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In silico and in vitro evaluation of oxypeucedanin-induced anticancer activity: Mitotoxicity?

Oksipösedanin kaynaklı antikanser aktivitenin *in siliko* ve in vitro değerlendirilmesi: Mitotoksisite?

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Abstract

Aim: This study aims to evaluate the alterations in Oxypeucedanin (OXY)-mediated anticancer activity in different media. Second aim is to predict the affinity of OXY to electron transfer chain (ETC) complexes.

Materials and Methods: MTT and LDH leakage assays were performed with OXY. Molecular docking studies were also conducted to predict the affinity of OXY to ETC complexes.

Results: 250 µM OXY reduced viability in glucose media. ≥50 µM OXY decreased viability in galactose media. ≥50 µM OXY increased membrane disruption in galactose media. Molecular docking studies also showed that OXY might possess the capacity to bind to the inhibition sites of Complex I and IV.

Conclusion: Galactose-conditioned media exacerbated the OXY-mediated cytotoxicity. Preliminary results suggested that mitotoxicity might take part in anticancer activity. Furthermore, OXY might cause ETC dysfunctions due to selective inhibition of Complex I and IV.

Keywords: Oxypeucedanin; Mitotoxicity; Anticancer activity; *In silico*.

Öz

Amaç: Çalışmanın amacı, farklı ortamlarda Oksipösedanin (OKS) aracılı antikanser aktivitedeki değişiklikleri değerlendirmektir. İkinci amaç, OKS'inin elektron transfer zincirine (ETZ) karşı afinitesini öngörmektir.

Gereç ve Yöntem: MTT ve LDH sızma deneyleri OKS ile gerçekleştirilmiştir. Ayrıca, OKS'inin ETZ komplekslerine karşı afinitesini öngörmek için moleküler kenetlenme çalışmaları uygulanmıştır.

Bulgular: Glukoz içeren ortamda 250 µM OKS canlılığı azaltmıştır. Galaktoz içeren ortamda ≥50 µM OKS hücre canlılığını azalmıştır. Galaktoz içeren ortamda ≥50 µM OKS membran parçalanmasını artırmıştır. Moleküler kenetlenme çalışmaları, OKS'inin Kompleks I ve IV'ün inhibisyon bölgelerine bağlanma kapasitesine sahip olabileceğini göstermektedir.

Sonuç: Galaktoz içeren ortam, OKS aracılı sitotoksisiteyi artırmıştır. Ön sonuçlar, antikanser aktivitede mitotoksisitenin yer alabileceğini göstermektedir. Ayrıca OKS, Kompleks I ve IV'ün seçici inhibisyonu nedeni ile ETZ disfonksiyonuna neden olabilmektedir.

Anahtar Kelimeler: Oksipösedanin; Mitotoksisite; Antikanser aktivite; *İn siliko*.

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Introduction

Natural plants are sources of phytochemicals such as bioactive secondary metabolites. Pharmacologically active phytochemicals have been used since ancient times in order to treat various diseases with the advantages of effectiveness and low occurrence of adverse effects. A wide range of phytochemicals have been isolated from medicinal plants to suppress several diseases, including cancer progression and development.[1,2] OXY is a derivative of furanocoumarin extracted and isolated from *Angelica*, *Ferulago*, and *Prangos* species. Over 50% of OXY has been isolated and characterized from the roots.[3,4] OXY was reported to have anti-mutagenic, cytotoxic, and antiproliferative activities against several cancer cells, including colon, breast, liver, and lung cancers.[5-7]

Cancer cells need more significant biosynthetic components and building blocks, including amino acids and nucleotides, than normal cells due to their uncontrolled and highly proliferative cell division characteristics. Also, the expression of proapoptotic proteins is lower in cancer cells than in normal cells, which makes cancer cells more resistant to anti-cancer treatments and molecules.[8,9] Mitochondria are one of the most targeted organelles for cancer treatment in drug discovery and development processes, as the mitochondria clearly play a pivotal role in cancer cells in that they take part in tumor initiation and promotion, regulation of energy homeostasis, intrinsic apoptosis, and the synthesis of biomass and building blocks.[9,10] The primary purpose of anticancer treatment relies on killing of cancer cells. Investigating the role of mitotoxicity in phytochemical-mediated anti-cancer activity sheds light on novel pathways and molecules for cancer treatment.[9-11]

It is complicated for scientists to investigate mitotoxicity directly. *In vivo* models, including in-bred rodent models and transgenic mice, are not properly effective in reflecting the mechanism of mitotoxicity.[12-14] *In vitro* studies are more likely to reveal the mechanism of mitotoxicity compared to *in vivo* models. Standard *in vitro* models use high glucose-conditioned media for cancer cells to uncover the mechanisms of mitotoxicity. However, cancer cells produce more than 50% of their energy via glycolytic pathway apart from oxidative phosphorylation (OXPHOS) due to the Crabtree effect, which reduces their sensitivity to mitochondrial toxicants (mitotoxicants).[15,16] Marroquin et al. (2007) proposed a model for HepG2 cells by replacing glucose with galactose. This model allows cancer cells to use galactose inefficiently via glycolysis, blocking ATP generation in the cytosol, and forcing the cell to produce ATP via OXPHOS.[17] This model was adopted by many *in vitro* studies in order to figure out the mitotoxicity by using several cell types.[18-20]

