FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food and Health 10(1), 85-95 (2024) • https://doi.org/10.3153/FH24008 Research Article

Green tea extracts and substantial catechin derivatives: Evaluation of their potential against breast cancer

Nadire ÖZENVER[1], **Sıla ÜNLÜ**[1,2], **Seren GÜNDOĞDU**[1], **L. Ömur DEMİREZER**[1]

Cite this article as:
Özenver, N., Ünlü, S., Gündoğdu, S., Demirezer, L.Ö. (2024). Green tea extracts and substantial catechin derivatives: Evaluation of their potential against breast cancer. *Food and Health,* 10(1), 85-95. https://doi.org/10.3153/FH24008

[1] Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Türkiye

[2] Güven Hospital, 06540, Çankaya, Ankara, Türkiye

ORCID IDs of the authors:
N.Ö. 0000-0001-9561-8994
S.Ü. 0009-0000-1007-1193
S.G. 0000-0003-4295-2520
O.D. 0000-0002-5284-0744

Submitted: 21.07.2023
Revision requested: 10.10.2023
Last revision received: 20.10.2023
Accepted: 24.11.2023
Published online: 30.12.2023

Correspondence:
Nadire ÖZENVER
E-mail: nadire@hacettepe.edu.tr

Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

Breast cancer is one of the most predominant types of cancer. Although assorted treatment options are available to cope with breast cancer (*e.g.* chemotherapy, radiotherapy, surgery, hormone therapy, targeted therapy), chemotherapy regimens still hold vital importance. Studies on the discovery of drug-candidate molecules that can create an alternative in the treatment of breast cancer continue at full speed. At this point, nature has a substantial place offering great diversity. Natural products may exhibit anticancer properties directly through molecular targets such as genes or indirectly through metabolic pathways. Moreover, they may be adjuvant agents and contribute to conventional therapy, and thus, they can enhance the efficacy of chemotherapeutics or even ease their side effects. Green tea, a critical dietary source of polyphenols and flavonoids, is obtained from the minimally fermented or unfermented leaves of the *Camellia sinensis* L. plant and is used in traditional Chinese medicine for many important conditions, including cancer. The phytochemical content of green tea is extremely rich, including (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC); (-)-epicatechin-3-gallate (ECG) and (-)-epicatechin (EC) as the main catechins in the composition of green tea. Within the scope of our study, we proposed the cytotoxicity and toxicity comparison of the water and 80% ethanolic extract of the green tea extracts as well as of (-)-epicatechin (EC) and (-)-epigallocatechin (EGC) in terms of their cytotoxicity and toxicity based on the structure-activity relationship on breast cancer. Therefore, we tested aqueous and 80% ethanolic extracts of green tea and EGC and EC on MDA-MB-231, MDA-BMB-468, MCF-7 and SK-BR-3 breast cancer cells. Their toxicity on healthy rat myoblastoma H9c2 cells was further examined. Resazurin reduction assay was used to detect cytotoxicity and toxicity. Both water and 80% ethanolic extract of green tea exhibited remarkable cytotoxicity on MCF-7 cancer cells deserving further investigation, including phytochemical characterization of the extract. Epigallocatechin was also cytotoxic on MCF-7 cells with an IC_{50} value of 20.07 µM. The possible therapeutic potentials of green tea extracts and their substantial catechin derivatives were assessed for breast cancer therapy.

Keywords: Breast cancer, Epicatechin, Epigallocatechin, Cytotoxicity, Green tea, Toxicity

Introduction

Breast cancer is among the most prevalent cancer types in the world causing 685000 deaths in 2020 (WHO, 2023). Assorted treatment regimens are available for breast cancer, such as targeted therapy, hormonal therapy, radiation therapy, surgery and chemotherapy (Akram et al., 2017; WHO, 2023). Chemotherapy, a substantial treatment option in cancer therapy, varies based on different factors such as the cancer type (*i.e.* if it is estrogen receptor (ER), progesterone receptor (PR) or human epidermal growth factor receptor 2 (HER2)/neu dependent or not), stage and grade of cancer, patient's overall health (WHO, 2023). Cyclophosphamide, cisplatin, and doxorubicin are among the common chemotherapeutics against breast cancer, but their effects on healthy cells also cause serious side effects (WHO, 2023; Waks & Winer, 2019). At this point, nature provides undeniable options for the discovery of new agents that can either become potential drug candidates against cancer or may support cancer therapy by alleviating the side effects of chemotherapeutics, boosting the immune system. The search for less toxic, more effective, and less costly natural compounds with anticancer therapeutic potential has been maintained for decades. Newman and Cragg (2020) reported that only 29% of small molecule drugs approved between 1981 and 2019 were totally synthetic drugs while others were natural products, their mimics or derivatives (Newman & Cragg, 2020). Therefore, phytochemicals hold great potential to prevent or cure cancer. They can act directly through molecular targets such as genes or indirectly through metabolic pathways (Saldanha & Tollefsbol, 2012).

