

Access this article online
Quick Response Code:

Website: www.jehp.net
DOI: 10.4103/jehp.jehp_1456_20

Evaluation of cytotoxicity and anticancer activity of kombucha and doxorubicin combination therapy on colorectal cancer cell line HCT-116

Latifeh Rasouli¹, Naheed Aryaeian², Mehran Gorjian³, Mitra Nourbakhsh³, Fatemehsadat Amiri⁴

Abstract:

BACKGROUND: Colorectal cancer is the third leading to death type of cancer in the world. The therapeutic guideline varied between different methods. As the main therapeutic guideline is chemotherapy, recent studies had shown utilization of natural products in combination with conventional medication, elevate the efficiency of chemotherapeutic methods. Kombucha is a traditional beverage obtained from the fermentation of green tea as a rich source of flavonoid medicinal plant. This study aimed to evaluate the natural potential of combination therapy of this natural product with doxorubicin as a chemotherapeutic agent.

MATERIALS AND METHODS: The study was performed as *in vitro* evaluation of biological activity of kombucha on HCT-116 cell line (human colon cancer cell line). The cytotoxic effect of different kombucha beverages (fermented green tea) in comparison with green tea extract was evaluated by dimethylthiazolyl tetrazolium bromide (MTT) assay. In the next step, anticancer activity of doxorubicin as a general guideline chemotherapeutic agent in combination with kombucha was evaluated by cell cycle analysis and apoptosis assay flow cytometry. Apoptotic genes expression pattern was determined using real-time polymerase chain reaction. The experiments were designed in three independent replications and statistically analyzed using SPSS software.

RESULTS: The results show that kombucha compared with the green tea extract caused more (1.2 fold) early apoptosis induction and G0/G1 phase arrest. Moreover, kombucha increased the expression levels of p21, p53, and B-cell leukemia/lymphoma 2 (Bcl-2)-associated X protein genes (2, 2.5, and 1.5 fold, respectively) while it decreased Bcl-2 gene expression level (5–8 fold) compared with doxorubicin alone. Combination of kombucha with doxorubicin shows 2-fold increased G0/G1 phase compared with the doxorubicin treatment.

CONCLUSION: This result indicated that kombucha caused boosted anticancer activity of doxorubicin agent. These findings suggest that kombucha may be has an assistor and useful role in colorectal cancer treatment align with chemotherapy.

Keywords:

Apoptosis, cell cycle, colorectal cancer, doxorubicin, kombucha

Introduction

One of the main worldwide causes of mortality is cancer and demonstrates a serious health problem. According to world health organization (WHO) reports,

17.5 million anticipated cancer deaths and 27 million new cancer cases will happen annually 2050.^[1] Accounting to 8% of all cancer-related deaths annually, colorectal cancers are the third leading cause of cancer death in both men and women.^[2,3]

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Rasouli L, Aryaeian N, Gorjian M, Nourbakhsh M, Amiri F. Evaluation of cytotoxicity and anticancer activity of kombucha and doxorubicin combination therapy on colorectal cancer cell line HCT-116. J Edu Health Promot 2021;10:376.

¹Department of Nutrition, School of Public Health, Iran University of Medical Sciences, International Campus, Tehran, Iran, ³Department of Biology, School of Sciences, Payame-Noor University of Mashhad, Mashhad, Iran, ⁴Department of Biochemistry, School of Medicine, Iran University of Medical Sciences, Tehran, Iran, ²Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

Address for correspondence:

Dr. Naheed Aryaeian, Department of Nutrition, School of Public Health, Iran University of Medical Sciences, P.O. Box 14155-6171, Tehran, Iran. E-mail: aryaeian.n@iums.ac.ir

Received: 29-10-2020
Accepted: 02-02-2021
Published: 29-10-2021

Asian lifestyle emerged by special dietary habits with higher intake of meat, fat, and total calories, along with increased life stresses, pressures, and expectancy, caused a remarkable increase in the burden of colorectal cancer prognosis.^[2,4]

