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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ISOIMPERATORIN-MEDIATED ANTICANCER ACTIVITY: ROLE OF MITOCHONDRIAL DYSFUNCTION IN HEPG2 CELLS

İZOİMPERATORİN ARACILIKLI ANTİKANSER AKTİVİTE: MİTOKONDRİYAL DİSFONKSİYONUN HEPG2 HÜCRELERİNDEKİ ROLÜ

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ABSTRACT

Objective: *The first goal of the present study is to investigate the role of mitochondria due to the Crabtree effect in HepG2 cells exposed to ISO in either glucose- or galactose-conditioned media. The second aim is to predict the interactions between electron transport chain (ETC) complexes and ISO, which might be the possible reason for mitochondrial dysfunction.*
Material and Method: *Cell viability and membrane damage for HepG2 cells exposed to ISO (12.5, 25, 50, 100, and 250 µM) were assessed by MTT and LDH leakage assays in either glucose- or*

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galactose-conditioned media. The affinity of ISO to ETC complexes was also determined by a molecular docking study.
Result and Discussion: *MTT assay showed that 250 µM ISO leads to cytotoxic activity in glucose-conditioned media, while 25 µM and higher concentrations of ISO decrease cell viability in galactose-conditioned media. A membrane damage assay conducted in a glucose-conditioned media assay revealed that 250 µM ISO disrupts the cell membrane. 100 and 250 µM ISO increased membrane damage in galactose-conditioned media. According to docking simulations, binding affinities of ISO to ETC complexes are in descending order: Complex IV > Complex I > Complex III > Complex II. Inhibition of complex IV by ISO inhibits the transfer of electrons from cytochrome c to oxygen, and the proton gradient collapses. The present study proposed that ISO leads to mitochondrial dysfunction via inhibition of the ETC.*
Keywords: *Anticancer, crabtree effect, electron transport chain, isoimperatorin, mitochondrial dysfunction*

ÖZ

Amaç: *Çalışma kapsamındaki ilk amaç, glukoz veya galaktoz içeren besiyerlerinde İzoimperatorin'e (İZO) maruz kalmış HepG2 hücrelerindeki mitokondrinin rolünü Crabtree etkisi nedeni ile araştırmaktır. İkinci amaç, mitokondriyal disfonksiyonun ortaya çıkmasında rolü olabilecek olan İZO ve elektron transfer zinciri arasındaki (ETZ) etkileşimi öngörmektir.*
Gereç ve Yöntem: *Glukoz veya galaktoz içeren besiyerlerinde İZO (12.5, 25, 50, 100 ve 250 µM) ile inkübe edilen HepG2 hücrelerin canlılığı ve membran hasarı MTT ve LDH sızma deneyleri ile gerçekleştirilmiştir. İZO'nun ETZ kompleksleri üzerine olan afinitesi moleküler kenetleme çalışması ile analiz edilmiştir.*
Sonuç ve Tartışma: *MTT deneyi sonuçlarına göre glukoz içeren besiyerinde 250 µM İZO sitotoksik etki gösterirken, galaktoz içeren besiyerinde 25 µM ve daha yüksek konsantrasyonlar hücre canlılığını azaltmıştır. Glukoz içeren besiyerinde gerçekleştirilen membran hasarı deneyi, 250 uM İZO'nun membran bütünlüğünü bozduğunu göstermiştir. Galaktoz içeren besiyerinde 100 ve 250 uM İSO membran hasarını artırmıştır. Moleküler kenetlenme çalışma sonuçlarına göre İZO'nun ETZ komplekleri üzerine olan afinitesi Kompleks IV > Kompleks I > Kompleks III > Kompleks II şeklindedir. İZO, elektronun sitokrom C'den oksijene aktarılmasını engelleyerek kompleks IV'ün inhibisyonunu yapmakta ve proton gradiyentinin azalmasına neden olmaktadır. Elde edilen sonuçlar, İZO'nun gerçekleştirdiği ETZ inhibisyonunun mitokondriyal disfonksiyona neden olabileceğini göstermektedir.*
Anahtar Kelimeler: *Antikanser, crabtree etkisi, elektron transfer zinciri, izoimperatorin, mitokondriyal disfonksiyon*

INTRODUCTION

Cancer is a global public health challenge. It ranks second in death cases after cardiovascular disorders in nations with high life quality, which means that cancer is an obstacle to increasing human life expectancy [1]. One of the most common cancer treatments is chemotherapy. Increasing cancer incidence and mortality has encouraged researchers to focus on novel strategies. Even though drug development studies for cancer treatment have gradually increased recently, there is no precise and successful treatment for all types of cancer. Original molecules and novel molecular pathways must be studied to cope with cancer treatment challenges such as resistance or drug-induced adverse effects. Taken together, novel mechanisms for marketed chemicals showing anticancer activity must also be defined *in vitro*, as new targets might contribute to developing more effective and safer anticancer molecules [2,3].