Green tea, an important dietary source of polyphenols and flavonoids, is obtained from the minimal or unfermented leaves of the plant *Camellia sinensis* L.. and its production is characterized by a heating process that kills the enzyme polyphenol oxidase, which is responsible for the conversion of leaf flavanols into dark polyphenolic compounds (Ranjan et al., 2019; Ruchika & Sehgal, 2022). Green tea consumption first started in China and spread quickly to different cultures. Its use increased when its health benefits became clear. In traditional Chinese medicine, green tea has been considered to have cardioprotective, anti-cancer, and anti-infective effects. The chemical composition of green tea includes proteins, amino acids, carbohydrates, lipids, sterols, vitamins, pigments such as chlorophyll and carotenoids, alcohols, esters, aldehydes, hydrocarbons, and lactones. It further consists of minerals and trace elements. Green tea is rich in catechins; the four main catechins in its composition, (-)-epicatechin-3-gallate (ECG) (about 13.6%), (-)-epicatechin (EC) (approximately 6.4%), (-)-epigallocatechin (EGC) (about 19%) and (-)-epigallocatechin-3-gallate (EGCG) (about 59%) (Chacko et al., 2010). Polyphenols, including catechins, directly activate copper bound to chromatin and induce the formation of reactive oxygen species, leading to cellular DNA breaks (Farhan et al., 2015). Moreover, due to their antioxidant capacity, catechins are also involved in the prevention/treatment of diseases associated with free radical damage, such as cancer, Alzheimer's disease, cardiovascular diseases, Parkinson's disease, and diabetes (Braicu et al., 2013).

Tea polyphenols are chelators of metal ions, and the polyphenolic structure provides scavenging of free radicals by electron delocalization. It suggests that both antioxidant and prooxidant activities of catechins are important against malignancy, especially for the prevention of cancer (Shirakami & Shimizu, 2018). The oxidant and pro-oxidant nature of EGCG is important in terms of its antitumor effect. EGCG leads the generation of reactive oxygen species to activate pro-oxidative pathways by covalently bonding with antioxidants and induces the death of cancer cells. EGCG has been shown to inhibit cell proliferation by suppressing STAT3, ERK1/2, Akt/PI3K, Wnt and NF-KB pathways (Alam et al., 2022). MCF-7 cells under the treatment of EGCG showed that both p21 and p27 proteins were overexpressed, with an increased proportion of cells arrested at G1. EGCG has been shown to be effective in both estrogen receptor (ER) negative and positive cell lines (Stuart et al., 2006). EGCG has also been found to exhibit significant chemopreventive effects and anti-cancer stem cell activity in a variety of ways (Abd El-Rahman et al., 2017). In another study, human breast cancer cells when treated with EGCG, the protein expression, mRNA expression level, and the activity of MMP-2 lessened. It is a beneficial situation. Because MMP-2 overexpression was reported to be linked to many cancers' malignant and invasive phenotypes (Chen et al., 2011).

Similar to EGCG, EGC and EC (Figure 1) are phenolic compounds, and their possible antioxidant potentials, as well as their structural similarities with EGCG, make us think that they may be important molecules in anticancer drug discovery. Within the scope of our project, we planned to evaluate the antitumor potentials of green tea extracts (water and 80% ethanolic extracts), EGC and EP on breast cancer cells, as well as their toxicity on healthy H9c2 rat myoblastoma cells.

In our project, the cytotoxic effects of aqueous (infusion) and 80% ethanolic extracts of green tea and EGC and EC, which

are intensely found in green tea, on breast cancer cells with different origin and their toxicity on healthy rat myoblastoma cells were investigated. Thus, we aimed to unravel the possible antitumor potentials of the extracts and catechin derivatives in breast cancer.

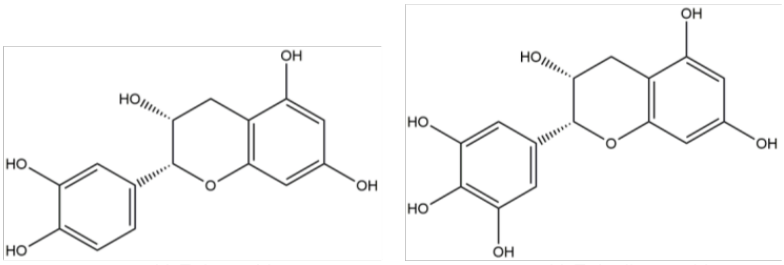

Figure 1. Chemical structures of (-)-epicatechin and (-)-epigallocatechin. The structures were drawn by using ChemDraw® (Cambridge, MA).