Previous results showed that OXY caused selective inhibition in a wide range of human cancer cells.[7,21-23] Nevertheless, the mechanism of OXY-mediated mitotoxicity has yet to be precisely uncovered. Furthermore, previous studies, including *in vitro* assays, used standard glucose-conditioned media, which can not fully indicate mitotoxicity due to the Crabtree effect. Thus, observing alterations in OXY-mediated anticancer activity as well as to possible mitotoxicity by comparing both glucose, and galactose conditions matters to investigate. The present study aims to investigate two primary purposes: i) to figure out the alterations in OXY-mediated anticancer activity in HepG2 cells made vulnerable to mitotoxicants by using either glucose- or galactose-conditioned media. ii) to predict the possible affinity of OXY to the ETC, which takes part in the inner membrane of mitochondria as structural and functional components, using molecular docking studies.

Materials and Methods

Materials and chemical reagents

All chemicals were obtained from Sigma-Aldrich (Darmstadt, Germany) except for cell culture reagents. Cell culture reagents were purchased from Thermo-Fisher Scientific (Loughborough, UK).

Cell line and cell culture

HepG2 cells were purchased from American Type Culture Collection (ATTC,

HB-8065, USA). HepG2 cells were maintained under high glucose and galactose conditions as described previously.[17] The passage numbers for HepG2 cells were maintained between 7 and 15.

Isolation and characterization of oxypeucedanin

The OXY used in this study was obtained from previous study.[24] The compound was isolated from the roots of *Prangos heyniae* H.Duman & M.F. Watson, an endemic species in Türkiye. The roots were collected from Hadim/ Konya city of Türkiye in 2016. The air-dried and crushed roots were sequentially extracted with *n*-hexane, chloroform, and methanol in an ultrasonic water bath for 24 h. The extracts were filtrated and evaporated to dryness separately at 40°C under low pressure, yielding *n*-hexane (25g), chloroform (9g), and methanol (39g) extracts. Column chromatography was used for purification studies. After several chromatographic column studies with the chloroform extract, oxypeucedanin (100 mg), was isolated and identified using 1D NMR and MALDI-TOF-MS.[24] The compound was stored at 20°C as frozen form.

MTT assay

The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to evaluate cell viability in HepG2 cells exposed to OXY in a dose-dependent manner in high glucose or galactose conditions as described in previous studies with minor modifications.[17,25] In brief, HepG2 cells (10^4 cells/well) exposed to OXY (6.25, 12.5, 25, 50, 100, and 250 µM) were incubated for 24 h at 37˚C with 5% CO_2. Final dimethyl sulfoxide (DMSO, solvent control) and Triton X-100 (positive control) concentrations were 1%. After treatment, MTT solution (5 mg/mL in phosphate buffer solution) was added to each well, and the well plate was incubated for 4 h. After incubation, formazan crystals were solubilized by using DMSO, and the color intensity was measured by a multi-plate reader. IC_{50} values were calculated as previously described in our study.[25]

LDH leakage assay

Lactate dehydrogenase (LDH) leakage assay was used to observe alterations in HepG2 cells exposed to OXY in a dose-dependent manner in high glucose or galactose conditions as described in previous studies with minor modifications.[17,26] In brief, HepG2 cells (10^4 cells/well) were exposed to OXY (6.25, 12.5, 25, 50, 100, and 250 µM) for 24 h at 37˚C with 5% CO_2. Final DMSO (solvent control) and Triton X-100 (positive control) concentrations were 1%. After treatment, LDH activity was determined by diluting media with pH 7.4 phosphate buffer (1:2) at 37 °C. Then, NADH (300 µM, final concentration) and sodium pyruvate (770 µM, final concentration) were added to the media. Absorbances of the media were measured by a multi-plate reader at 340 nm for 4 minutes as previously described in our study.[26] IC_{50} values were calculated as described in our previous study.[25]

Docking simulation

An *in silico* docking analysis of OXY with ETC complexes was carried out in this study using MOE 2020 (Molecular Operating Environment 2020). The structure of OXY was drawn in the ChemDraw 19.1 (Perkin Elmer Informatics) program, optimized by MOE, and subjected to energy minimization using the MMFF 94x (Merck Molecular Force Field) package program. The RCSB website (http://www.rcsb.org/pdb) was used to obtain ETC complex structures in PDB format [Complex I (PDB ID: 5XTD)[27], Complex II (PDB ID: 8GS8)[28], Complex III (PDB ID: 5XTE)[27], and Complex IV (PDB ID: 5Z62)[29]]. Since the human crystal structure of Complex V was not found, it was not used in this study. Crystal ligands and water molecules were removed from the enzyme complexes before docking. The surfaces of the complexes were scanned to identify the active sites of the enzymes. Hydrogen atoms and charges were added, while default values for other properties were used. The docking score was used to compare the capacity of affinity of OXY to ETC Complexes. Since a well-known inhibitor with organic structure of Complex IV was not found by molecular modeling study, Complex I inhibitor, Rotenone (ROT), was used as a

positive control[30] ROT was used as a reference molecule for molecular docking studies.