Mitochondria maintain many critical cellular processes. It plays a central role in energy homeostasis in normal cells, producing more than 90% of adenosine triphosphate (ATP). Nevertheless, cancer cells produce approximately 50-60% of adenosine triphosphate (ATP) in the cytosol with increased glucose uptake and lactate production, called Crabtree or Warburg effects [4]. *In vitro* anticancer studies are performed with immortalized cancer cells using high glucose-conditioned media to illuminate new pathways. However, mechanistic studies investigating the role of mitochondria cannot reflect reliable results in cancer cells cultured in high glucose-conditioned media due to the Crabtree

effect, as the mitochondria are not sufficiently active for energy homeostasis in cancer cells in high glucose-conditioned media. Marroquin et al. (2007) proposed a model of HepG2 cells by replacing glucose with galactose in cell culture media. This model forces HepG2 cells to produce ATP by shifting from the cytosol to mitochondria, consequently making HepG2 cells vulnerable to mitochondrial toxicity [5]. Several studies adopted this model to investigate mitochondrial dysfunction in several cell types [6-9]. When taking into account that mitochondria play an essential role in biomass synthesis, including fatty acids, amino acids, and nucleotides, which are required for the growth and proliferation of cancer cells, it is noteworthy for scientists to investigate the interactions of anticancer molecules with possible regions or structures in mitochondria by using HepG2 cells vulnerable to mitochondrial toxicity [8-10].

Phytochemicals and their synthetic derivatives have been increasingly used to treat and manage diseases. Several phytochemicals have been identified and characterized to date in order to suppress cancer progression and development [11,12]. Isoimperatorin (ISO) is a secondary plant metabolite belonging to natural furanocoumarins. It is one of the most abundant compounds in the Apiaceae family, which includes Angelica, Notopterygium, Peucedanum, Ferula, Ferulago, and Prangos species [13-15].

Previous studies reported that ISO shows various pharmacological activities, including antitumor, anti-inflammatory, antibacterial, anti-hypertensive, analgesic, and antiviral properties. ISO has been commonly used in traditional Chinese medicine (TDC) due to its various biological activities [16-19]. ISO was reported to have anticancer activities *in vitro* [20,21]. However, the mitochondrial pathway of ISO-induced anticancer activity using human hepatocellular carcinoma (HepG2) cells vulnerable to mitochondrial toxicants in different conditioned media remained ambiguous. Therefore, the main goal of the present study is twofold; i) to assess the role of mitochondria in HepG2 cells exposed to ISO in either glucose- or galactose-conditioned media ii) to predict the possible targets for ISO in mitochondria by molecular docking studies.

MATERIAL AND METHOD

Materials and Cell Culture

All chemicals and compounds were purchased from Sigma-Aldrich (Darmstadt, Germany) except for cell culture reagents. All cell culture reagents and supplements were obtained from Thermo-Fisher Scientific (Loughborough, UK). HepG2 cells were cultured under high glucose and galactose-conditioned media, as described previously [5].

Isolation and Characterization of Isoimperatorin

ISO was isolated and identified from endemic *Prangos heyniae* H.Duman & M.F.Watson in our previous studies [22,23]. The plant roots were collected from Konya province (1580 m altitude) in Türkiye on 3 June 2016. The collected plant was authenticated by authors and deposited in Ege University. Air-dried roots of the plant (399g) were extracted with *n*-hexane (3×3l), chloroform (3×3l), and methanol (3×3l), sequentially in a sonicator (Bandelin-Sonorex RK-514-BH, Germany) at room temperature. After filtration and evaporation (Heidolph Laborota-4000, Germany) of three extracts, *n*-hexane (25g), chloroform (9g), and methanol extracts were yielded. Chloroform extract was suspended in MeOH (250 ml) at 4°C for 24 h in oreder to precipitate the compounds. The mix was filtered and separated into two fractions. The precipitated portion (630mg) was applied to a silica gel column (50g; column size, 3id×900 mm) with an isocratic elution of n-hexane/ethyl acetate (7:3, 300 ml). The compound (200mg) was purified and yielded after this process, and its structure was elucidated as ISO by 1D NMR and LC-MS [22].