Materials and Methods

Chemicals

(-)-Epicatechin, (-)-epigallocatechin and doxorubicin were purchased from Cayman Chemical, Türkiye. The media used in the present study were commercially purchased. Dulbecco's Modified Eagle's Medium (DMEM) and Minimum Essential Medium (MEM) were obtained from Sigma, Türkiye. McCoy's 5A media, Fetal Bovine Serum (FBS) and Penicillin-Streptomycin Solution were purchased from Biowest, Türkiye.

Cell Culture

The cell lines used in the present work, their origins, and sustainment were previously reported (ATTC, 2023). In brief, various breast cancer cells with different origins (MCF-7, MDA-MB-231, MDA-MB-468 and SKBR-3) and healthy rat myoblastoma cells (H9c2) were used for experimental studies.

Preparation of the Extracts

Green tea is commercially obtained from the market. To obtain 80% ethanolic extract of green tea leaves, green tea leaves (20 g) were extracted with 80% ethanol (2 x 100 mL) in a water bath at 40 °C, concentrated to dryness under decreased pressure and lyophilized *in vacuo*.

To acquire the water extract from green tea leaves, boiling water (2 x 100 mL) was poured on green tea leaves (20 g) for 30 min. each, concentrated to dryness under decreased pressure and lyophilized *in vacuo*.

The extracts were dissolved in DMSO to prepare the stock solutions. The stock solutions of the extracts and catechin derivatives were prepared as 10 mM. Then, the required concentrations were prepared via stock solution.

Cytotoxicity and Toxicity Determination

We performed a resazurin reduction assay to test the cytotoxicity. In this case, the resazurin is reduced to resorufin by viable cells (O'Brien et al., 2000). Non-viable cells do not display blue staining due to losing their metabolic capacity. Briefly, aliquots of 5×10^5 adherent cells were seeded in 96-well plates and were allowed to attach overnight. In the following, the cells were incubated with or without the addition of varying concentrations of the test substance to get a total volume of 200 µL/well. After 72 h incubation and the addition of resazurin (Sigma-Aldrich, Turkey) for 4 h, staining was measured by an Infinite 200 M Plex plate reader (Tecan, Turkey) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Each assay was independently performed thrice, with six parallel replicates each. The protocol has been recently reported (Kuete et al., 2016). GraphPad Prism v6.0 software (GraphPad Software Inc., San Diego, CA, USA). It was used to prepare dose–response curves of the extracts and catechin derivatives. The 50% inhibition concentrations (IC_{50}) were calculated by nonlinear regression using Microsoft Excel. The results were expressed as ± standard deviations (SD).

Results and Discussion

Cytotoxicity of the Green Tea Extracts and of Catechin Derivatives in a Variety of Breast Cancer Cells

We investigated the cytotoxic activity of the water and 80% ethanolic extracts of green tea as well as of epicatechin and epigallocatechin on assorted breast cancer cells with different origins. Therefore, we tested the cytotoxicity of the water and 80% ethanolic extracts of green tea at ranging concentrations from 1 to 100 µg/mL. Moreover, we treated the breast cancer cells with ranging concentrations of epicatechin and epigallocatechin from 1 to 100 µM to evaluate their potential cytotoxic activity on these cells. We treated the breast cancer cells MDA-MB-231, MDA-MB-468, MCF-7 and SK-BR-3. The cell viability percentages of the breast cancer cells under the treatment of the water and 80% ethanolic extracts of green tea as well as of epicatechin and epigallocatechin are shown in Figures (2-4) below.