Statistics

The data were shown as the mean ± SD from three experiments (n:3). Data were analyzed by Mann-Whitney U test using GraphPad Prism version 8.4.2 for Windows. Statistical significance was accepted when $p \leq 0.05$.

Results

MTT assay demonstrated that lower than 250 µM concentrations did not cause any alterations in cell viability in high glucose-conditioned media (Figure 1). 250 µM OXY reduced cell viability to 79% compared to control. Predicted IC_{50} value for OXY-induced cytotoxicty in glucose conditioned media was 548 ± 16 µM (Table 1). In galactose-conditioned media, 6.25, 12.5, and 25 µM OXY did not cause any cytotoxicity, however 50 µM and higher concentrations of OXY decreased cell viability by 21, 39, and 55% compared to control (Figure 1). IC_{50} value for OXY-induced cytotoxicty in galactose conditioned media was 211 ± 8 µM (Table 1). 50, 100, and 250 µM OXY in galactose-conditioned media gave rise to significant decrease of cell viability compared to glucose-conditioned media (Figure 1). Triton X-100 used as a positive control reduced cell viability by 92 and 93% in high glucose and galactose-conditioned media, respectively (data not shown).

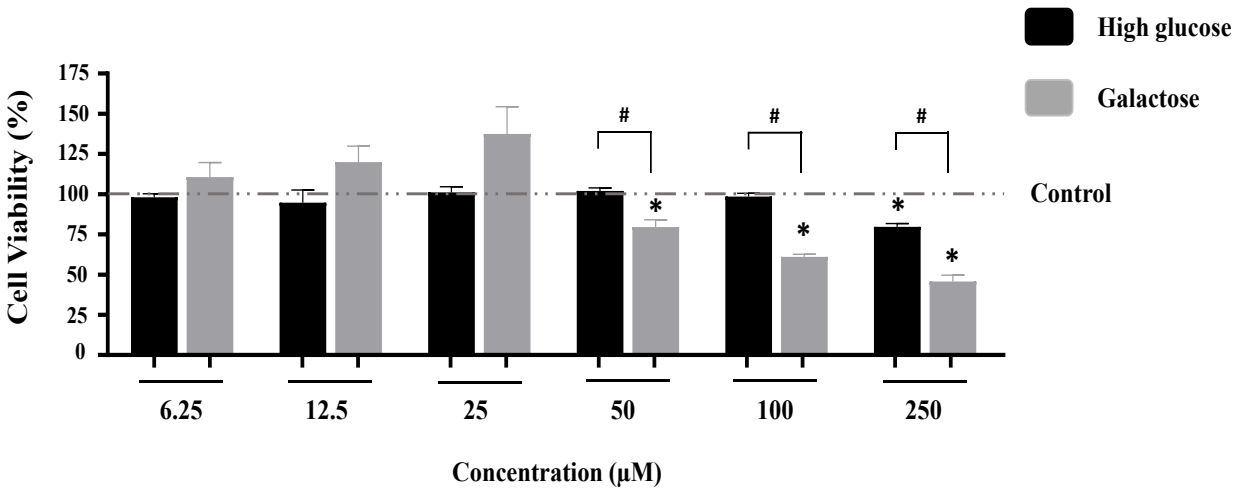

Figure 1. Cell viability in HepG2 cells exposed to OXY. MTT assay was performed in order to determine the cytotoxicity of HepG2 cells exposed to OXY in a dose-dependent manner in high glucose (black) or galactose (gray) conditioned media after 24 hours of incubation. Values are the mean ± SD from three independent experiments (n:3). The data were expressed as a percent of the solvent (1% DMSO) control. (*) significantly different ($p<0.05$) than the solvent control (1% DMSO)

Table 1. IC_{50} values (µM) ± SD of OXY against HepG2 cells cultured in glucose and galactose conditions for 24 h.

	IC50 (µM)	
Assay	**Glucose**	**Galactose**
MTT	548 ± 16#	211 ± 8*
LDH Leakage	744± 24#	227± 9*

IC_{50}: The concentrations (µM) that inhibited 50% of cell viability and increased 50% of LDH enzyme activity for MTT, and LDH leakage assays, respectively.
#: Predicted IC_{50} values.
*: IC_{50} value is significantly different ($p<0.05$) than glucose.