In Iran, colorectal cancer is one of the most common prognostic cancers. This type of cancer is the third most common cancer in Iranian men (standardized incidence: 8.1–8.3/100,000) and the fourth most common cancer in women with a standardized incidence of 6.5–7.5/100,000.^[5-7] As known, this is a multifactorial disease. Low physical activity, high body mass index (BMI), high-fat diet, alcohol consumption, low intake of vegetables and fruit, tobacco smoking, a family history, and use of certain medications, including contraception pills and nonsteroidal anti-inflammatory drugs, are risk factors for cancer.^[7-9]

Disruption of the apoptosis process, mutations in proto-oncogenes, mutations in repairing genes, and tumor suppressors are the main cause's of colorectal cancer.^[10,11] Other than surgery, radiotherapy, endocrine therapy, and immunotherapy, chemotherapy is the main treatment for cancer.^[12] However, conventional cancer chemotherapy is associated with high systemic toxicity and low therapeutic effectiveness. Therefore, reducing the side effects of chemotherapy has become a focus in the management of cancer.^[13]

Natural product and medicinal herb use in combination with conventional medication is a new manner to help reduce the side effects of chemotherapy.^[14] Green tea as a medicinal plant is one of the richest sources of flavonoids. Green tea is rich in flavonoids such as catechin, epicatechin, epigallocatechin, and epigallocatechin gallate, which have been recognized as anti-inflammatory, antioxidant, anticancer, and antimutagenic agents.^[15] Based on practical studies, consumption of green tea can help weight loss, decreases BMI, and decreases insulin resistance.^[16] Despite the huge evidence on the green tea benefits, this type of tea has very low popularity. Among the available black tea brands, the imported ones are more popular than the homemade products.^[17]

Kombucha is a traditional beverage usually obtained from the fermentation of black or green tea (sweetened with 5%–8% of sugar) by a symbiotic microbial consortium, which is mainly composed of acetic acid bacteria and osmophilic yeasts.^[16,17] Kombucha is known for its nutraceutical properties and consumed in Asia for thousands of years. History of preparation of this drink dating back to approximately 220 BC. In last decades, the market interest has spread to the whole world, and also, home brewing is now a common practice.^[18,19] Today, kombucha is commonly consumed worldwide

as a medicinal health-promoting beverage.^[20,21] Many researches demonstrated that kombucha constitutes a potent therapeutic supplement that improved resistance against cancer, prevented cardiovascular diseases, promoted digestive functions, stimulated the immune system, and reduced inflammatory problems.^[20,22,23] The beneficial effects of kombucha are attributed to the presence of polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, amino acids, antibiotics, and a variety of micronutrients produced during fermentation.^[24,25] The anticancer mechanisms of green tea polyphenols include inhibition of gene mutation, inhibition of cancer cell proliferation, induction of cancer apoptosis, and termination of metastasis.^[24,26,27] In the traditional medicine, the anticancer activity of kombucha had reported with no scientific knowledge background. Recent studies as Jaya Balan group investigations, take a new overview for anticancer specification of kombucha especially in liver and renal carcinoma.^[22] Hence, the present study aimed to evaluate the anticancer potential of kombucha on the HCT-116 cell line (human colon cancer cell line) and therapeutic effectiveness in combination with doxorubicin as a common chemotherapeutic agent.

Materials and Methods

Study design and setting

The study was performed as *in vitro* evaluation of biological activity of kombucha on HCT-116 cell line (human colon cancer cell line). Two different kombucha beverages (pasteurized fermented green tea) were purchased from Delestan Health Tea Factory: kombucha beverage was fermented for 2 weeks (kombucha 1) and 3 weeks (kombucha 2). For comparison, green tea extract was prepared from boiled green tea (12 g) for 15 min, then filtered through a sterile sieve, and cooled to room temperature. Doxorubicin was purchased from Sigma-Aldrich Chemicals (USA). The HCT-116 cell line was purchased from Pasteur Institute of Iran, Tehran, Iran.