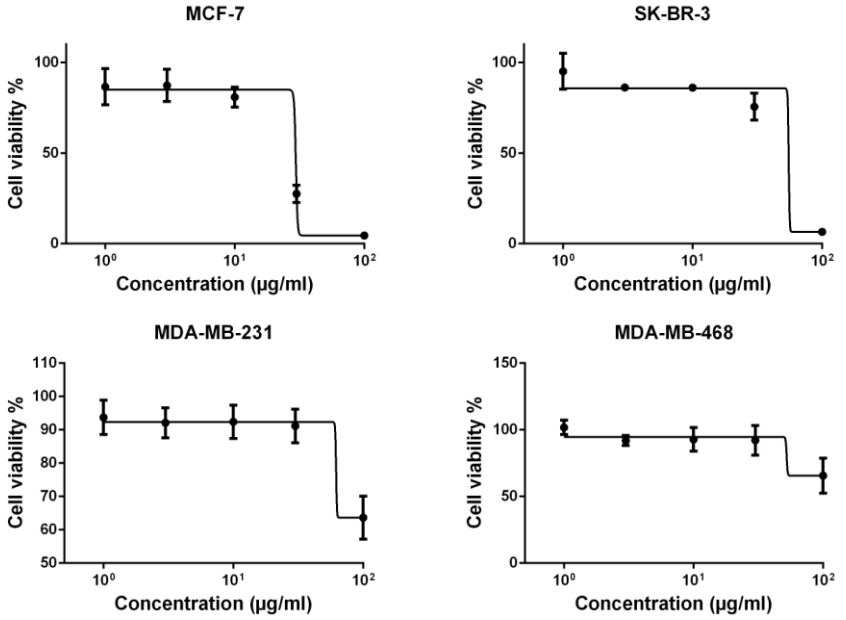

Figure 2. The effect of 80% ethanolic extract of green tea on a variety of breast cancer cells. GraphPad Prism v6.0 software (GraphPad Software Inc., San Diego, CA, USA) was used to generate dose–response curves of the extracts. The results were expressed as ± standard deviations (SD).

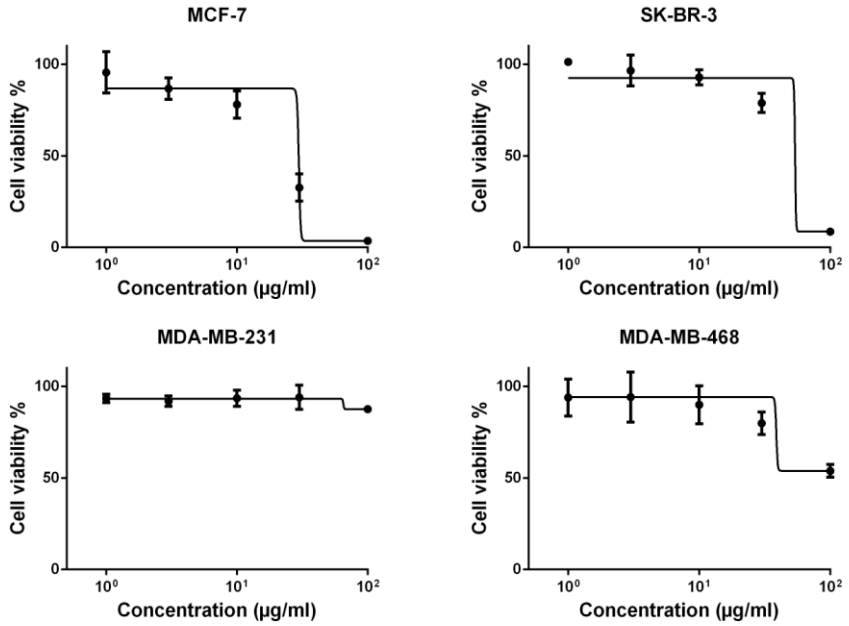

Figure 3. The effect of green tea's water extract on various breast cancer cells. GraphPad Prism v6.0 software (GraphPad Software Inc., San Diego, CA, USA) generated dose–response curves for the green tea extracts. The results were expressed as ± standard deviations (SD).

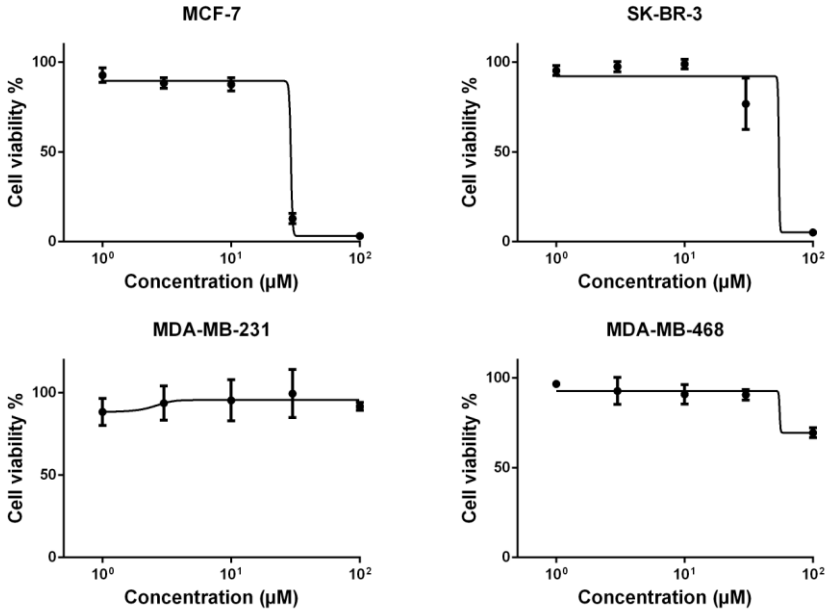

Figure 4. The effect of epigallocatechin on a variety of breast cancer cells. GraphPad Prism v6.0 software (GraphPad Software Inc., San Diego, CA, USA) was used to generate dose–response curves of epigallocatechin. The results were expressed as ± standard deviations (SD).