As shown in Figure 2, OXY did not cause any increase in LDH activity in high glucose-conditioned media. Predicted IC_{50} value for OXY-induced membrane damage in glucose conditioned media was 744 ± 24 µM (Table 1). In galactose-conditioned media, 6.25, 12.5, and 25 µM OXY did not increase the LDH activity (Figure 2). 50 µM and higher concentrations of OXY led to an increase in LDH activity and membrane disruption compared to control. IC_{50} value for OXY-induced membrane damage in galactose conditioned media was 227 ± 9 µM (Table 1). In addition, 50, 100, and 250 µM OXY increased membrane damage in galactose-conditioned media compared to glucose-conditioned media (Figure 2). Triton X-100 is a kind of detergent and is used as a positive control for membrane disruption. Triton X-100 increased membrane damage by 47 and 138% in high glucose and galactose-conditioned media, respectively (data not shown).

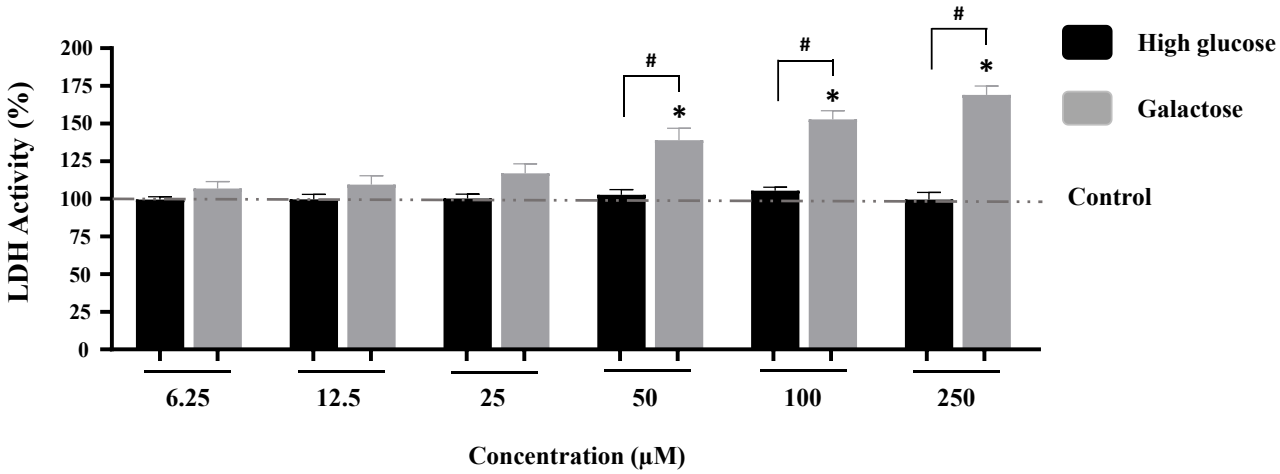

Figure 2. LDH activity resulting from membrane damage in HepG2 cells exposed to OXY. LDH leakage assay was performed in order to observe the membrane of HepG2 cells exposed to OXY in a dose-dependent manner in high glucose (black) or galactose (gray) conditioned media after 24 hours of incubation. Values are the mean ± SD from three independent experiments (n:3). The data were expressed as a percent of the solvent (1% DMSO) control. (*) significantly different ($p<0.05$) than the solvent control (1% DMSO).

In silico binding affinity of OXY with ETC complexes showed that docking scores for Complex I, Complex II, Complex III, and Complex IV ranged from - 6.46 to - 7.3 kcal/mol. OXY showed significant docking score with Complex IV (-7.3 kcal/mol, RMSD: 1.1646), Complex III (-6.94 kcal/mol, RMSD: 1.5761), Complex I (-6.79 kcal/mol, RMSD: 1.1327) and Complex II (-6.46 kcal/mol, RMSD: 1.6117) (Table 2). The highest docking score resulted from combination of OXY and Complex IV (-7.3 kcal/mol), indicating that it was properly positioned inside the Complex IV binding site. Table 2 and Figure 3 demonstrated that this enzyme possessed a greater affinity for OXY. The Aren (π)-H, Aren (π)- Aren (π), and H-Aren (π) interactions with the residues (Trp 126, Tyr 129, Trp 236, His 291, and Phe 377) led to the establishment of the maximum binding energy between OXY and Complex IV (Table 2 and Figure 3). Positive control ROT showed remarkable docking score with Complex I (-7.49 kcal/mol, RMSD: 0.9158) (data not shown).

Table 2. Docking result of OXY with the ETC complexes.

	Ligand = Oxypeucedanin			
Targets	Binding energy (kcal/mol)	RMSD values	Binding site amino acids	Interactions
Complex I (5XTD)	-6.79	1.1327	Phe 64, Gly 63, Asp 205	Aren (π)-H, H-bond acceptor, Ligand exposure
Complex II (8GS8)	-6.46	1.6117	Asn 81, Arg 512, Leu 513, Gln 516	Aren (π)-H, Aren (π)-cation, H-bond acceptor, H-bond donör, Ligand exposure
Complex III (5XTE)	-6.94	1.5761	Ala 84, Gly 130, Tyr 131	Aren (π)-H, Ligand exposure,
Complex IV (5Z62)	-7.3	1.1646	Trp 126, Tyr 129, Trp 236, His 291, Phe 377	Aren (π)-H, Aren (π)- Aren (π), H-Aren (π), Ligand exposure