Cell Viability Assay

The cell viability was determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay in high glucose or galactose conditioned media as described previous studies with minor modifications [5,24]. In brief, HepG2 cells (10^4 cells/well) were exposed to ISO (12.5, 25, 50, 100, and 250 μM) 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 was added for each

well, and the plate was incubated for 4 h. At the end of the incubation, formazan crystals were dissolved with DMSO, and the colour of intensity was measured by a multi-plate reader as described in our previous study [24].

Membrane Integrity Assay

Lactate dehydrogenase (LDH) enzyme exists in cytosol of mammary cells. LDH enzyme releases from the cytosol into the medium when the cell membrane is damaged. Measurement of the extracellular LDH enzyme activity is used to detect membrane damage, which means that increased LDH enzyme activity is proportional to membrane disruption [25].

Membrane damage was investigated by LDH leakage assay in HepG2 cells in high glucose or galactose-conditioned media as described previous studies with minor modifications [5,26]. In brief, HepG2 cells (10^4 cells/well) were exposed to ISO (12.5, 25, 50, 100, and 250 μM) 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 described in previous study [26].

Molecular Docking Study

Molecular docking studies were conducted using the MOE 2020 (Molecular Operating Environment 2020) program to determine the interactions of ISO with electron transfer system enzyme complexes. The crystal structures of Complex I (PDB ID: 5XTD) (Guo et al., 2017), Complex II (PDB ID: 8GS8) (Du et al., 2023), Complex III (PDB ID: 5XTE) (Guo et al., 2017), and Complex IV (PDB ID: 5Z62) (Zong et al., 2018) were obtained from the RCSB Protein Data Bank (www.rcsb.org). However, the human crystal structure of Complex V could not be used as it has not yet been defined. To prepare the enzymes for docking, they were energy-minimized, water molecules and ligands used for crystallization were removed, and the surfaces of the complexes were scanned to identify the active sites.

The 2D structure of ISO 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.

In the docking study, the binding sites used during enzyme crystallization were selected and modeled as the binding sites for ISO. The modeling process was limited to the 30 most stable conformers with different torsion angles for ISO. The obtained data were analyzed based on the Root Mean Square Deviation (RMSD) and binding energy to interpret the results.

Statistics

Data were expressed as the mean ± SD from three experiments in triplicate. GraphPad Prism Software version 8.4.2 (San Diego, CA, USA) for Windows were used for statistical analyses. Statistical comparisons were made using the Mann-Whitney U test with a confidence level of 95%. Accordingly, significance was accepted when $p \leq 0.05$.

RESULT AND DISCUSSION

The basic purpose of anticancer therapy depends on killing cancer cells having uncontrolled and high proliferation rates. One of the most used targets in cancer cells is to inhibit mitochondrial function, as the mitochondria play a key role in maintaining genomic stability and generating building blocks by synthesizing constitutional nucleotides, amino acids, and enzymes [27]. Immortalized cancer cells cultured in glucose-conditioned media make cells to mitochondrial toxicants inconvenient for illuminating novel anticancer mechanisms due to the Crabtree effect [28,29].

In this present study, HepG2 cells were cultured in either high glucose or galactose-conditioned media to overcome the Crabtree effect and investigate the possible role of mitochondria in the anticancer

activity of ISO. Molecular docking studies were also performed to predict the binding affinities of ISO in ETC.

Cell Viability Assay

Previous studies reported that ISO led to anticancer and antiproliferative activities in various types of cancer cells, including lung, ovarian, skin, colon, gastric, breast, and glioblastoma, which indicated a wide range of IC_{50} values ranging between approximately 50 µM and 1.1 mM [10,30,31]. However, only a few studies have mentioned the mechanism of mitochondrial alterations in ISO-induced anticancer activity. Tong et al. (2017) revealed that ISO induces apoptosis in SGC-7901 cells by altering the expression levels of apoptotic proteins [22]; in contrast, another study suggested that ISO improves mitochondrial function and shows a protective effect against carbon tetrachloride-induced liver injury [32]. These mechanistic studies revealed data about mitochondrial activity; however, they estimated the Crabtree effect and did not make the cells vulnerable to mitochondrial toxicants to uncover the mechanism of mitochondrial alterations.

