DJS Vol. 48(2)(2024) –pp. 182-192 ISSN: 1012-5965

Delta Journal of Science

Available online at
https://djs.journals.ekb.eg/

Research Article

Zoology

Enhancement of Docetaxel therapeutic efficacy by catechin from black grapes (*Vitis vinifera L.*) in (PC3) cells

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Received: 11/ 7 / 2024 **Accepted:** 2 /9/2024

KEY WORDS

DNA toxicity, Prostate cancer, SRB assay, *Vitis vinifera L.*

ABSTRACT

In the present investigation, the potential cytotoxic impact of catechin (Cat) combination therapy was examined, a highly polyphenolic compound extracted from black grapes (*Vitis Vinifera L.*), and Docetaxel (DTX) against prostate cancer cells (PC-3 cells) using Sulforhodamine B (SRB) assay. After extraction and characterization with HPLC chromatography, and evaluation of the Cat extraction, the IC_{50} doses of treatment groups were identified in the PC-3 cell line: DTX therapy, Cat therapy, DTX + Cat treatment (1:1 volume), and untreated cells used as controls. The results show that the treatment with DTX, Cat, or their combination dramatically reduced the total number of PC-3 cells that survived. Also, the treatments induced DNA breakdown as indicated by DNA fragmentation assay causing clear cellular toxicity and apoptosis to the prostate cancer cells. The morphological changes seen after treatment with each drug alone were cell shrinkage, cell rounding, detaching, floating and obvious cell swelling and rupture indicating apoptotic features. Treatment with the combination of both compounds has shown more prominent rounded cells with more obvious apoptotic features than each treatment alone. In conclusion, Cat induced strong cellular cytotoxicity to prostate cancer cells, while it promotes the chemotherapeutic activity of DTX when administered in combination.

Introduction

One of the malignant diseases is prostate cancer (PC) that is becoming more common among men **(Okamoto *et al.*, 2020**). Prostate cancer ranks fourth globally among malignant neoplasms that cause mortality in men, according to epidemiological statistics. Due to smoking, it is becoming more common in male patients. According to Globocan 2022, prostate cancer is anticipated to be the fourth most common malignant neoplasm in Egypt, accounting for 2,102 fatalities and 5,181 new cases per year (**Sung *et al.*, 2021**).

Chemotherapy, surgery, and natural products are approaches for cancer therapy (**Wang *et al.*, 2018**). Chemotherapy is the most popular cancer treatment, despite having plenty of side effects. Organic compounds can be used as a treatment on their own or in conjunction with traditional chemotherapeutic drugs (**Lin *et al.*, 2020**). Several natural substances have demonstrated in clinical trials the efficacy in killing malignant cells, including flavonoids, phenols, terpenes, and alkaloids (**Bisol *et al.*, 2020**).

The Mediterranean region frequently grows *Vitis vinifera L*. (black grapes); a crop that is rich in bioactive substances that support the body's defense processes (**Haseeb *et al.*, 2019**). The polyphenolic molecule Cat, which is most prevalent in black grapes, has demonstrated therapeutic effects against many cancer types *in vivo* and *in vitro*, making it a prospective candidate for anticancer therapy **(Cheng *et al.*, 2020**). The main pro-apoptotic gene *p53* (tumor suppressor) is responsible for inducing apoptosis. It also triggers DNA repair and activates G1/S and G2/M phases of cell cycle checkpoints. The Bcl-2 gene family is responsible for inducing apoptosis. Bax, a member of this gene family, releases cytochrome C from the inner-membrane gap in the mitochondria, acting as one of the downstream mediators of the apoptotic dependent on the tumor suppressor gene p53. It moves from the cytosolic to the mitochondrial as well as initiates the caspase cascade, which is the preparatory step toward the final stage of apoptosis **(Huska *et al.*, 2019).**

Docetaxel (DTX) is an anti-tumor antibiotic that is made from Yew tree needles and is a member of the toxoids class of anti-cancer drugs (**Montero *et al.*, 2005)**. Because of its broad antitumoral action, it is effective as a chemotherapeutic drug for treating a variety of carcinomas, such as stomach, prostate and soft tissue (**Lin *et al.*, 2012**). This prevents the normal assembling of

microtubules into the mitotic spindle, which terminates the cell process during G2/M. Moreover, tumor cells frequently express the Bcl-2 protein to prolong their life, and inhibiting this gene makes it simpler for tumor cells to undergo apoptosis (**Jiang *et al*., 2018**). Recently, combination therapy referred to the treatment of a disease with two or more medications **(Salim *et al*., 2023)**.