Discussion

Mitochondria play a pivotal role in maintaining biomass synthesis including nucleotides, fatty acids, and amino acids, in highly proliferative cells such as cancer. Mitochondria also control programmed cell death or apoptosis. However, apoptosis is inhibited in cancer cells. Therefore, mitochondrial dysfunction is one of the most targeted mechanisms in the treatment of cancer.[9] In addition to synthetic drugs or chemicals, pharmacologically active phytochemicals are also used to lead to mitochondrial dysfunction and consequently mitotoxicity to destroy the cancer cells.[31,32]

Nevertheless, most studies estimated the Crabtree effect and used high glucose-conditioned media required for cancer cells to investigate the mitotoxicity.[7,21-23]

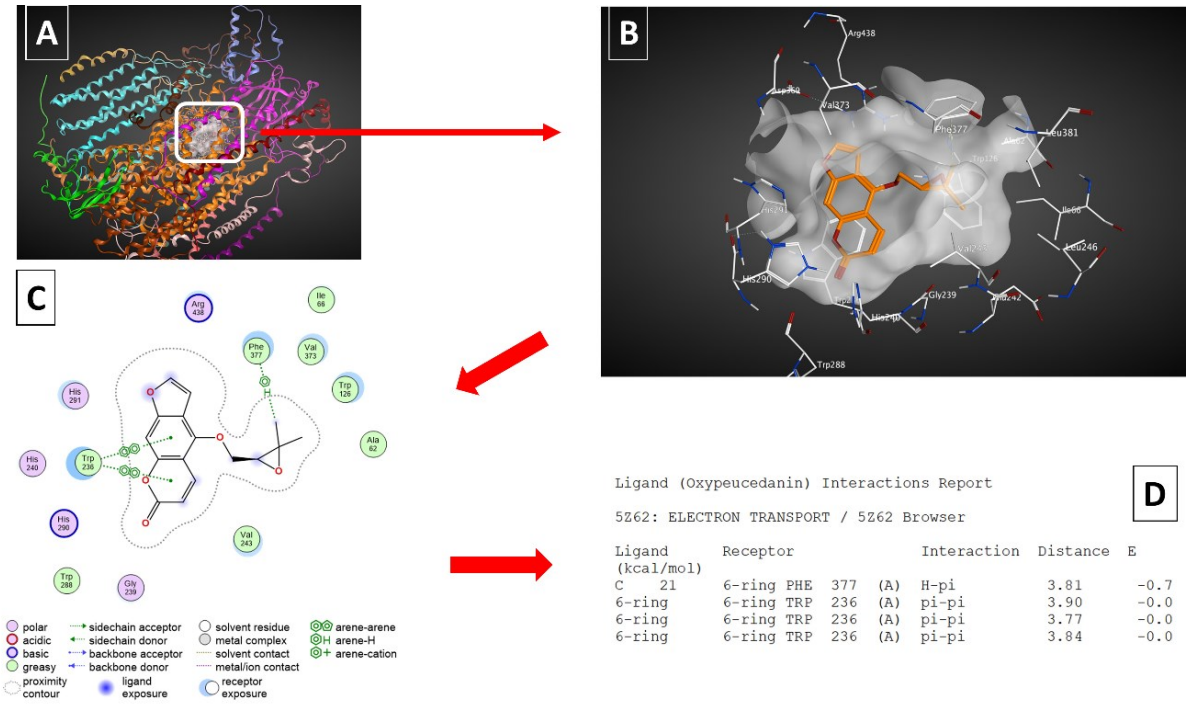

Figure 3. 2D binding pose of OXY with the human Complex IV [cytochrome c oxidase (PDB ID: 5Z62)] active site. Receptor cites (A), binding site amino acids (B), interactions (C), and ligand interaction report (D).

Several *in vitro* studies have been reported for isolation, and antiproliferative activities of OXY. Kim et al. (2007) isolated OXY from the root of *Angelica dahurica* and determined antitumor properties by using sulforhodamine B (SRB) assay in various cell lines. IC_{50} values for A549 (human lung carcinoma), SK-OV-3 (human ovarian cancer), SK-MEL-2 (human melanoma cancer), XF498 (human central nervous system) and HCT-15 (human colon adenocarcinoma) found as approximately 32, 68, 58, 57, and 12 µM, respectively.[22] In another study Mottaghipisheh et al. (2018) isolated the OXY and other furocoumarins from flower, leaves and stem of *Ducrosia anethifolia*. Furocoumarins were subjected to MTT assay for anticancer activities by using L5178Y mouse T-cell lymphoma cells (IC_{50}: 26 µM), ABCB1-expressing L5178Y cell line (IC_{50}: 29 µM).[23] Tavakoli et al. (2017) isolated a wide range of OXY and its analogs from the root of *Ferulago trifida* Boiss and antitumor potantial was also investigated using MTT assay. IC_{50} values for MDA-MB-231 (human breast adenocarcinoma), A-549, HT-29 (human colon adenocarcinoma), and MRC-5 (human fetal lung fibroblast) were reported as 1190, 800, 1280, and 1790 µM, respectively.[21] A recent study isolated OXY from the root of *Angelica dahurica* and evaluated anticancer activity by using SRB assay. This study indicated that OXY led to selective inhibiton towards SK-Hep-1 (human hepatic adenocarcinoma, IC_{50}: 32.4 µM) and HepG2 (IC_{50}: 43.8 µM) cells rather than MDA-MB-231 (IC_{50}: 50.8 µM), T47D (ductal carcinoma, IC_{50}: 95.5 µM), SNU-638 (gastric carcinoma, IC_{50}: 50.4 µM), A549 (IC_{50}: 46.3 µM).[7]

