resistance, radiographic and cytogenetic studies, *Cancer Res* 30 (1970) 1174–1184.
- [12] V. Ling, L.H. Thompson, Reduced permeability in CHO cells as a mechanism of resistance to colchicine, *J. Cell. Physiol.* 53 (1974) 103–111.
- [13] M. Inaba, H. Kobayashi, V. Sakurai, R.K. Johnson, Active efflux of daunorubicin and Adriamycin in sensitive and resistant sublines of P388 leukemia, *Cancer Res.* 39 (1979) 2200–2203.
- [14] J. Kasaian, F. Mosaffa, J. Behravan, M. Masullo, S. Piacente, M. Ghandadi, M. Iranshahi, Reversal of P-glycoprotein-mediated multidrug resistance in MCF-7/Adr cancer cells by sesquiterpene coumarins, *Fitoterapia* 103 (2015) 149–154.
- [15] J. Wei, Y. Zhou, G.Q. Jiang, D. Xiao, Silencing of ETS1 reverses adriamycin resistance in MCF-7/ADR cells via downregulation of MDR1, *Cancer Cell Int.* 14 (2014) 22.
- [16] M. Russo, C. Spagnuolo, I. Tedesco, G.L. Russo, Phytochemicals in cancer prevention and therapy: truth or dare? *Toxins (Basel)* 2 (2012) 517–551.
- [17] S. Prasad, B.B. Aggarwal, Chronic diseases caused by chronic inflammation require chronic treatment: anti-inflammatory Role of dietary spices, *Clin Cellular Immunol.* 5 (2014) 1–11.
- [18] A. Lacy, R. O'Kennedy, Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer, *Curr. Pharm. Des.* 10 (2004) 3797–3811.
- [19] P.K. Jain, H., J. Joshi, Coumarin: chemical and pharmacological profile, *App. Pharm. Sci.* 2 (6) (2012) 236–240.
- [20] S.J. Lee, U.S. Lee, W.J. Kim, S.K. Moon, Inhibitory effect of esuletin on migration, invasion and matrix metalloproteinase-9 expression in TNF- α -induced vascular smooth muscle cells, *Mol. Med. Rep.* 4 (2011) 337–341.
- [21] B. Xu, L. Wang, L. Gonzalez-Molleda, Y. Wang, J. Xu, Y. Yuan, Antiviral activity of (+)-Rutamarin against Kaposi's sarcoma-associated herpesvirus by inhibition of the catalytic activity of human topoisomerase II, *Antimicrob. Agents Chemother.* 58 (2014) 563–573.
- [22] I. Kostova, S. Bhatia, P. Grigorov, S. Balkansky, V.S. Pramar, A.K. Prasad, L. Saso, Coumarins as antioxidants, *Curr. Med. Chem.* 18 (2011) 3929–3951.
- [23] P. Anand, B. Singh, N. Singh, *Bioorg. Med. Chem.* 20 (2012) 1175–1180.
- [24] A. Sanchez-Recillas, G. Navarrete-Vazquez, S. Hidalgo-Figueroa, M.Y. Rios, M. Ibarra-Barajas, S. Estrada-Soto, Semisynthesis, ex vivo evaluation, and SAR studies of coumarin derivatives as potential antiasthmatic drugs, *Eur. J. Med. Chem.* 77 (2014) 400–408.
- [25] K.V. Sashidhara, R.K. Modukuri, S. Singh, K.B. Rao, G.A. Teja, S. Gupta, S. Shukla, Design and synthesis of new series of coumarin-aminopyran derivatives possessing potential anti-depressant-like activity, *Bioorg. Med. Chem. Lett* 15 (2015) 337–341.
- [26] C. Chia-Hsiung, L. Ku-Chung, W. Wai-Theng, X. Yen-Fang, C. Lin, Induction of ROS-independent JNK-activation-mediated apoptosis by a novel coumarin derivative, DMAC, in human colon cancer cells, *Chem. Bio. Int.* 218 (2014) 42–49.
- [27] N. Saidu, S. Valente, B. Emilie, K. Gilbert, B. Denyse, M. Mathias, Coumarin polysulfides inhibit cell growth and induce apoptosis in HCT116 colon cancer cells, *Bioorg. Med. Chem.* 4 (2012) 1584–1593.
- [28] G. Jurgen, S. Hironobu, C. Shiuian, P. Atul, Steroid sulfatase inhibitors: promising new tools for breast cancer therapy? *J. Steroid Biochem. Mol. Biol.* 125 (2011) 39–45.
- [29] P. Sonneveld, Multidrug resistant cells with high proliferative capacity determine response to therapy in acute myeloid leukemia, identification and modulation of drug resistance in acute myeloid leukemia 9 (1025) (2014) 63.
- [30] I. De Kouchkovsky, M. Abdul-Hay, Acute myeloid leukemia: a comprehensive review and 2016 update, *Blood Canc. J.* 6 (7) (2016) e441.
- [31] H. Liu, W. Jiang, M. Xie, Flavonoids: recent advances as anticancer drugs, *Recent Pat. Anti-Cancer Drug Discov.* 5 (2) (2010) 152–164.
- [32] R. Bertramann, G. Alth, M. Kreiter, A. Kubin, Natural products derived from plants as potential drugs for the photodynamic destruction of tumor cells, *J. Photochem. Photobiol. B Biol.* 36 (2) (1996) 95–97.