Evaluation of cytotoxicity

Determination of half maximal inhibitory concentration (IC50)

The cytotoxic effect of different kombucha beverages (fermented green tea) in comparison with green tea extract was evaluated by dimethylthiazolyl tetrazolium bromide (MTT) assay. Briefly, HCT-116 cells were seeded in Dulbecco's Modified Eagle's Medium, supplemented with 10% fetal bovine serum, 100 µg/mL of penicillin, and 100 µg/mL of streptomycin in 96-well plate sat 10,000 cells per well for 24 h at 37°C in a humidified atmosphere of 95% air and 5% CO₂. After 24 h, cells were exposed for 48 h at 37°C to known concentrations of the kombucha beverage, green tea

extract, and doxorubicin. Then, the culture medium was removed and 200 µl of MTT reagent (diluted in culture medium, 0.5 mg/ml) was added. Following incubation for 4 h, the MTT/medium was removed and replaced with dimethyl sulfoxide (200 µl) to dissolve the formazan crystals. The absorbance of each well was recorded by EPOCH microplate spectrophotometer (BioTek, USA) at 570 nm. The cells without culture medium treatment were defined as a control group. The cell viability was calculated using the following formula:

$$\text{Growth inhibition percent} = \frac{\text{A570 nm of treated cells}}{\text{A570 nm of control cells}} \times 100.[1,13]$$

Evaluation of anticancer activity

Cell cycle analysis

HCT-116 cells were seeded at a density of 2×10^5 cells into 24-well tissue culture plates. After culturing for 24 h, the medium was changed and the desired concentrations of the kombucha beverages 1 (3.21 mg) and 2 (3.83 mg), combination of kombucha beverages 1 + doxorubicin (2.1 mg) and kombucha beverages 2 + doxorubicin (1.43 mg), green tea extract (1.9 mg), and doxorubicin (0.0018 mg) added. After 48 h of incubation, the cells were harvested and fixed gently with 80% ethanol in the freezer for 2 h and then treated with 0.25% Triton X-100 for 5 min in ice bath. Cells were resuspended in 150 µL of PBS containing 40 µg/mL propidium iodide (PI) and 0.1 mg/mL RNase and were incubated in a dark room for 20 min at room temperature. Cell cycle analysis was performed using a FACScan flow cytometer. The data were analyzed using FlowJo 9.0 software (Tree Star, Ashland, OR, USA). Untreated cells were used as a control group.^[28,29]

Apoptosis assay

HCT-116 cells were seeded in 24-well tissue culture plates (2×10^5 cells/well). After 48 h, the medium was changed and treated with the desired concentrations as above. After 48 h, the cells floating in the medium were collected and added to the detached adherent cells with 0.05% trypsin. After gentle pipetting, the cells were centrifuged for 5 min at $1500 \times g$. The cell pellet was stained with annexin V-fluorescein isothiocyanate (FITC) and PI according to the manufacturer's instructions. Briefly, the supernatant was removed, and cell pellet was washed twice with ice-cold PBS (pH 7.4) and resuspended in 500 µL PBS. The cells were incubated with 5 µL of annexin V-FITC and 10 µL of PI in the dark for 20 min at room temperature. Finally, the stained cells were subjected to fluorescence intensity assay in FL-1 (FITC) and FL-2 (PI) channels, using FACScan flow cytometer (Mindray, China). Annexin V-FITC detects translocation of phosphatidylinositol from the inner to the outer cell membrane during early apoptosis, and PI

can enter the cell in late apoptosis or necrosis. Untreated cells were used as a control for the double staining. The data were analyzed using FlowJo 9.0 software (Tree Star, Ashland, OR, USA).^[28,29]