In addition to anticancer and antiproliferative activities, OXY was also found to have protective effects towards drug-induced cytotoxicity. OXY isolated from the root of *Angelica dahurica* reversed Tacrin-mediated cytotoxicity in HepG2 cells (EC_{50}: 286 µM).[5] Another study revealed that 10 µM OXY alleviated Sunitinib induced apoptosis.[33] 280 µM OXY was also suggested to inhibit doxorubicin-induced apoptosis in PC12 (rat adrenal pheochromocytoma) cells. In same study, MTT assay displayed that 350 µM, the highest dose, OXY did not cause any cytotoxicity in PC12 cells [34]

Even though OXY was reported to display anticancer, antiproliferative, and protective

activities, limited mechanisms have been proposed to uncover the mechanism of cytotoxicity and mitotoxicity in OXY-mediated anticancer activity in hepatoma cells. Park et al. (2020) reported that OXY-mediated anticancer activity might be result from induction of cell cycle arrest and p53-mediated signaling.[7] However, there is no study applied in galactose-conditioned media. For this reason, experiments must also be performed in galactose-conditioned media to make cells more sensitive to mitotoxicity as well as to high glucose-conditioned media. Hence, we first planned to investigate and compare the alterations of anticancer and cytotoxic activities of OXY in HepG2 cells by utilizing frequently used end-point assays (MTT and LDH leakage) in glucose and galactose-conditioned media; second, molecular docking studies were performed to predict the possible affinity for OXY in ETC Complexes. MTT and LDH leakage assays displayed that galactose-conditioned media altered response of HepG2 cells exposed to OXY. 50, 100, and 250 µM OXY in galactose-conditioned media gave rise to significant decrease of cell viability, and increase of membrane disruption compared to glucose-conditioned media (Figure 1 and 2). These preliminary data propose that anticancer activity of OXY might depend on mitotoxicity in HepG2 cells.

ETC (Complex I-V) is a functional and structural components in mitochondria. In addition to the production of energy and membrane potential, ETC also maintains the synthesis of enzymes and intermediates including aspartase and pyrimidine, in highly proliferative cells such as cancer. Therefore, ETC inhibition is one of the most commonly used mechanisms in mitotoxicity to reduce cancer cell proliferation and growth.[35] Since no molecular modeling study indicating the possible interactions with OXY and ETC complexes existed, molecular docking study was also applied to predict the affinity of OXY to ETC complexes. Although OXY showed high affinity for the inhibition site of Complex IV (Table 2 and Figure 3), it was also found that RMSD values for Complex I (RMSD: 1.1327) and IV (RMSD: 1.1646) were close for OXY. Furthermore, OXY (-6.79 kcal/mol, RMSD: 1.1327) showed close activity to ROT (-7.49 kcal/mol, RMSD: 0.9158) for Complex I thanks to high score and low RMSD value. This data might suggest that OXY have a potential for Complex I and Complex IV inhibition. Consequently, inhibition of Complex I and IV by OXY might result in collapse of proton gradient and energy production.[36] This data need to be supported with enzymatic assays to claim that OXY is a Complex I and IV inhibitor.

Conclusion

Our study showed that significant alterations in OXY-mediated anticancer activity were observed in glucose, and galactose-conditions. Our preliminary data suggest that mitotoxicity might take part in OXY-mediated anticancer activity. Also, *in silico* studies supported our hypothesis. Molecular docking studies proposed that OXY might show high affinity to complex I and IV, and OXY might be a potential candidate for Complex inhibition. Further studies including oxygene consumption assay, measurement of cellular and mitochondrial energy status, membrane potential, complex activity assay require to make certain of the role of mitotoxicity in OXY-mediated anticancer activity in glucose and galactose conditioned media.

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Ethics Committee Approval

There was no data obtained from animal or human experiments for this article.

Informed Consent

The consents were obtained from all of the authors for this article.

Author Contributions

All of the authors contributed at every stage of the study.

Conflict of Interest

The authors declare that there is no conflict of interest for this article.

Financial Disclosure

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Statements

These data have not been presented or published anywhere previously.