Cytotoxic concentrations of ISO were determined by MTT assay in HepG2 cells cultured in high glucose (Figure 1A) or galactose (Figure 1B) conditioned media. Although ISO did not cause cytotoxicity between the 12,5 and 100 µM range in glucose-conditioned media, 250 µM of ISO moderately reduced cell viability by 33% compared to the control (IC_{50}>250 µM). ISO caused dose-dependent manner cytotoxicity in galactose-conditioned media. 12.5, 25, 50, 100, and 250 µM ISO reduced cell viability approximately by 34, 42, 45, and 55%, respectively, compared to the control (IC_{50}:161 µM). Triton X-100 decreased cell viability by approximately 92% compared to control in both media (Figures 1A and 1B)

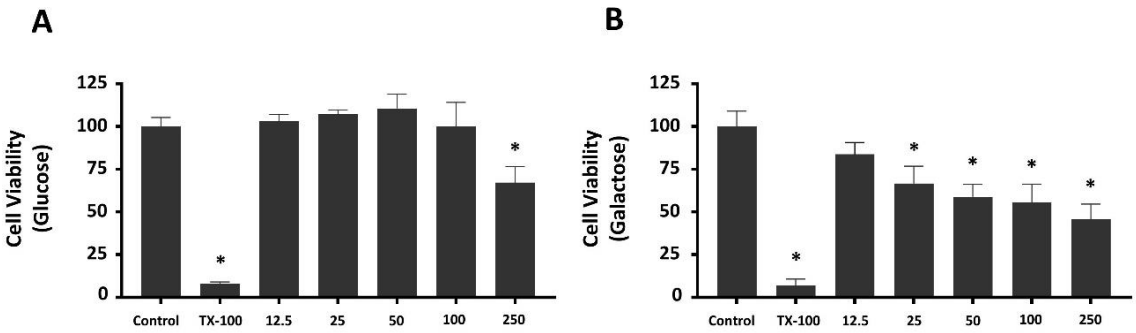

Figure 1. Cytotoxicity of ISO was determined by MTT assay in high glucose (A) or galactose (B) conditioned media in HepG2 cells after 24 hours incubation. Values are mean ± SD from three independent experiments. Experimental data were expressed percent of solvent (1% DMSO) control. (*) significantly different ($p<0.05$) than the solvent control (1% DMSO)

Membrane Integrity Assay

MTT assay indicated that replacing glucose with galactose exacerbated the cytotoxicity of ISO in HepG2 cells (Figures 1A and 1B). Then, an LDH leakage assay was also performed to observe alterations in cell membrane integrity in HepG2 cells exposed to ISO in high glucose (Figure 2A) or galactose (Figure 2B) conditioned media. ISO did not cause any alteration in membrane integrity between the range of 12.5 and 100 µM (Figure 2A); nevertheless, LDH activity statistically increased by 33% at only 250 µM ISO compared to the control in high glucose-conditioned media (IC_{50}>250 µM). Galactose-conditioned media made HepG2 cells vulnerable to ISO-induced membrane damage (Figure 2B). Although 12.5, 25, and 50 µM ISO did not alter LDH activities, 100 and 250 µM ISO caused membrane damage and increased LDH activities by 41% and 69%, respectively (IC_{50}: 195 µM). Triton X-100 increased LDH activity by 42% and 106% in glucose and galactose-conditioned media, respectively.