Accordingly, the current investigation aimed to investigate the potency of Cat and DTX combined therapy compared to the potency of each medication separately. Furthermore, using biochemical and molecular parameters, the investigation assessed the underlying processes related to the anticancer effects of Cat, DTX, and their combinations.

Material and Methods

Chemicals: Docetaxel **(**DTX) (Sanofi/Aventis U.S.A LLC Bridgewater, Newjersy 08807A) was extracted as a pale yellow to brownish yellow, sterilized non-pyrogenic fluid.

Cell line and cell culture

Nawah Scientific Inc. provided the human PC-3 prostatic cancer cell line (Mokatam, Cairo, Egypt). The cells were cultured in DMEM medium with 10.0 percent heat-inactivated fetal bovine serum added as a supplementary component, streptomycin (100.0 mg/mL), and penicillin (100.0 units /mL) were used in a humidified 5.0 percent (v/v) CO_2 incubator (37°C). The viability of cells has been assessed using the SRB test. The 96-well plates containing aliquots of 100.0 µL suspended cells (5x10^3) were incubated for 24 hours with a full medium. After that, the cells were given an additional 100 µL aliquot of media with various drug doses (0.01, 0.1, 1.0, 10.0 and 100.0 µm).

Following a 48-hour drug treatment, after an hour of incubation at 4 degrees Celsius, the cells were fixed by adding 150.0 µL of ten percent TCA to the solution. The cells are rinsed five times with distilled water after the TCA solution is removed. Following the addition of 70.0 µL of SRB solution (0.4 percent w/v), the mixture was incubated for 10 minutes at room temperature in the dark. Following the elimination of the TCA solution, the cells are washed five times with distilled water. After adding 70.0 µL of SRB solution (0.4 percent w/v), then incubated at room temperature in darkness for ten minutes. The used plates were thoroughly washed 3 rounds in one percent acetic acid and then were left to air dry for a full night. Following that, 150.0 µL of TRIS (10.0 mM) was used to liquefy the SRB-bounded protein staining and a BMGLABTECH®-FLUO star Omega microplate reader (Ortenberg, Germany)

was used to detect the absorbance at 540 nm.

HPLC qualitative analysis

Following Singh's approach, the cat was obtained via a Waters 2690 Alliance HPLC system that included the Waters array detector (996 photodiode) and high/performance liquid chromatography (HPLC-DAD) **(Raja *et al*., 2017)**.

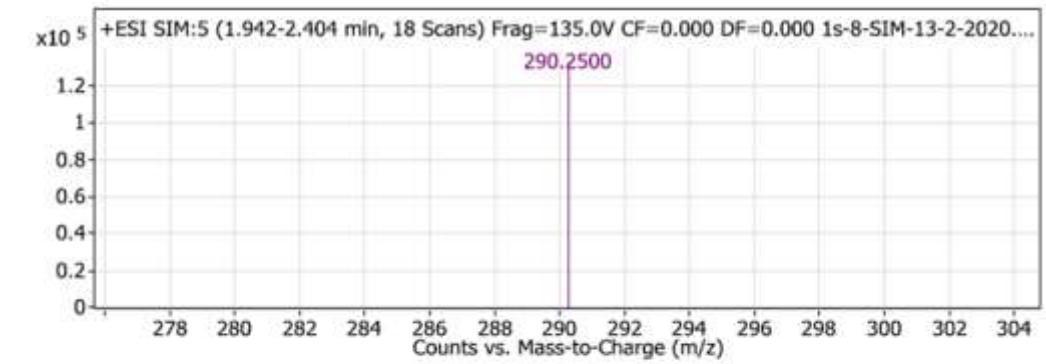
DNA extraction & DNA fragmentation assay