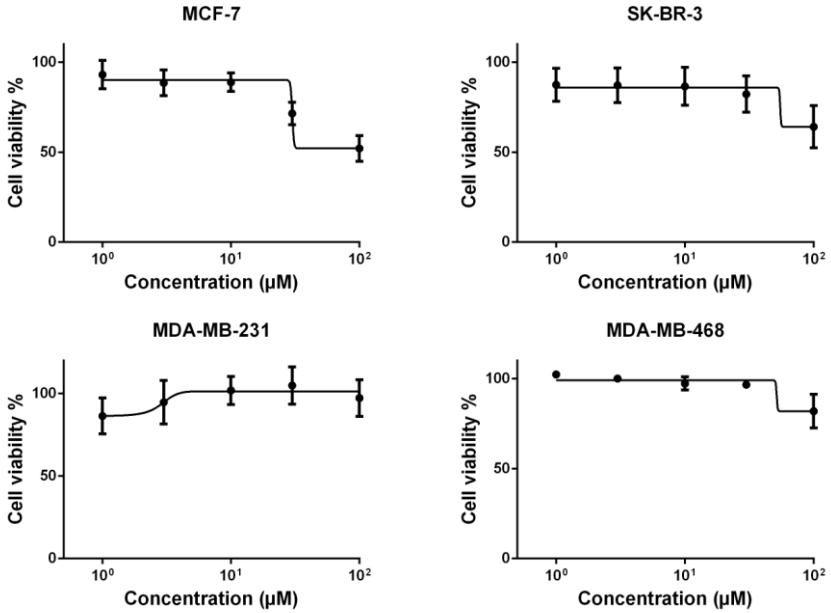

Figure 5. The effect of epicatechin on a variety of breast cancer cells. GraphPad Prism v6.0 software (GraphPad Software Inc., San Diego, CA, USA) was used to generate dose–response curves of epicatechin. The results were expressed as ± standard deviations (SD).

As seen in Table 1 and Figure 2, 80% ethanolic extract of green tea exhibited greater cytotoxicity than that of water extract (Table 1, Figure 3). The hydroethanolic extract of green tea displayed the strongest cytotoxicity on MCF-7 cells with an IC_{50} value of 21.58 µg/mL in addition to possessing moderate cytotoxicity on SK-BR-3 cells with an IC_{50} value of 55.94 µg/mL. Likewise, the water extract of green tea presented greater cytotoxicity on MCF-7 and SK-BR-3 cells, respectively with IC_{50} values of 22.38 (MCF-7) and 58.81 µg/mL (SK-BR-3). The green tea extracts were not cytotoxic enough on MDA-MB-231 and MDA-MB-468 cells that the cell viability of these cells did not even go down below 50% at 100 µg/mL. The comparison of the cytotoxic properties of the catechin derivatives indicated the cytotoxic activity of epigallocatechin (Table 1, Figure 4) on MCF-7 and SK-BR-3 cells, while no cytotoxic activity profile was observed in epicatechin (Table 1, Figure 5). The IC_{50} values of the extracts and catechin derivatives are summarized in Table 2.

Toxicity of the Green Tea Extracts and of Catechin Derivatives in a Variety of Breast Cancer Cells

We also demonstrated the toxicity of the water and 80% ethanolic extracts of green tea as well as of epicatechin and epigallocatechin on healthy rat cardiac H9c2 myoblastoma cells. Therefore, we treated the healthy rat cardiac H9c2 myoblastoma cells with diverse concentrations of the extracts (1 to 100 µg/mL) and catechins (1 to 100 µM). We observed that neither extracts nor catechin derivatives exhibited potent toxicity on H9c2 cells (Table 1, Figure 6), confirming their safety profile in comparison to clinically used doxorubicin which killed nearly half of the cells at the lowest concentrations tested (0.003 µM) (Figure 6).

Cardiotoxicity is a critical adverse effect of clinically used chemotherapeutics such as doxorubicin (Chatterjee et al., 2010; Rawat et al., 2021; Volkova & Russell, 2011). Therefore, discovering potential anticancer agents with remarkable cytotoxicity and minimal toxicity is a primary objective in anticancer drug development. Drug discovery is a long and tough process in which preclinical studies hold great importance. Identification of agents with potent cytotoxic activity and lessened toxicity is vital in that they may hold potency as potential drug leads or as a part of drug combination regimens in cancer therapy.