Apoptotic gene expression assay

The HCT-116 cells were seeded in 6-well plates at 1×10^6 cells per well for 24 h and then treated as previous. After incubation for 48 h of incubation, total RNA was extracted using RNX-Plus reagent (RNX-Plus™ CinnaGen Co). Total extracted RNA was quantified by optical density measurement (A260/A280 ratio) with NanoDrop 2000c Spectrophotometer. The cDNA synthesis was performed using QuantiTect Reverse Transcription Kit (Qiagen, USA). The cDNA was utilized as a template for subsequent quantitative polymerase chain reaction (qPCR) amplification using primers specific for B-cell leukemia/lymphoma 2 (Bcl-2)-associated X protein (Bax), Bcl-2, p21, p53, and β-actin genes. The β-actin gene was used as an internal control gene [Table 1]. The primer sequences were designed using Gene Runner software version 6.4.08 Beta (Hastings Software Inc., NY, US).

q-PCR reaction was carried out on step one real-time PCR system (Thermo Scientific, USA) using SYBR Green PCR Master Mix. The reaction mixture consisted of 1 µl of cDNA, 5.75 µM of each primer, and 12.5 µ M of 1X qPCR master mix. The qPCR program performed with following steps: The first step was initial denaturation at 95°C for 2 min, followed by 35-cycle denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 40 s, and the final extension at 72°C for 7 min. The results were expressed as $2^{-\Delta\Delta Cq}$.^[30,31]

Table 1: Primer sequences used for evaluation of apoptotic gene expression by quantitative polymerase chain reaction

Gene name	Gene sequence (5'→3')	Product length (bp)
Bax		
Forward	CCCGAGAGGTCTTTTTCCGAG	144
Reverse	TGGTTCTGATCAGTCCGGC	
Bcl-2		
Forward	GTTCCGCGTGATTGAAGA	191
Reverse	CCCGTTATCGTACCCT	
p21		
Forward	GGCACCCTAGTCTACCTCA	245
Reverse	CTCCTTGTTCCGCTGCTAAT	
p53		
Forward	CGTGTGGAGTATTGGATGAC	101
Reverse	TTGTAGTGGATGGTGGTACAGTC	
β-actin		
Forward	AAACTGGAACGGTGAAGGT	130
Reverse	AACAACGCATCTCATATTGGAA	

Data collection tool and technique

All of the experiments were in a minimum of three independent replicates. The results were expressed as mean \pm standard deviation. Differences between groups were assessed by one-way ANOVA. The significance of difference between particular treatment groups was analyzed using SPSS software (version 20.0; IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Results

Cell viability

The cell viability assay was assessed by MTT method. After evaluation of half-maximal inhibitory concentration (IC₅₀) concentration of each agent individually, HCT-116 cells were exposed for 48 h at 37°C to known serial concentrations of kombucha beverage, green tea extract, and doxorubicin. The IC₅₀ values of each one are presented in Table 2. According to IC₅₀ concentration, the treatment groups were selected as follows: kombucha beverage 1 (3.21 mg), kombucha beverage 2 (3.83 mg), kombucha beverage 1 + doxorubicin (2.1 mg), kombucha beverage 2 + doxorubicin (1.43 mg), green tea extract (1.9 mg), and doxorubicin (0.0018 mg). The results show that kombucha beverages could significantly increase the cytotoxicity of doxorubicin, in this state, the IC₅₀ of doxorubicin diminished to ~1.5-fold in HCT-116 cells.

Cell cycle

The cell cycle analysis of treated HCT-116 cells with each group is shown in Table 3. The results showed that administration of kombucha compared with the green tea extract significantly increased the G₀/G₁ phase compared with green tea extract (i.e., 60.5% and 55.7% at kombucha 2 and kombucha 1 vs. 55.6% green tea, respectively), with a concomitant decrease in S phase cells. Therefore, during kombucha treatment, transition of the cells to S phase could not performed and they remain in G₀/G₁ phase of the cell cycle.