After being re-suspended in 0.5 ml of lysis buffer, the cells were centrifuged at 14,000 rpm/RT for 5 minutes after incubating for 1.5 hours at 37°C. The supernatants have been transferred into fresh tubes together with 25 ml of 4 M NaCl (a final concentration of 100 mM) and an equivalent volume of isopropanol. After incubation overnight at -20°C, tubes were centrifuged for 20–25 minutes at 14,000 rpm/RT. Then the DNA pellets were dissolved in 30.0 - 50.0 ml of ddH2O containing 1.0 - 2.0 ml of RNase. The DNA concentration was determined spectrophotometrically after 1 hour of incubation at 37°C, and 5.0 µl of DNA per lane was run on a 1% agarose gel to evaluate the DNA fragmentation degrees. Non-fragmented DNA displayed a smear shape on the gel sheet following DNA extraction, whereas fragmented DNA following treatment revealed itself as fragments separated by a clear distance from the cathode based on base pairs.

Results

HPLC qualitative analysis

HPLC-DAD methodology was developed and validated to assess the molecular weight of Cat in black grapes extract. The spectrum source shows that the molecular weight of catechin is (290.2 g/mol) **(Fig.1)**.



Peak List

m/z	Abund
290.25	133075.59

Fig.(1): Grape extract HPLC chromatograms displaying molecular weight. The Source of the Spectrum Peak (1) in "+TIC SIM", Ionization Mode ESI, Fragmentor Voltage: 135, Collision Energy: 0.

Cell viability data by SRB

The cell viability of PC3 cells after treatment with Cat in **Fig. (2)** shows the percentage of cell viability after 48 hrs incubating of PC3 cells with various concentrations of Cat. The findings demonstrate that, in a dose-dependent way, the fraction of surviving cells decreases as Cat concentrations increase. The data presented in **Fig. (2)** show that the IC_{50} of Cat-treated cells were calculated to be about 100µg/ml.

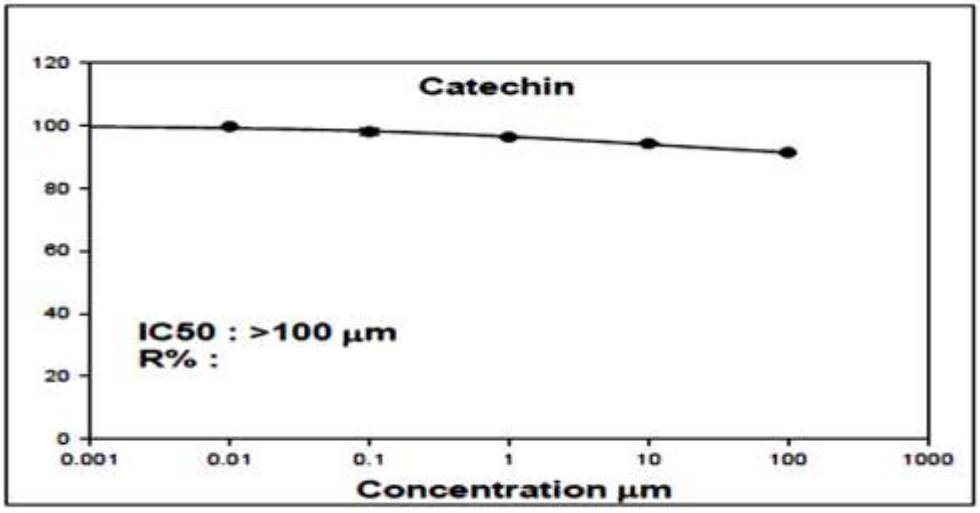


Fig. (2): The percentages of cell viability after 48hrs incubation of PC-3 cells with different concentrations of Cat.

Morphological changes after 48 h incubation with IC_{50} treatment dosages of DTX and Cat

In general, the morphological change of PC-3 cells treated via DTX, Cat, and 1:1 v/v combination drug show mainly apoptotic and cytotoxic changes as well as unusual features compared to untreated control cells **(Fig. 3)**. The modifications seen were generally as follows: cells gradually shrank, were rounded off, detached from the tissue culture flask surface, and floated in the culture medium. Eventually, they displayed pronounced cell swelling and rupture, which suggested apoptotic characteristics. Treatment via a combination of two compounds has shown other prominent rounded cells with more obvious apoptotic features as compared with each treatment alone.