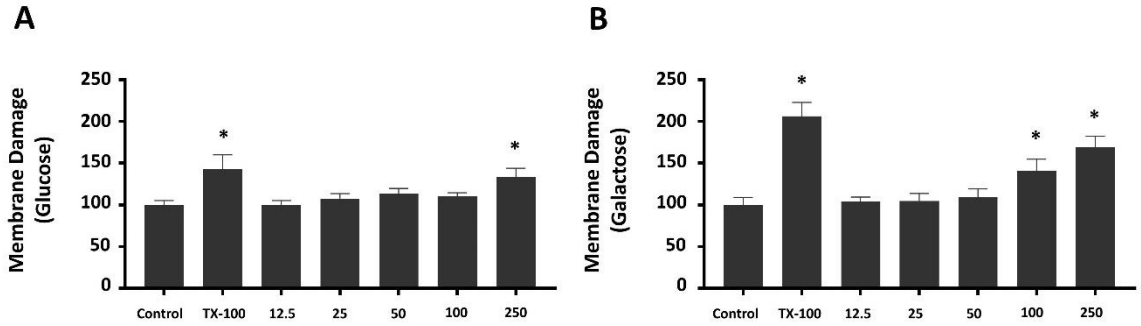

Figure 2. Membrane damage of ISO was determined by LDH leakage assay in high glucose (A) or galactose (B) conditioned media in HepG2 cells after 24 hours incubation. Values are mean ± SD from three independent experiments. Experimental data were expressed percent of solvent (1% DMSO) control. (*) significantly different ($p<0.05$) than the solvent control (1% DMSO)

MTT assay indicated that IC_{50} of glucose conditioned media (>250 μM) is at least 0.55 fold more than IC_{50} of galactose conditioned media (161 μM) (Figures 1). LDH leakage assay revealed that IC_{50} of glucose conditioned media (>250 μM) at least 0.28 fold more than IC_{50} of galactose conditioned media (195 μM) (Figures 2). Lower IC_{50} values calculated from galactose conditioned media mean that HepG2 cells are more prone and vulnerable to cytotoxicity. Given these findings we might conclude that replacing glucose with galactose uncovered the role of mitochondria in ISO-mediated anticancer activity.

Molecular Docking Studies

Mitochondria are double-membrane organelles. The outer membrane transfers ions and molecules of less than 1.5 kDa via anion channels and pores, while the inner membrane is impermeable and allows only tiny molecules via specialized transporters. The inner membrane also contains five protein complexes (I, II, III, IV, and V), which produce ATP and membrane potential required for mitochondrial homeostasis and function. ETC synthesizes constitutional nucleotides, amino acids, and enzymes such as aspartase, pyrimidine, and proline. These building blocks are essential and required for highly proliferative cells such as cancer cells. Inhibition of ETC was reported to contribute to the decrease in cancer cell growth [27,33,34]. Also, MTT and LDH leakage assays proposed that mitochondrial pathways play a primary role in ISO-induced anticancer activity (Figures 1 and 2). For this reason, molecular docking studies were also applied to investigate whether ISO shows affinity to ETC in mitochondria, which is the possible reason for mitochondrial dysfunction (Table 1 and Figure 3).

Table 1. Docking result of ISO with the complexes

Targets	Ligand = ISO			
	Binding energy (kcal/mol)	**RMSD values**	**Binding site amino acids**	**Interactions**
Complex I (5XTD)	-6.9640	1.4263	Phe 64, Gly 63, Asp 205	Aren (π)-H, Ligand exposure
Complex II (8GS8)	-6.5313	1.6468	Asn 81, Arg 512, Leu 513, Gln 516	Aren (π)-H, Aren (π)-cation, H-bond acceptor, H-bond donor, Ligand exposure
Complex III (5XTE)	-6.7658	1.1445	Ala 84, Gly 130, Tyr 131	Ligand exposure
Complex IV (5Z62)	-7.1741	1.2043	Trp 126, Tyr 129, Trp 236, His291, Val 373	Aren (π)-H, Aren (π)- Aren (π), Ligand exposure