Table 2: The IC₅₀ values of kombucha beverage 1 (3.21 mg), kombucha beverage 2 (3.83 mg), kombucha beverage 1+doxorubicin (2.1 mg), kombucha beverage 2+doxorubicin (1.43 mg), green tea extract (1.9 mg), and doxorubicin (0.0018 mg) on HCT-116 cells

Treatment	IC ₅₀ value (mg/ml)
Kombucha beverage 2+doxorubicin	0.586 \pm 0.46
Kombucha beverage 1+doxorubicin	0.615 \pm 0.28
Doxorubicin	0.879 \pm 0.38
Kombucha beverage 2	0.937 \pm 0.19
Kombucha beverage 1	0.964 \pm 0.29
Green tea extract	1.24 \pm 0.1

Data represent the mean \pm SD. SD=Standard deviation

Moreover, the co-administration of kombucha with doxorubicin increased the G₀/G₁ phase compared with the doxorubicin alone (i.e., 71.2% vs. 61.5%, respectively) [Table 3].

Apoptosis

Apoptosis progress was analyzed using double-staining annexin V-FITC/PI in the treated HCT-116 cells using flow cytometry. The scatter plot of double-variable flow cytometry illustrated living cells in the Q1 quadrant (FITC-/PI-), early apoptotic cells in the Q2 quadrant (FITC+/PI-), late apoptotic cells in the Q3 quadrant (FITC+/PI+), and necrotic cells in the Q4 quadrant (FITC-/PI+). As shown in Figure 1, treatment with kombucha compared with the green tea extract caused significantly increasing in the rate of early apoptosis [i.e., 15.6% and 10% at kombucha 2 and kombucha 1 vs. 6.08% green tea, Figure 1a-f, respectively]. Moreover, the co-administration of kombucha with doxorubicin drug increased the cells in the early phase apoptosis rate compared with the doxorubicin individually (i.e., 20.1% vs. 17.4%, respectively).

Apoptotic genes expression

Expression of genes that involved in cell survival and apoptosis (Bax, Bcl-2, p21, and p53) evaluated by real-time PCR. As illustrated in Figures 2-5, the administration of kombucha compared with the green tea extract increased the expression levels of p21, p53, and Bax while it decreased the expression level of Bcl-2 in HCT-116 cells during 48 h. Moreover, the treatment HCT-116 cells with kombucha compared with the green tea extract increased the ratio of Bax/Bcl-2 expression. Moreover, the co-administration of kombucha with doxorubicin increased the p21, p53, Bax, and the ratio of Bax/Bcl-2 expression while it decreased the expression level of Bcl-2 compared with the doxorubicin treatment alone [Table 4].

Discussion

In the traditional medicine according to epidemiological studies, green tea is known as strong protective against certain human cancers. The main constructors of chemical composition of green tea are catechin polyphenols that demonstrated inhibition activity against proliferation of colon carcinoma, lung carcinoma, breast carcinoma, melanoma, and leukemic cells. Chemoprevention using a combination of dietary phytochemicals with diverse mechanisms has proposed as a successful approach to control different types of cancer with fewer side effects.^[22,32]

The anticancer and antiapoptotic effect of kombucha beverage prepared from green tea on human colon cancer

Table 3: Cell cycle analysis results of kombucha beverage 1 (3.21 mg), kombucha beverage 2 (3.83 mg), kombucha beverage 1+doxorubicin (2.1 mg), kombucha beverage 2+doxorubicin (1.43 mg), green tea extract (1.9 mg), and doxorubicin (0.0018 mg) on HCT-116 cells

Cell cycle stage	Kombucha beverage 2+doxorubicin	Kombucha beverage 1+doxorubicin	Doxorubicin	Kombucha beverage 2	Kombucha beverage 1	Green tea extract	Control
G0/G1	71.2	66.1	61.5	60.5	55.7	55.6	51.2
S	12.4	12.7	13	13.2	14.4	14.8	20.9
G2/M	8.92	14	22.5	22.7	28.3	28.5	29.3

Table 4: Relative expression of Bax/Bcl2 in HCT-116 human colorectal cancer cell treated with kombucha beverage 1 (3.21 mg), kombucha beverage 2 (3.83 mg), kombucha beverage 1+doxorubicin (2.1 mg), kombucha beverage 2+doxorubicin (1.43 mg), green tea (1.9 mg), and doxorubicin (0.0018 mg) on expression levels of Bax, Bcl-2, p21, and p53