DNA fragmentation assay

DNA extraction & DNA fragmentation test

It was determined using a DNA fragmentation technique whether the potential of treatment drugs, Cat, DTX, or their v/v combination at the IC_{50} concentration dosages could influence the activation of cell death in PC-3. **Fig. (4)** demonstrated that there was essentially no apparent DNA fragmentation when comparing the non-treated cells at lane 1 to the other

treatment groups. In comparison to the control, the treated cells' DNA displayed signs of DNA degradation. Importantly, the gel documentation assay revealed that there was a clear post-treatment DNA fragmentation pattern indicated by a strong ladder configuration. The DNA ladder was stronger and more deleterious in lane 4 of the v/v combination of Cat and DTX than that of lane 3 of Cat treatment which is in turn higher than lane 4 of DTX treatment.

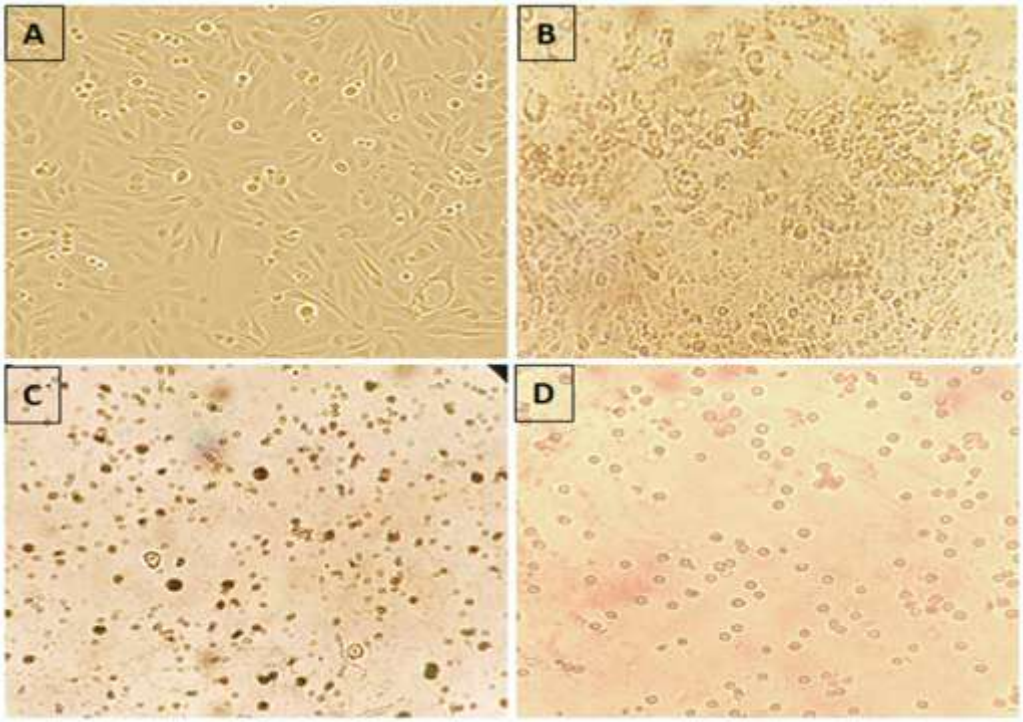


Fig. (3): Morphological changes of PC3 cells: (**A**) untreated-PC3 cells, (**B**) cells administered with DTX, (**C**) cells treated via Cat, and (**D**) cells treated via IC_{50} dose of v/v (1:1) combination of DTX and Cat. Note morphological changes such as rounding of cells, shrinkage, and swelling.