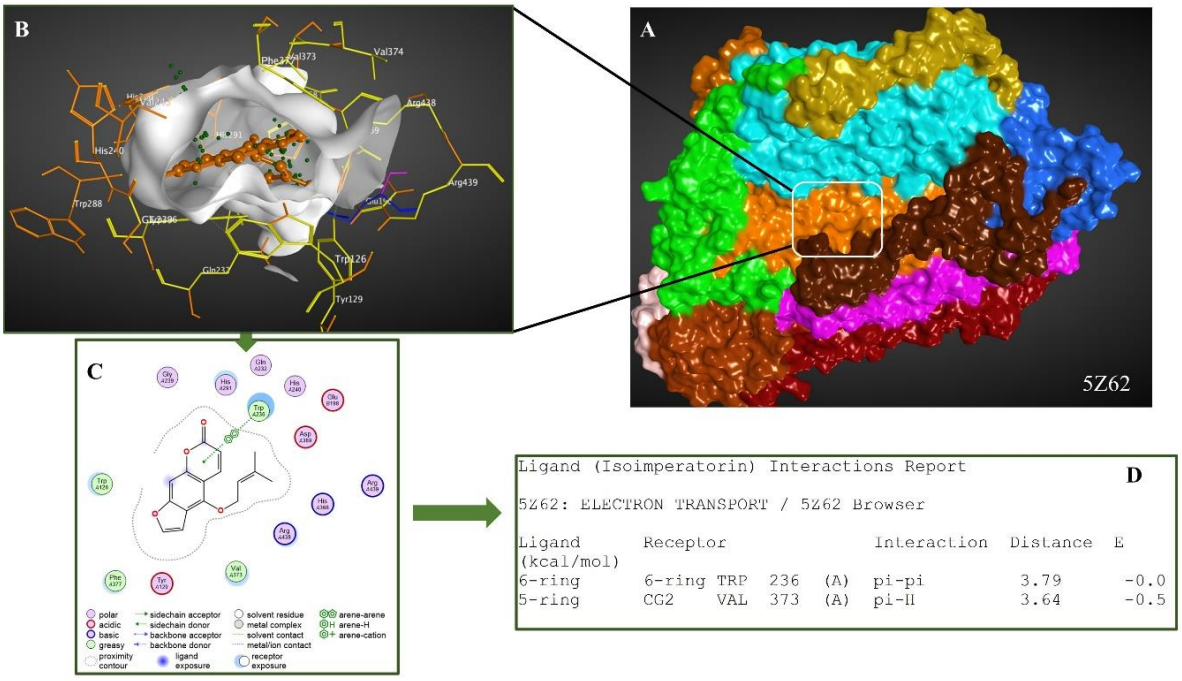

Figure 3. 2D binding pose of ISO with human cytochrome c oxidase (PDB ID: 5Z62) active site. Receptor cite (A), binding site amino acids (B), interactions (C), and ligand interaction report (D)

The binding energy in MOE 2020 shows the bonding relationship between ISO and amino acids in the enzymes. According to docking simulations, binding affinities of ISO to ETC complexes are in descending order: Complex IV > Complex I > Complex III > Complex II. Since the human three-dimensional crystal structure of complex V has not been defined, it could not be used in the docking study. ISO showed better binding with Complex IV (-7.1741 kcal/mol) than other complexes, considering the binding energy and RMSD values. It is observed that hydrophobic amino acids (tryptophan, tyrosine, valine, etc.) play a significant role in the interaction at the receptor site and increase the capacity to bond with unsaturated bonds (Table 1). The amino acids Trp 126, Tyr 129, Trp 236, and Val 373 in the receptor region of the A chain of this protein were essential in the better binding energy of ISO with complex IV (Figure 3). The function of this complex IV is to transfer electrons from cytochrome c to oxygen, creating a proton gradient. Inhibiting the complex IV by ISO might suppress ATP generation by blocking the proton gradient.

The present study assessed the role of mitochondria in ISO-induced anticancer activity in HepG2 cells vulnerable to mitochondrial toxicants. Our data suggest that ISO primarily causes anticancer activities via mitochondrial dysfunction with the highest binding energy to complex IV. Further studies need to be implemented to determine which molecular pathways play a role in ISO-induced mitochondrial dysfunction in HepG2 cells vulnerable to mitochondrial toxicants.

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AUTHOR CONTRIBUTIONS

Concept: A.E.; Design: A.E., E.A., F.K.; Control: A.E., E.A., G.A., F.K., H.O., Ş.B.; Sources: A.E., E.A., G.A., F.K., H.O., Ş.B.; Materials: A.E., E.A., G.A., F.K., H.O., Ş.B.; Data Collection and/or Processing: A.E., E.A., G.A., F.K., H.O., Ş.B.; Analysis and/or Interpretation: A.E., E.A., G.A., F.K.,