Type of treatment	Kombucha beverage 2+doxorubicin	Kombucha beverage 1+doxorubicin	Doxorubicin	Kombucha beverage 2	Kombucha beverage 1	Green tea extract	Control
Ratio Bax/Bcl2	0.326	0.197	0.019	0.016	0.002	0.000000083	1

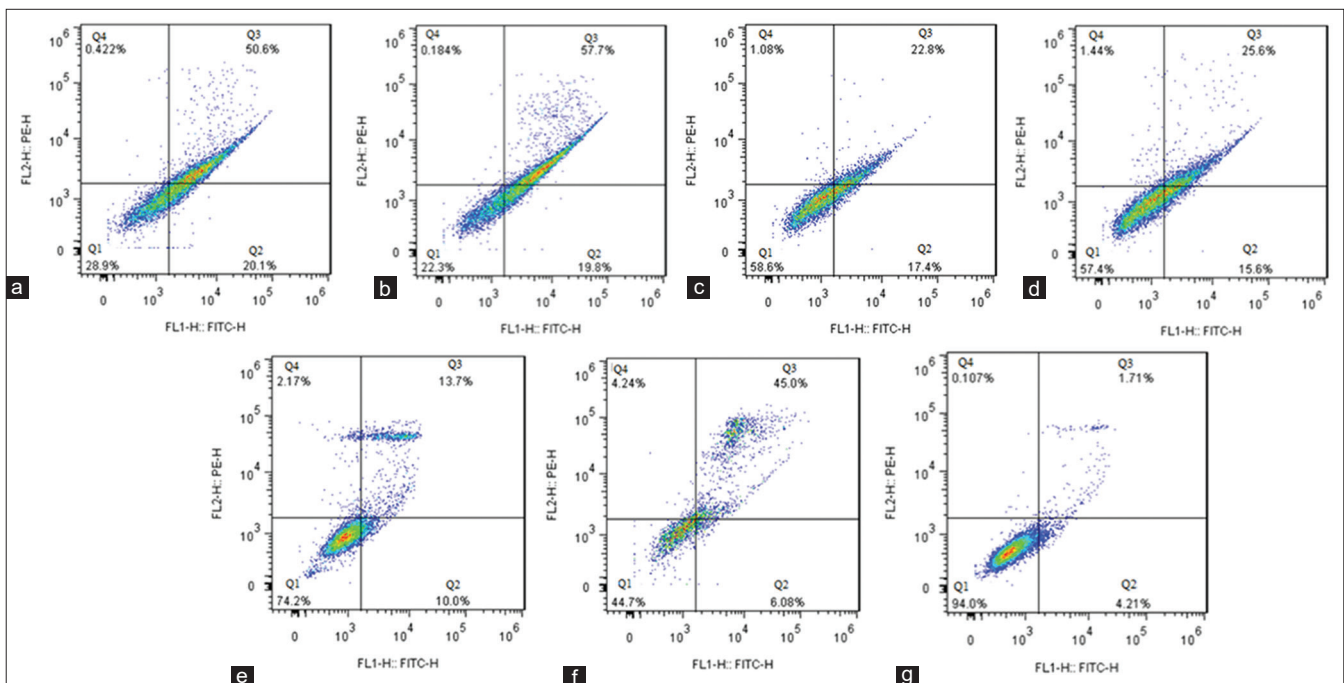


Figure 1: Apoptosis assay using flow cytometry following the treatment of cells for 48 h. (a) Kombucha beverage 2 + doxorubicin (1.43 mg); (b) Kombucha beverage 1 + doxorubicin (2.1 mg); (c) doxorubicin (1.43 mg); (d) Kombucha beverage 2 (3.83 mg); (e) Kombucha beverage 1 (3.21 mg); (f) Green tea extract (1.9 mg); (g) Control