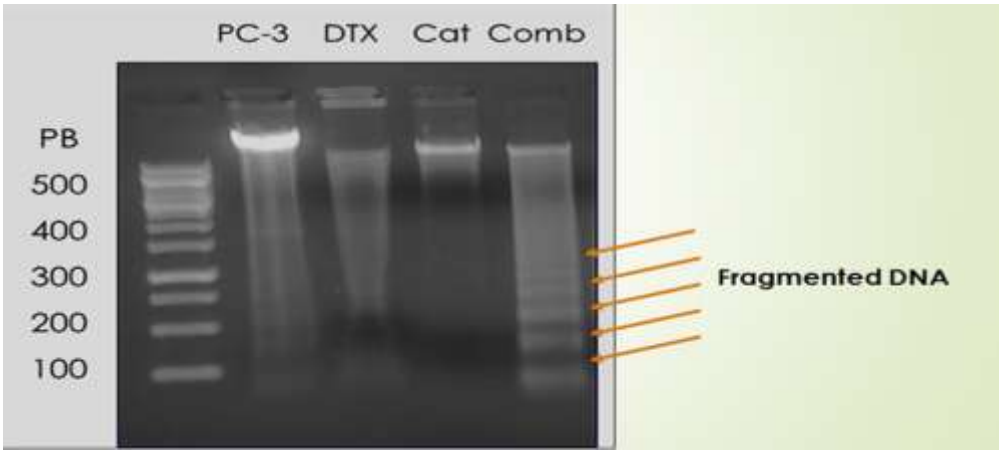


Fig. (4): A photomicrograph of agarose gelhours. Note the absence of a DNA ladder in lane 1 documentation demonstrating the assessment ofcontrol cells (CC). Also, note the stronger DNA fragmentation of DNA using the electrophoreticladder fragments in the combination treatment in examination of DNA obtained from PC-3 cells thatLane 4 (Mix) as compared with Lane 3 of Cat were either treated or not treated with varyingtreatment (T1) followed by Lane 2 of DTX.
amounts of DTX and Cat, as well both, during 24

Discussion

The most prevalent bioactive compounds found in fruits, vegetables, seeds, and other foods are called polyphenolic substances. They have a variety of uses in the prevention and cure of diseases, notably cancer (**Yessenkyzy *et al*., 2020**). A class of chemicals known as catechins is polyphenols that are mostly found in natural plants. They have anti-inflammatory, antioxidant, anticancer, and microvascular properties (**Omori *et al*., 2017**). Furthermore, multiple studies showed that catechin caused apoptosis in several cell lines (**Sari *et al*., 2019**). In vitro studies on several cancerous cells, including breast, melanoma, and cervix revealed that tea catechins inhibited growth and triggered apoptosis (**Payen *et al*., 2017**).

In our study, high-performance liquid chromatography determined the molecular weight of Cat being 290.2 g/mol. In line with the molecular weight of the Cat that was computed via PubChem 2.2 (Pub-Chem release of 2021.10.14) (290.27 g/mol). Also, the obtained results of SRB of Cat could inhibit the growth of PC3 tumor cells, with the half maximal inhibitory concentration being 100µg/ml, in line with a previous result that showed the IC_{50} values of Cat were 146.7±5.1 µg/ml (**Song *et al*., 2014**). Conversely, research has indicated that Cat has both anti-proliferative and apoptotic properties on cancer cells have shown that the treated cells undergo morphological changes such as nuclei fragmentation, shrinking and floating of the cell membrane, and intense DNA condensation (**Gottesman *et al*., 2002**). The DNA extracted from all the treated cells displayed DNA damage when compared with the control. The combination of DTX and Cat was more potent and destructive to DNA than the Cat treatment, and higher than in the DTX treatment. These results are consistent with other studies that show the flavonoid's ability to cause DNA fragmentation (**Papież *et al*., 2010**).