H.O., Ş.B.; Literature Review: A.E., E.A., G.A., F.K., H.O., Ş.B.; Manuscript Writing: A.E., E.A., G.A., F.K., H.O., Ş.B.; Critical Review: A.E., E.A., G.A., F.K., H.O., Ş.B.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 71(3), 209-249. [CrossRef]
2. Zugazagoitia, J., Guedes, C., Ponce, S., Ferrer, I., Molina-Pinelo, S., Paz-Ares, L. (2016). Current challenges in cancer treatment. Clinical Therapeutics, 38(7), 1551-1566. [CrossRef]
3. Tsimberidou, A.M., Fountzilas, E., Nikanjam, M., Kurzrock, R. (2020). Review of precision cancer medicine: Evolution of the treatment paradigm. Cancer Treatment Reviews, 86, 102019. [CrossRef]
4. Liberti, M.V., Locasale, J.W. (2016). The warburg effect: How does it benefit cancer cells? Trends in Biochemical Sciences, 41(3), 211-218. [CrossRef]
5. Marroquin, L.D., Hynes, J., Dykens, J.A., Jamieson, J.D., Will, Y. (2007). Circumventing the crabtree effect: Replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants. Toxicological sciences: An Official Journal of the Society of Toxicology, 97(2), 539-547. [CrossRef]
6. Pereira, C.V., Oliveira, P.J., Will, Y., Nadanaciva, S. (2012). Mitochondrial bioenergetics and drug-induced toxicity in a panel of mouse embryonic fibroblasts with mitochondrial DNA single nucleotide polymorphisms. Toxicology and Applied Pharmacology, 264(2), 167-181. [CrossRef]
7. Swiss, R., Will, Y. (2011). Assessment of mitochondrial toxicity in HepG2 cells cultured in high-glucose- or galactose-containing media. Current Protocols in Toxicology, Chapter 2, Unit2.20. [CrossRef]
8. Will, Y., Dykens, J. (2014). Mitochondrial toxicity assessment in industry-A decade of technology development and insight. Expert Opinion on Drug Metabolism & Toxicology, 10(8), 1061-1067. [CrossRef]
9. Dott, W., Mistry, P., Wright, J., Cain, K., Herbert, K.E. (2014). Modulation of mitochondrial bioenergetics in a skeletal muscle cell line model of mitochondrial toxicity. Redox Biology, 2, 224-233. [CrossRef]
10. Liu, Y., Shi, Y. (2020). Mitochondria as a target in cancer treatment. MedComm, 1(2), 129-139. [CrossRef]
11. González-Vallinas, M., González-Castejón, M., Rodríguez-Casado, A., Ramírez de Molina, A. (2013). Dietary phytochemicals in cancer prevention and therapy: A complementary approach with promising perspectives. Nutrition Reviews, 71(9), 585-599. [CrossRef]
12. Wong, S.C., Kamarudin, M.N.A., Naidu, R. (2021). Anticancer mechanism of curcumin on human glioblastoma. Nutrients, 13(3), 950. [CrossRef]
13. Danchul, T.Y., Kuznetsova, G., Sokolova, E., Kuz'mina, L. (1979). Coumarins from the roots of *Prangos lamellata*. Chemistry of Natural Compounds 15, 753.
14. Kuznetsova, G., Danchul, T.Y., Sokolova, E., Kuz'mina, L. (1979b). Coumarins from the roots of *Prangos arcis-romanae*. Chemistry of Natural Compounds, 15, 751-752.
15. Wei, Y., Ito, Y. (2006). Preparative isolation of imperatorin, oxypeucedanin and isoimperatorin from traditional Chinese herb "bai zhi" *Angelica dahurica* (Fisch.ex Hoffm) Benth. et Hook using multidimensional high-speed counter-current chromatography. Journal of Chromatography. A, 1115(1-2), 112-117. [CrossRef]
16. Baek, N.I., Ahn, E.M., Kim, H.Y., Park, Y.D. (2000). Furanocoumarins from the root of *Angelica dahurica*. Archives of Pharmacal Research, 23(5), 467-470. [CrossRef]
17. Bruni, R., Barreca, D., Protti, M., Brighenti, V., Righetti, L., Anceschi, L., Mercolini, L., Benvenuti, S., Gattuso, G., Pellati, F. (2019). Botanical sources, chemistry, analysis, and biological activity of furanocoumarins of pharmaceutical interest. Molecules, 24(11), 2163. [CrossRef]
18. Moon, T.C., Jin, M., Son, J.K., Chang, H.W. (2008). The effects of isoimperatorin isolated from *Angelicae dahuricae* on cyclooxygenase-2 and 5-lipoxygenase in mouse bone marrow-derived mast cells. Archives of Pharmacal Research, 31(2), 210-215. [CrossRef]