cells had not studied yet. Hence, in the present study, we evaluated the effects of kombucha on the carcinogenic factors in HCT-116 cells (human colorectal cancer cell line) as *in vitro* model. The study was performed in comparative mode between kombucha beverage, green tea extract, and doxorubicin drug individually or combined. As many researches have investigated the antiproliferative properties of kombucha, the current study demonstrated that kombucha has a significant antiproliferative activity *in vitro*, against HCT-116 human colon cancer cells. This effect suggested that correlated to the kombucha rich matrix of polyphenols, D-saccharic acid-1,4-lactone, vitamins, gluconic acid, glucuronic acid, and lactic acid.^[33] On the other hand, catechin that

was found in green tea has antioxidant, anticancer, antidiabetic, and anti-atherosclerosis properties, which were better exerted under an environment containing acetic acid and glucuronic acid. The increase of anticancer effects of catechin available in green tea may be because of the existence of acetic acid and gluconic acid in kombucha.^[15,34]

In the recent study, the estimation of anticancer activity, evaluated by cell cycle analysis in HCT-116 cells treated with the desired concentrations of the kombucha beverages, kombucha beverage + doxorubicin, green tea extract, and doxorubicin, demonstrated that the co-administration of kombucha with doxorubicin

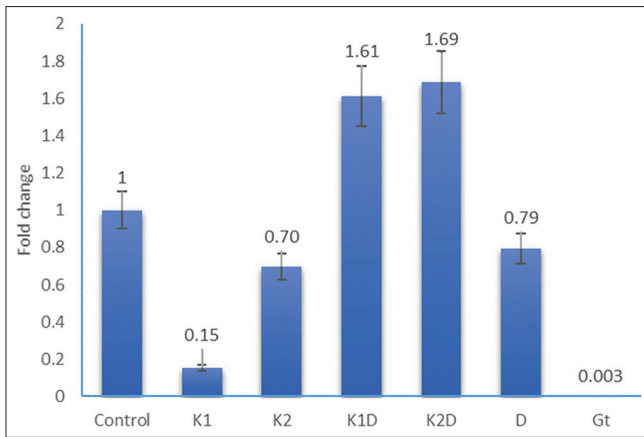


Figure 2: Expression of B-cell leukemia/lymphoma 2-associated X protein gene following the treatment of cells for 48 h. Control; K1 = Kombucha beverage 1 (3.21 mg), K2 = Kombucha beverage 2 (3.83 mg), K1D = Kombucha beverage 1 + doxorubicin (2.1 mg), K2D = Kombucha beverage 2 + doxorubicin (1.43 mg), D = doxorubicin (1.43 mg), GT = Green tea extract (1.9 mg)

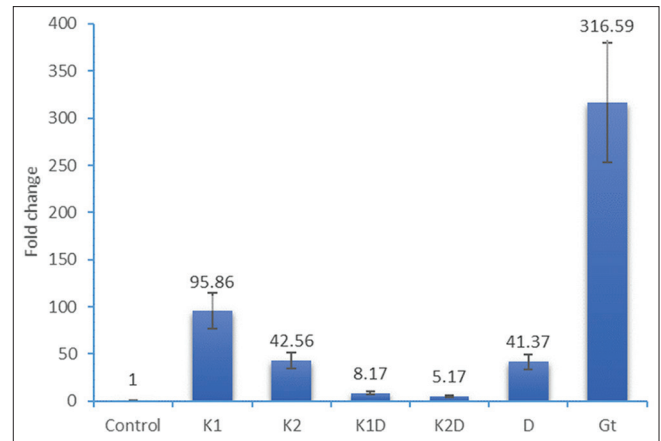


Figure 3: Expression of B-cell leukemia/lymphoma 2 gene following the treatment of cells for 48 h. Control; K1 = Kombucha beverage 1 (3.21 mg), K2 = Kombucha beverage 2 (3.83 mg), K1D = Kombucha beverage 1 + doxorubicin (2.1 mg), K2D = Kombucha beverage 2 + doxorubicin (1.43 mg), D = Doxorubicin (1.43 mg), GT = Green tea extract (1.9 mg)