Emerging literature data has pointed out that botanicals are considered efficient cytotoxic agents if their IC_{50} values are lower than 20 μg/mL following the 72 h incubation period (Mbaveng et al., 2019). Besides, according to the National Cancer Institute USA (NCI), botanicals yielding IC_{50} values below or around 30 μg/mL should be applied to purification to acquire cytotoxic substances (Mbaveng et al., 2019; Stiffness et al., 1990). Assessed the cytotoxicity of the water and 80% ethanolic extracts of green tea, both extracts exhibited strong cytotoxicity on MCF-7 cells with the IC_{50} values of 22.38 μg/mL and 21.58 μg/mL, respectively, deserving further purification steps to obtain remarkable drug leads. Having identical IC_{50} values on MCF-7 cells makes us think their chemical composition should be similar, probably due to the polarity of the solvents used for the extraction. On the other hand, unfortunately, the water and 80% ethanolic extracts of green tea displayed modest to slight cytotoxic activity on other breast cancer cells. Green tea infusion exhibited moderate cytotoxic activity on SK-BR-3 cells (IC_{50}: 58.81 μg/mL) while possessing slight cytotoxicity on MDA-MB-231 and MDA-MB-468 cells ($IC_{50} > 100$ μg/mL).

Regarding catechin derivatives, epigallocatechin substantially repressed the cell viability% of MCF-7 cells with the IC_{50} value of 20.07 μM. It inhibited SK-BR-3 cells with the IC_{50} value of 56.19 μM, unlike its slight cytotoxicity on MDA-MB-468 and MDA-MB-231 cells ($IC_{50} > 100$ µM). On the other hand, epicatechin was not cytotoxic enough to affect the tested breast cancer cells. It lessened the cell viability of MCF-7 and SK-BR-3 up to 52.08% and 64.11%, respectively, while almost no cytotoxic activity was observed in MDA-MB-468 and MDA-MB-231 cells.

To sum up the cytotoxic activity profiles of the extracts and catechin derivatives from a general perspective, the extracts and catechin derivatives displayed slight or no cytotoxicity on MDA-MB-231 and MDA-MB-468 cells that are both triple-negative breast cancer cells and are not ER, PR, or HER2-dependent cancer cells. On the other hand, assessing the cytotoxic activities of green tea extracts and catechin derivatives, MCF-7 was the most sensitive cell line whose cell viability was significantly inhibited when treated with green tea extracts and catechin derivatives. The gallo form of epicatechin, epigallocatechin, demonstrated a greater cytotoxic capacity, indicating the importance of the presence of the galloyl group for enhanced cytotoxic activity. MCF-7 cells are ER-dependent and HER2-independent cells (Holliday & Speirs, 2011), pointing out their anticancer potential on ER-positive and HER2-negative breast cancer cells.

Table 1. The effect of the green tea extracts/catechin derivatives on a variety of breast cancer cells and healthy cells.

The tested concentrations	Cell survival (%) MDA-MB-468 cell line	Cell survival (%) MDA-MB-231 cell line	Cell survival (%) MCF-7 cell line	Cell survival (%) SK-BR-3 cell line	Cell survival (%) H9c2 cell line
80% ethanolic extract					
1 µg/mL	101.87 ± 5.48	93.72 ± 5.14	86.82 ± 10.02	95.09 ± 9.93	93.70 ± 5.98
3 µg/mL	91.74 ± 3.71	92.12 ± 4.48	87.34 ± 8.84	86.23 ± 1.74	87.82 ± 3.02
10 µg/mL	92.87 ± 8.85	92.40 ± 5.04	80.90 ± 5.53	86.16 ± 1.96	82.32 ± 2.27
30 µg/mL	92.18 ± 11.10	91.16 ± 5.05	27.52 ± 4.72	75.60 ± 7.39	69.49 ± 6.46
100 µg/mL	65.64 ± 13.09	63.66 ± 6.45	4.55 ± 0.38	6.52 ± 0.39	47.04 ± 4.39
Water extract					
1 µg/mL	93.89 ± 10.15	93.44 ± 2.28	95.58 ± 11.32	101.37 ± 1.99	97.95 ± 3.95
3 µg/mL	94.18 ± 13.67	91.92 ± 2.90	86.73 ± 5.88	96.61 ± 8.48	91.96 ± 4.46
10 µg/mL	89.95 ± 10.35	93.55 ± 4.36	78.05 ± 7.39	92.87 ± 4.07	74.55 ± 0.73
30 µg/mL	79.86 ± 6.16	94.04 ± 6.66	32.72 ± 7.39	78.90 ± 5.23	56.93 ± 6.86
100 µg/mL	53.89 ± 3.53	87.56 ± 1.99	3.60 ± 0.29	8.67 ± 1.31	34.38 ± 4.19
Epicatechin					
1 µM	102.32 ± 2.12	86.47 ± 10.83	93.09 ± 7.93	87.47 ± 9.15	91.29 ± 13.20
3 µM	100.01 ± 2.04	94.75 ± 13.22	88.48 ± 7.74	87.19 ± 9.70	87.47 ± 8.37
10 µM	97.32 ± 3.71	101.94 ± 8.47	88.87 ± 5.12	86.59 ± 10.54	78.64 ± 5.89
30 µM	96.65 ± 1.66	104.91 ± 11.3	71.54 ± 6.23	82.24 ± 10.07	74.33 ± 8.12
100 µM	81.92 ± 9.38	97.34 ± 11.11	52.08 ± 7.11	64.11 ± 11.75	51.26 ± 9.90
Epigallocatechin					
1 µM	96.69 ± 1.85	88.42 ± 8.19	92.71 ± 4.05	95.33 ± 2.75	92.99 ± 8.46
3 µM	92.75 ± 7.58	93.77 ± 10.45	88.33 ± 2.98	97.49 ± 2.87	91.48 ± 7.77
10 µM	90.85 ± 5.44	95.48 ± 12.54	87.63 ± 3.59	98.98 ± 2.66	85.74 ± 10.62
30 µM	90.60 ± 2.98	99.60 ± 14.54	12.92 ± 2.80	76.77 ± 14.27	78.92 ± 10.18
100 µM	69.47 ± 2.69	91.79 ± 2.44	3.17 ± 0.42	5.22 ± 0.77	53.43 ± 6.28