19. Park, H.Y., Kwon, S.B., Heo, N.K., Chun, W.J., Kim, M.J., Kwon, Y.S. (2011). Constituents of the stem of Angelica gigas with rat lens aldose reductase inhibitory activity. Journal of the Korean Society for Applied Biological Chemistry, 54(2), 194-199. [CrossRef]
20. Kim, Y.K., Kim, Y.S., Ryu, S.Y. (2007). Antiproliferative effect of furanocoumarins from the root of *Angelica dahurica* on cultured human tumor cell lines. Phytotherapy Research, 21(3), 288-290. [CrossRef]
21. Tong, K., Xin, C., Chen, W. (2017). Isoimperatorin induces apoptosis of the SGC-7901 human gastric cancer cell line via the mitochondria-mediated pathway. Oncology Letters, 13(1), 518-524. [CrossRef]
22. Albayrak, G. (2020). PhD Thesis. Investigation on phytochemistry and bioactivity of endemic *Prangos heyniae* H. Duman & M.F. Watson and *Prangos uechtritzii* Boiss. & Hausskn. Species naturally distributed in Anatolia. Department of Pharmaceutical Botany, Faculty of Pharmacy, Ege University, İzmir, Turkey.
23. Albayrak, G., Demir, S., Koyu, H., Baykan, S. (2023). Anticholinesterase and antityrosinase activities of endemic *Prangos heyniae* H. Duman & M.F. Watson and its metabolites. İstanbul Journal of Pharmacy, 53 (1), 51-57. [CrossRef]
24. Kuzu, B., Ergüç, A., Karakuş, F., Arzuk, E. (2023). Design, synthesis, and antiproliferative activities of novel thiazolyl-pyrazole hybrid derivatives. Medicinal Chemistry Research, *in press*. [CrossRef]
25. Adan, A., Kiraz, Y., Baran, Y. (2016). Cell proliferation and cytotoxicity assays. Current Pharmaceutical Biotechnology, 17(14), 1213-1221. [CrossRef]
26. Hassoun, E.A., Roche, V.F., Stohs, S.J. (1993). Release of enzymes by ricin from macrophages and Chinese hamster ovary cells in culture. Toxicology Methods, 3(2), 119-129. [CrossRef]
27. Desler, C., Munch-Petersen, B., Stevnsner, T., Matsui, S., Kulawiec, M., Singh, K.K., Rasmussen, L.J. (2007). Mitochondria as determinant of nucleotide pools and chromosomal stability. Mutation Research, 625(1-2), 112-124. [CrossRef]
28. Kamalian, L., Chadwick, A.E., Bayliss, M., French, N.S., Monshouwer, M., Snoeys, J., Park, B.K. (2015). The utility of HepG2 cells to identify direct mitochondrial dysfunction in the absence of cell death. Toxicology in Vitro, 29(4), 732-740. [CrossRef]
29. Vaupel, P., Schmidberger, H., Mayer, A. (2019). The warburg effect: Essential part of metabolic reprogramming and central contributor to cancer progression. International Journal of Radiation Biology, 95(7), 912-919. [CrossRef]
30. Shokoohinia, Y., Sajjadi, S.E., Gholamzadeh, S., Fattahi, A., Behbahani, M. (2014). Antiviral and cytotoxic evaluation of coumarins from Prangos ferulacea. Pharmaceutical Biology, 52(12), 1543-1549. [CrossRef]
31. Bruno, M., Ilardi, V., Lupidi, G., Quassinti, L., Bramucci, M., Fiorini, D., Venditti, A., Maggi, F. (2019). The nonvolatile and volatile metabolites of *Prangos ferulacea* and their biological properties. Planta Medica, 85(11-12), 815-824. [CrossRef]
32. Yang X., Liang L., Wang X. (2018). Protective effects of isoimperatorin on CCL4 induced acute liver injury in mice. Chinese Journal of Hospital Pharmacy, 38(13), 19-23.
33. Nolfi-Donegan, D., Braganza, A., Shiva, S. (2020). Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. Redox Biology, 37, 101674. [CrossRef]
34. Kühlbrandt, W. (2015). Structure and function of mitochondrial membrane protein complexes. BMC Biology, 13, 89. [CrossRef]