One class of anticancer medications that targets cancers including prostate and breast cancer is called docetaxel (DTX), which is derived from taxanes (**Wang *et al*., 2015**). DTX is the first line of treatment for prostate cancer, which has an anticancer effect on PC3 (**Nevedomskaya *et al*., 2018**). Epigallocatechin gallate (EGCG), which makes up approximately 50–60% of Cat compounds, that are found in green tea, is not harmful to normal cells or tissues; however, EGCG lowers oxidative damage, which is beneficial for treating and preventing tumors (**Aggarwal *et al*., 2022**). Additionally, EGCG is demonstrated to enhance the sensitivity of chemotherapy drugs and stop cancer stem cells from becoming resistant to

them, hence reducing the dosage needed and adverse side effects like nephrotoxicity and gastrointestinal disturbances (**Li *et al.*, 2020**). When EGCG and DTX bind to different tubulin sites in prostate cancer, they have synergistic suppressive actions on the targeted tubulin that cause cell cycle arrest (**Núñez *et al.*, 2021**). Additionally, the anticancer drug's transportation and absorption DTX were significantly enhanced when combined with EGCG (**Jackson *et al.*, 2016**).

In this study, we found that the antiproliferative impact of Cat in the prostate cancer cells was accompanied by obvious DNA breakdown indicating a high level of apoptosis induction. Also, this present work confirmed that Cat combined with DTX, clearly enhances its chemotherapeutic effect. Further, *in vivo* studies are warranted before application for human trials. In conclusion, the present work has shown Cat-enhanced induction of apoptosis alone, or combined with DTX chemotherapeutic drug. This work offers new mechanistic insights into the anti-proliferative action of DTX and further supports the potential advantages of improving its efficacy as prostate cancer chemotherapy.

Conclusion:

In conclusion, the present study has shown the potency of the Catechin, combination on inhibiting PC-3 prostate cancer cells in vitro associated with apoptosis. Cat induced strong cellular cytotoxicity to prostate cancer cells, while it promotes the chemotherapeutic activity of DTX when administered in combination.

ACKNOWLEDGMENTS

The authors wish to thank the researchers in the Research Lab. of Molecular Carcinogenesis. Faculty of Science, Tanta University of Professor Elsayed. I. Salim for technical help. The authors declare that they received no funds throughout this work.

References

Aggarwal V, Tuli HS, Tania M, Srivastava S, Ritzer EE, Pandey A, Aggarwal D, Barwal TS, Jain A, Kaur G, Sak K, Varol M, Bishayee A. (2022). Molecular mechanisms of action of epigallocatechin gallate in cancer: Recent trends and advancement. *Semin Cancer Bio.l,* 80: 256-275. doi: 10.1016/j.semcancer.2020.05.011.

Bisol Â, de Campos PS, Lamers ML. (2020). Flavonoids as anticancer therapies: A systematic review of clinical trials. *Phytother Res*., 34 (3):568-582. doi: 10.1002/ptr.6551.

Cheng Z, Zhang Z, Han Y, Wang J, Wang Y, Chen X, Shao Y, Cheng Y, Zhou W, Lu X, Wu Z. (2020). A review on anti-cancer effect of green tea catechins. *JFF*., 1(74):104-172. doi: 10.1016/j.jff.2020.104172.

Chou TC, Talalay P. (1984). Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme*

Regul.; 22:27-55. doi: 10.1016/0065-2571(84)90007-4.

Du Q, Jiang G, Li S, Liu Y, Huang Z. (2018). Docetaxel increases the risk of severe infections in the treatment of non-small cell lung cancer: a meta-analysis. *Oncoscience*., 5(7-8):220-238. doi: 10.18632/oncoscience.444.

Gottesman MM, Fojo T, Bates SE. (2002). Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer*., 2(1):48-58. doi: 10.1038/nrc706.

Haseeb S, Alexander B, Santi RL, Liprandi AS, Baranchuk A. (2019). What's in wine? A clinician's perspective<sup/>. *Trends Cardiovasc Med.*, 29(2):97-106. doi: 10.1016/j.tcm.2018.06.010.

Huska JD, Lamb HM, Hardwick JM. (2019). Overview of BCL-2 Family Proteins and Therapeutic Potentials. *Methods Mol Biol*., 1877:1-21. doi: 10.1007/978-1-4939-8861-7_1.

Jackson JK, Letchford K. (2016). The Effective Solubilization of Hydrophobic Drugs Using Epigallocatechin Gallate or Tannic Acid-Based Formulations. *J Pharm Sci*,. 105(10):3143-3152. doi: 10.1016/j.xphs.2016.06.027.