Peer-review

Externally peer-reviewed

References

1. González-Vallinas M, González-Castejón M, Rodríguez-Casado A, Ramírez de Molina A. Dietary phytochemicals in cancer prevention and therapy: a complementary approach with promising perspectives. *Nutrition Reviews*. 2013;71(9):585-599. doi: 10.1111/nure.12051
2. Wong SC, Kamarudin MNA, Naidu R. Anticancer Mechanism of Curcumin on Human Glioblastoma. *Nutrients*. 2021;13(3):950. doi:10.3390/nu13030950
3. Mottaghipisheh J, Kiss T, Tóth B, Csupor D. The Prangos genus: a comprehensive review on traditional use, phytochemistry, and pharmacological activities. *Phytochemistry Reviews*. 2020;19(6):1449-1470. doi: 10.1007/s11101-020-09688-3
4. Mottaghipisheh J. Oxypeucedanin: Chemotaxonomy, Isolation, and Bioactivities. *Plants (Basel)*. 2021;10(8):1577. doi:10.3390/plants10081577
5. Oh H, Lee HS, Kim T, et al. Furocoumarins from Angelica dahurica with hepatoprotective activity on tacrine-induced cytotoxicity in HepG2 cells. *Planta Medica*. 2002;68(5):463-464. doi:10.1055/s-2002-32075
6. Jalilian F, Moieni-Arya M, Hosseinzadeh L, Shokoohinia Y. Oxypeucedanin and isoimperatorin extracted from *Prangos ferulacea* (L.) Lindl protect PC12 pheochromocytoma cells from oxidative stress and apoptosis induced by doxorubicin. *Research in Pharmaceutical Sciences*. 2021;17(1):12-21. doi:10.4103/1735-5362.329922
7. Park SH, Hong JY, Park HJ, Lee SK. The Antiproliferative Activity of Oxypeucedanin via Induction of G_2/M Phase Cell Cycle Arrest and p53-Dependent MDM2/p21 Expression in Human Hepatoma Cells. *Molecules*. 2020;25(3):501. doi:10.3390/molecules25030501
8. Pfeffer CM, Singh ATK. Apoptosis: A Target for Anticancer Therapy. *International Journal of Molecular Sciences*. 2018;19(2):448. doi:10.3390/ijms19020448
9. Liu Y, Shi Y. Mitochondria as a target in cancer treatment. *MedComm*. 2020;1(2):129-139. doi:10.1002/mco2.16
10. Fulda S, Galluzzi L, Kroemer G. Targeting mitochondria for cancer therapy. *Nature Reviews. Drug Discovery*. 2010;9(6):447-464. doi:10.1038/nrd3137
11. Badrinath N, Yoo SY. Mitochondria in cancer: in the aspects of tumorigenesis and targeted therapy. *Carcinogenesis*. 2018;39(12):1419-1430. doi:10.1093/carcin/bgy148
12. Pereira CV, Oliveira PJ, Will Y, Nadanaciva S. Mitochondrial bioenergetics and drug-induced toxicity in a panel of mouse embryonic fibroblasts with mitochondrial DNA single nucleotide polymorphisms. *Toxicology and Applied Pharmacology*. 2012;264(2):167-181. doi:10.1016/j.taap.2012.07.030
13. Ong MM, Latchoumycandane C, Boelsterli UA. Troglitazone-induced hepatic necrosis in an animal model of silent genetic mitochondrial abnormalities. *Toxicological Sciences*. 2007;97(1):205-213. doi:10.1093/toxsci/kfl180
14. Ramachandran A, Lebofsky M, Weinman SA, Jaeschke H. The impact of partial manganese superoxide dismutase (SOD2)-deficiency on mitochondrial oxidant stress, DNA fragmentation and liver injury during acetaminophen hepatotoxicity. *Toxicology and Applied Pharmacology*. 2011;251(3):226-233. doi:10.1016/j.taap.2011.01.004
15. Diaz-Ruiz R, Rigoulet M, Devin A. The Warburg and Crabtree effects: On the origin of cancer cell energy metabolism and of yeast glucose repression. *Biochimica et Biophysica Acta*. 2011;1807(6):568-576. doi:10.1016/j.bbabio.2010.08.010
16. Pascale RM, Calvisi DF, Simile MM, Feo CF, Feo F. The Warburg Effect 97 Years after Its Discovery. *Cancers (Basel)*. 2020;12(10):2819. doi:10.3390/cancers12102819
17. Marroquin LD, Hynes J, Dykens JA, Jamieson JD, Will Y. Circumventing the Crabtree effect: replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants. *Toxicological Sciences*. 2007;97(2):539-547. doi:10.1093/toxsci/kfm052
18. Swiss R, Will Y. Assessment of mitochondrial toxicity in HepG2 cells cultured in high-glucose- or galactose-containing media. *Current Protocols in Toxicology*. 2011;49(1):2-20. doi:10.1002/0471140856.tx0220s49
19. Will Y, Dykens J. Mitochondrial toxicity assessment in industry-a decade of technology development and insight. *Expert Opinion on Drug Metabolism Toxicology*. 2014;10(8):1061-1067. doi:10.1517/17425255.2014.939628