- [33] A. Lacy, R. O'Kennedy, Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer, *Curr. Pharmaceut. Des.* 10 (30) (2004) 3797–3811.
- [34] J. Pan, Q. Zhang, C. Zhao, R. Zheng, Redifferentiation of human hepatoma cells induced by synthesized coumarin, *Cell Biol. Int.* 28 (5) (2004) 329–333.
- [35] C. Zwergel, S. Valente, A. Salvato, Z. Xu, O. Talhi, A. Mai, A. Silva, L. Altucci, G. Kirsch, Novel benzofuran-chromone and -coumarin derivatives: synthesis and biological activity in K562 human leukemia cells, *Med. Chem. Commun.* 4 (2013) 1571–1579.
- [36] C. Seidel, M. Schneckeburger, C. Zwergel, F. Gaascht, A. Mai, M. Dicato, G. Kirsch, S. Valente, M. Diederich, Novel inhibitors of human histone deacetylases: Design, synthesis and bioactivity of 3-alkenylcoumarins, *Bioorg. Med. Chem. Lett* 24 (16) (2014) 3797–3801.
- [37] L. Zhang, Z. Wang, T. Khishignyam, T. Chen, C. Zhou, Z. Zhang, M. Jin, R. Wang, Y. Qiu, D. Kong, In vitro anti-leukemia activity of dual PI3K/mTOR inhibitor Voxelisib on HL60 and K562 cells, as well as their multidrug resistance counterparts HL60/ADR and K562/A02 cells, *Biomed. Pharmacother. = Biomedecine & pharmacotherapie* 103 (2018) 1069–1078.
- [38] Q. Zhou, Y. Chen, L. Zhang, Y. Zhong, Z. Zhang, R. Wang, M. Jin, M. Gong, Y. Qiu, D. Kong, Antiproliferative effect of ZSTK474 alone or in combination with chemotherapeutic drugs on HL60 and HL60/ADR cells, *Oncotarget* 8 (24) (2017) 39064–39076.
- [39] M. Hirose, E. Hosoi, S. Hamano, A. Jalili, Multidrug resistance in hematological malignancy, *J. Med. Invest.* 50 (3–4) (2003) 126–135.
- [40] D.M. van der Kolk, E.G. de Vries, M. Muller, E. Vellenga, The role of drug efflux pumps in acute myeloid leukemia, *Leuk. Lymphoma* 43 (4) (2002) 685–701.
- [41] S.Y. Kim, S.J. Kim, B.J. Kim, S.Y. Rah, S.M. Chung, M.J. Im, U.H. Kim, Doxorubicin-induced reactive oxygen species generation and intracellular Ca²⁺ increase are reciprocally modulated in rat cardiomyocytes, *Exp. Mol. Med.* 38 (5) (2006) 535.
- [42] B.K. Sinha, E.G. Mimnaugh, S. Rajagopalan, C.E. Myers, Adriamycin activation and oxygen free radical formation in human breast tumor cells: protective role of glutathione peroxidase in adriamycin resistance, *Cancer Res* 49 (1989) 3844–3848.
- [43] P. Ubezio, F. Civoli, Flow cytometric detection of hydrogen peroxide production induced by doxorubicin in cancer cells, *Free Radic. Biol. Med.* 16 (1994) 509–516.
- [44] V. Gouaze, M.E. Mirault, S. Carpentier, R. Salvayre, T. Levade, N. Andrieu-Abadie, Glutathione peroxidase-1 overexpression prevents ceramide production and partially inhibits apoptosis in doxorubicin-treated human breast carcinoma cells, *Mol. Pharmacol.* 60 (2001) 488–496.
- [45] C. Holze, C. Michaudel, C. Mackowiak, D.A. Haas, C. Benda, P., Hubel, D.W. Leung, Oxoeiptosis, a ROS-induced caspase-independent apoptosis-like cell-death pathway, *Nat. Immunol.* 19 (2) (2018) 130.
- [46] M.L. Agarwal, W.R. Taylor, M.V. Chernov, O.B. Chernova, k G.R. Star, The p53 network, *J. Biol. Chem.* 273 (1998) 1–4.
- [47] J.L. Ko, C. Prives, p53: puzzle and paradigm, *Genes Dev* 10 (1996) 1054–1072.
- [48] S. Wang, E.A. Konorev, S. Kotamraju, J. Joseph, S. Kalivendi, B. Kalyanaram, Doxorubicin induces apoptosis in normal and tumor cells via distinctly different mechanisms intermediacy of H2O2-and p53-dependent pathways, *J. Biol. Chem.* 279 (24) (2004) 25535–25543.
- [49] N. Pilco-Ferreto, G.M. Calaf, Influence of doxorubicin on apoptosis and oxidative stress in breast cancer cell lines, *Int. J. Oncol.* 49 (2) (2016) 753–762.
- [50] D.W. Nicholson, N.A. Thornberry, Caspases: killer proteases, *Trends Biochem. Sci.* 22 (1997) 299–306.
- [51] M. Ueno, Y. Kakinuma, K.I. Yuhki, N. Murakoshi, M. Iemitsu, T. Miyachi, I. Yamaguchi, Doxorubicin induces apoptosis by activation of caspase-3 in cultured cardiomyocytes in vitro and rat cardiac ventricles in vivo, *J. Pharmacol. Sci.* 101 (2) (2006) 151–158.