Li P, Liu A, Xiong W, Lin H, Xiao W, Huang J, Zhang S, Liu Z. (2020). Catechins enhance skeletal muscle performance. *Crit Rev Food Sci Nutr*., 60(3):515-528. doi: 10.1080/10408398.2018.1549534.

Lin JT, Lai GM, Chang TH, Liu MT, Bi CP, Wang JW, Chen MK. (2012). Chemotherapy with modified docetaxel, cisplatin, and 5-fluorouracil in patients with metastatic head and neck cancer. *Adv Ther*. 29(1):71-7. doi: 10.1007/s12325-011-0085-2.

Lin SR, Chang CH, Hsu CF, Tsai MJ, Cheng H, Leong MK, Sung PJ, Chen JC, Weng CF. (2020). Natural compounds as potential adjuvants to cancer therapy: Preclinical evidence. *BJP*., 177(6):1409-1423. doi: 10.1111/bph.14816.

Montero A, Fossella F, Hortobagyi G, Valero V. (2005). Docetaxel for treatment of solid tumours: a systematic review of clinical data. *Lancet Oncol*., 6(4):229-39. doi: 10.1016/S1470-2045(05)70094-2.

Nevedomskaya E, Baumgart SJ, Haendler B. (2018). Recent Advances in Prostate Cancer Treatment and Drug Discovery. *Int. J. Mol. Sci.*, 19(5):1359. doi: 10.3390/ijms19051359.

Núñez-Iglesias MJ, Novio S, García C, Pérez-Muñuzuri ME, Martínez MC, Santiago JL, Boso S, Gago P, Freire-Garabal M. (2021). Co-Adjuvant Therapy Efficacy of Catechin and Procyanidin B2 with Docetaxel on Hormone-Related Cancers In Vitro. *Int. J. Mol. Sci*., 22(13):7178. doi: 10.3390/ijms22137178.

Okamoto T, Hatakeyama S, Narita S, Arai Y, Habuchi T, Ohyama C. (2020). Validation and development of the CHAARTED criteria in patients with hormone-naïve metastatic prostate cancer: A multi-institutional retrospective study in Japan. *Int. J. Urol.*, 27(1):90-91. doi: 10.1111/iju.14136.

Omori G, Yamamoto N, Fushimi T, Kawasaki K, Oota N. (2017). Effects of combined intervention of quadriceps exercise and green tea catechins on physical function in medial knee osteoarthritis. *Osteoarthritis and Cartilage.*, 1(25):S398. doi.org/10.1016/j.joca.2017.02.683

Papiez MA, Baran J, Bukowska-Straková K, Wiczkowski W. (2010). Antileukemic action of (-)-epicatechin in the spleen of rats with acute myeloid leukemia. *FCT.,* 48(12):3391-7. doi: 10.1016/j.fct.2010.09.010.

Payen VL, Porporato PE, Danhier P, Vazeille T, Blackman MCNM, May BH, Niebes P, Sonveaux P. (2017). (+)-Catechin in a 1:2 Complex with Lysine Inhibits Cancer Cell Migration and Metastatic Take in Mice. *Front Pharmacol*., 8:869. doi: 10.3389/fphar.2017.00869.

Raja Singh P, Sugantha Priya E, Balakrishnan S, Arunkumar R, Sharmila G, Rajalakshmi M, Arunakaran J. (2017). Inhibition of cell survival and proliferation by nimbolide in human androgen-independent prostate cancer (PC-3) cells: involvement of the PI3K/Akt pathway. *Mol Cell Biochem.,* 427(1-2):69-79. doi: 10.1007/s11010-016-2898-4.

Salim EI, Mosbah AM, Elhussiny FA, Hanafy NAN, Abdou Y. (2023). Preparation and characterization of cetuximab-loaded egg serum albumin nanoparticles and their uses as a drug delivery system against Caco-2 colon cancer cells. *Cancer Nanotechnol.,* 14(1):4. doi:10.1186/s12645-022-00153-8

Sari L.M. (2019). Catechin: Molecular mechanism of Anti-Cancer Effect: Katekin: Mekanisme Molekular Efek Antikanker. *Dentika Dent. J*., 22:20–25. doi: 10.32734/dentika.v22i1.683.