20. Dott W, Mistry P, Wright J, Cain K, Herbert KE. Modulation of mitochondrial bioenergetics in a skeletal muscle cell line model of mitochondrial toxicity. *Redox Biology*. 2014;2:224-233. doi:10.1016/j.redox.2013.12.028
21. Tavakoli S, Delnavazi MR, Hadjiaghaee R, et al. Bioactive coumarins from the roots and fruits of Ferulago trifida Boiss., an endemic species to Iran. *Natural Product Research*. 2018;32(22):2724-2728. doi:10.1080/14786419.2017.1375915
22. Kim YK, Kim YS, Ryu SY. Antiproliferative effect of furanocoumarins from the root of Angelica dahurica on cultured human tumor cell lines. *Phytotherapy Research*. 2007;21(3):288-290. doi:10.1002/ptr.2043
23. Mottaghipisheh J, Nové M, Spengler G, Kúsz N, Hohmann J, Csupor D. Antiproliferative and cytotoxic activities of furocoumarins of Ducrosia anethifolia. *Pharmaceutical Biology*. 2018;56(1):658-664. doi:10.1080/13880209.2018.1548625
24. Albayrak G, Demir S, Kose FA, Baykan S. New coumarin glycosides from endemic *Prangos heyniae* H. Duman & M.F. Watson. *Natural Product Research*. 2023;37(2):227-239. doi:10.1080/14786419.2021.1961138
25. Kuzu B, Ergüç A, Karakuş F, Arzuk E. Design, synthesis, and antiproliferative activities of novel thiazolyl-pyrazole hybrid derivatives. *Medicinal Chemistry Research*. 2023;32:1690-1700. doi:10.1007/s00044-023-03090-2
26. Ergüç A, Karakuş F, Arzuk E, Mutlu N, Orhan H. Role of Oxidative Stress and Reactive Metabolites in Cytotoxicity & Mitotoxicity of Clozapine, Diclofenac and Nifedipine in CHO-K1 Cells In Vitro. *Endocrine, Metabolic & Immune Disorders Drug Targets*. 2023;(in press). doi: 10.2174/1871530323666230419084613
27. Guo R, Zong S, Wu M, Gu J, Yang M. Architecture of Human Mitochondrial Respiratory Megacomplex $I_2III_2IV_2$. *Cell*. 2017;170(6):1247-1257. doi:10.1016/j.cell.2017.07.050
28. Du Z, Zhou X, Lai Y, et al. Structure of the human respiratory complex II. *Proceedings of the National Academy of Sciences of the United States of America*. 2023;120(18). doi:10.1073/pnas.2216713120
29. Zong S, Wu M, Gu J, Liu T, Guo R, Yang M. Structure of the intact 14-subunit human cytochrome c oxidase. *Cell Research*. 2018;28(10):1026-1034. doi:10.1038/s41422-018-0071-1
30. Heinz S, Freyberger A, Lawrenz B, Schladt L, Schmuck G, Ellinger-Ziegelbauer H. Mechanistic Investigations of the Mitochondrial Complex I Inhibitor Rotenone in the Context of Pharmacological and Safety Evaluation. *Scientific Reports*. 2017;7:45465. doi:10.1038/srep45465
31. Patra S, Pradhan B, Nayak R, et al. Apoptosis and autophagy modulating dietary phytochemicals in cancer therapeutics: Current evidences and future perspectives. *Phytotherapy Research*. 2021;35(8):4194-4214. doi:10.1002/ptr.7082
32. Sitarek P, Synowiec E, Kowalczyk T, et al. Anticancer Properties of *Plectranthus ornatus*-Derived Phytochemicals Inducing Apoptosis via Mitochondrial Pathway. *International Journal of Molecular Sciences*. 2022;23(19):11653. doi:10.3390/ijms231911653
33. Xiao J, Wang J, Yuan L, Hao L, Wang D. Study on the mechanism and intervention strategy of sunitinib induced nephrotoxicity. *European Journal of Pharmacology*. 2019;864:172709. doi:10.1016/j.ejphar.2019.172709
34. Jalilian F, Moieni-Arya M, Hosseinzadeh L, Shokoohinia Y. Oxypeucedanin and isoimperatorin extracted from *Prangos*

ferulacea (L.) Lindl protect PC12 pheochromocytoma cells from oxidative stress and apoptosis induced by doxorubicin. *Research in Pharmaceutical Sciences*. 2021;17(1):12-21. doi:10.4103/1735-5362.329922

35. Birsoy K, Wang T, Chen WW, Freinkman E, Abu-Remaileh M, Sabatini DM. An Essential Role of the Mitochondrial Electron Transport Chain in Cell Proliferation Is to Enable Aspartate Synthesis. *Cell*. 2015;162(3):540-551. doi:10.1016/j.cell.2015.07.016
36. Li Y, Park JS, Deng JH, Bai Y. Cytochrome c oxidase subunit IV is essential for assembly and respiratory function of the enzyme complex. *Journal of Bioenergetics and Biomembranes*. 2006;38(5-6):283-291. doi:10.1007/s10863-006-9052-z