Table 2. IC_{50} values of the green tea extracts/catechin derivatives on a variety of breast cancer cells.

Extracts/catechin derivatives	MCF-7	SK-BR-3	MDA-MB-231	MDA-MB-468
80% Ethanolic extract	21.58 µg/mL	55.94 µg/mL	>100 µg/mL	>100 µg/mL
Water extract	22.38 µg/mL	58.81 µg/mL	>100 µg/mL	>100 µg/mL
Epicatechin	>100 µM	>100 µM	>100 µM	>100 µM
Epigallocatechin	20.07 µM	56.19 µM	>100 µM	>100 µM

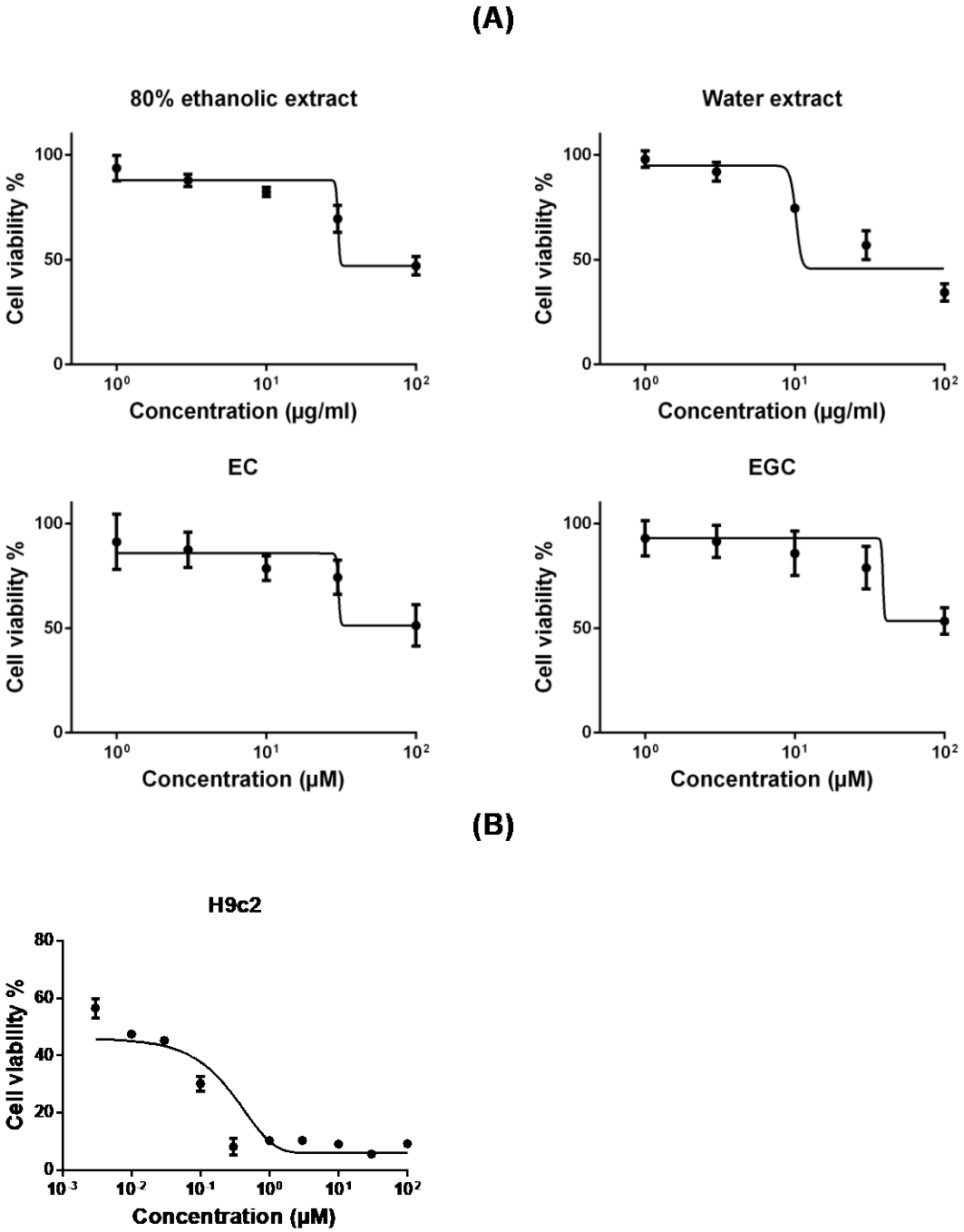

Figure 6. The effects of **(A)** green tea extracts and catechin derivatives **(B)** doxorubicin on H9c2 rat cardiac myoblastoma cells at various concentrations. GraphPad Prism v6.0 software (GraphPad Software Inc., San Diego, CA, USA) was used to generate dose–response curves of the extracts and catechin derivatives. The results were expressed as ± standard deviations (SD).

We further evaluated the toxicity profiles of green tea extracts and catechin derivatives on H9c2 healthy rat myoblastoma cells. Although the toxicity profiles of both extracts and catechin derivatives appeared similar, the water extract of green tea extracts displayed the highest toxicity in all, inducing 34.38% cell viability at 100 µg/mL. Still, compared to green tea extracts, epigallocatechin exhibited the lowest toxicity profile, and the cell viability of H9c2 cells was above 80% under the treatment of its corresponding IC_{50} value on MCF-7 cells, indicating its relative safety.

In this research, we assessed the cytotoxicity and toxicity profiles of water and 80% ethanolic extract of green tea extracts and their containing catechin derivatives epicatechin and epigallocatechin. We worked on various breast cancer cells with different origins to test the cytotoxicity. Emerging evidence has ascertained the anticancer potential of green tea extracts and catechin derivatives (Chen et al., 2011; Chen et al., 2022; Cheng et al., 2020; Yu et al., 2019). Even more, ongoing clinical studies exist about the effects of green tea extracts and green tea catechins on various cancers, including breast cancer (ClinicalTrials.gov, 2023). Still, the investigations mostly focus on either green tea or EGCG. Therefore, in the present research, our primary focus was investigating and comparing the cytotoxic and toxic effects of water and 80% ethanolic extracts of green tea and other catechin derivatives (EP and EGC) rather than EGCG. Our study also holds importance because tested breast cancer cells have different origins. Examining the extracts and the catechin derivatives towards these cell lines is a rationale approach enabling the assessment of their efficiency and selectivity in breast cancer therapy. The breast cancer cells were selected based on their origin (*i.e.