Song Q, Li D, Zhou Y, Yang J, Yang W, Zhou G, Wen J. (2014). Enhanced uptake and transport of (+)-catechin and (-)-epigallocatechin gallate in niosomal formulation by human intestinal Caco-2 cells. *Int J Nanomedicine.,* 9: 2157-65. doi: 10.2147/IJN.S59331.

Sung H, Ferlay J, Siegel RL, 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 Cancer J Clin.,* 71(3):209-249. doi: 10.3322/caac.21660.

Wang J, Li S, Han Y, Guan J, Chung S, Wang C, Li D. (2018). Poly (Ethylene Glycol)-Polylactide Micelles for Cancer Therapy. *Front Pharmacol.*, 9:202. doi: 10.3389/fphar.2018.00202.

Wang P, Henning SM, Heber D, Vadgama JV. (2015). Sensitization to docetaxel in prostate cancer cells by green tea and quercetin. *J Nutr Biochem.,* 26(4):408-15. doi: 10.1016/j.jnutbio.2014.11.017.

Wang P, Henning SM, Heber D, Vadgama JV. (2015). Sensitization to docetaxel in prostate cancer cells by green tea and quercetin. *J Nutr Biochem.,* 26(4):408-15. doi: 10.1016/j.jnutbio.2014.11.017.

Yessenkyzy A, Saliev T, Zhanaliyeva M, Masoud AR, Umbayev B, Sergazy S, Krivykh E, Gulyayev A, Nurgozhin T. (2020). Polyphenols as Caloric-Restriction Mimetics and Autophagy Inducers in Aging *Research. J Nutr.,* 8;12(5):1344. doi: 10.3390/nu12051344.

Yessenkyzy A, Saliev T, Zhanaliyeva M, Masoud AR, Umbayev B, Sergazy S, Krivykh E, Gulyayev A, Nurgozhin T. (2020). Polyphenols as Caloric-Restriction Mimetics and Autophagy Inducers in Aging *Research. J Nutr.,* 8;12(5):1344. doi: 10.3390/nu12051344.

تعزيز فعالية العلاج بالدوسيتاكسيل باستخدام الكاتيكين من العنب الأسود على خلايا سرطان البروستاتا

أ..د. السيد إبراهيم سالم - صفاءإبراهيم أبو الدهب - أ.د إيمان السيد النحاس

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يتناول هذا البحث ، فحص التأثير السام المحتمل للعلاج المركب بالكاتكين (Cat)، وهو مركب عالي البوليفينول مستخلص من العنب الأسود ، ودوسيتاكسيل ضد خلايا سرطان البروستاتا باستخدام اختبار سلفورودامين . بعد الاستخلاص والتوصيف باستخدام كروماتوغرافيا HPLC، وتقييم استخلاص Cat، تم تحديد جرعات IC50 لمجموعات العلاج في خط خلايا PC-3: علاج DTX، علاج Cat، علاج DTX + Cat (حجم ١:١)، والخلايا غير المعالجة المستخدمة كضوابط. تظهر النتائج أن العلاج باستخدام DTX أو Cat أو مزيج منهما قلل بشكل كبير من العدد الإجمالي لخلايا PC-3 التي نجت. كما تسببت العلاجات في انهيار الحمض النووي كما هو موضح بواسطة اختبار تجزئة الحمض النووي مما تسبب في سمية خلوية واضحة وموت الخلايا المبرمج لخلايا سرطان البروستاتا. كانت التغيرات المورفولوجية التي شوهدت بعد العلاج بكل عقار على حدة هي انكماش الخلايا، وتقريب الخلايا، وانفصالها، وطفوها، وتورم الخلايا الواضح وتمزقها مما يشير إلى سمات موت الخلايا المبرمج. أظهر العلاج بمزيج من المركبين خلايا مستديرة أكثر بروزًا مع سمات موت الخلايا المبرمج الأكثر وضوحًا مقارنة بكل علاج على حدة. وفي الختام، تسبب كات في سمية خلوية قوية لخلايا سرطان البروستاتا، في حين أنه يعزز النشاط الكيميائي لـ DTX عند إعطائه معًا.