* whether they carry ER, PR, or HER2/neu or not), and thereby, unlike much of the existing knowledge in the literature, which encompasses the cytotoxic activity of green tea extracts or EGCG on assorted cancer cells which are usually randomly selected. We intended to give a general overview of the cytotoxic evaluation of the extracts and catechin derivatives on breast cancer cells and their safety profile with a rationale approach.

In addition to the existing literature data, which puts special emphasis on either green tea or EGCG, we studied and made a comparable evaluation about the anticancer potential of water and 80% ethanolic green tea extracts as well as EC and EGC rather than EGCG on a variety of breast cancer cells with different origins. Detecting both extracts and EGC with remarkable cytotoxicity and relative safety, specifically on hormone-dependent breast cancer cells, makes us think they are prospective natural agents worthy of being studied in more detail against future breast cancer regimens connected to ER/PR.

Conclusion

The present study gave a perspective on the cytotoxic and toxic characteristics of green tea extracts and catechin derivatives. Green tea extracts and epigallocatechin exhibited anticancer potential against hormone-dependent breast cancer. Our findings were expected to significantly contribute to assessing and comparing the bioactivities of green tea extracts and catechin derivatives.

Compliance with Ethical Standards

Conflict of interests: The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

Ethics committee approval: Authors declare that this study includes no experiments with human or animal subjects. Ethics committee approval is not required for this study.

Data availability: Data will be made available on request.

Funding: This study was supported by TÜBİTAK 2209-A University Students Research Projects Support Program (Project No: 1919B012211398).

Acknowledgements: -

Disclosure: -

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