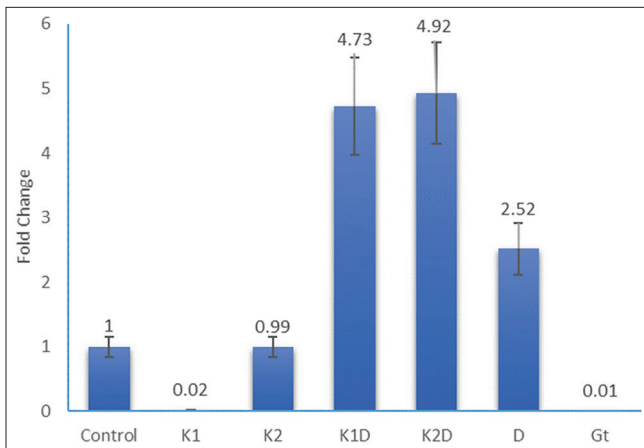


Figure 4: Expression of p53 gene following the treatment of cells for 48 h. Control; K1 = Kombucha beverage 1 (3.21 mg), K2 = Kombucha beverage 2 (3.83 mg), K1D = Kombucha beverage 1 + doxorubicin (2.1 mg), K2D = Kombucha beverage 2 + doxorubicin (1.43 mg), D = Doxorubicin (1.43 mg), GT = Green tea extract (1.9 mg)

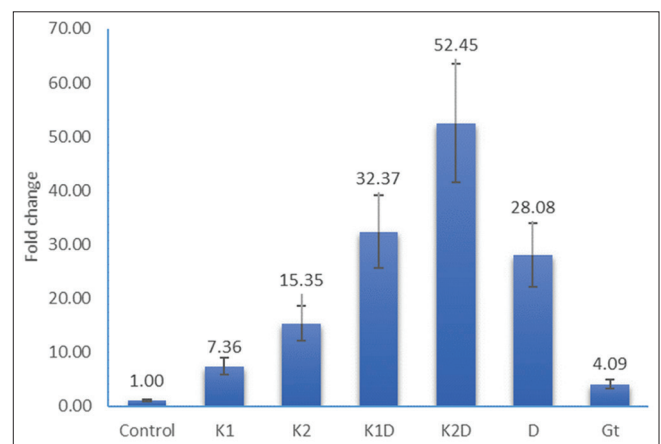


Figure 5: Expression of p21 gene following the treatment of cells for 48 h. Control; K1 = Kombucha beverage 1 (3.21 mg), K2 = Kombucha beverage 2 (3.83 mg), K1D = Kombucha beverage 1 + doxorubicin (2.1 mg), K2D = Kombucha beverage 2 + doxorubicin (1.43 mg), D = Doxorubicin (1.43 mg), GT = Green tea extract (1.9 mg)

drug increased the cells in the G0/G1 phase compared with the doxorubicin treatment alone. Therefore, in other words, kombucha beverages enhanced activity of antitumor agents that is new signing anti-colorectal activity of *in vitro* studies and provides the possibility that kombucha beverage acts as a biochemical modulator. It could expect that kombucha beverages promote cancer chemotherapy.

A regulated form of cellular suicide is apoptosis that occurs in multicellular organisms due to endogenous and exogenous death stimuli, including death ligands such as tumour necrosis factor alpha (TNF- α), FasL/CD95/Apo1, and TNF-related apoptosis-inducing ligand, as well as chemotherapeutic agents such as cisplatin, doxorubicin, and 5'FU.^[35-37] Ability of cellular mechanisms to suppress the tumor formation and

response to many types of cancer therapy is due to p53 proteins that induce apoptosis. Following cellular stresses, p53 protein was stabilized and binds to DNA as a tetramer, in a sequence-specific manner that results in the transcriptional activation of a series of apoptosis-associated proteins including p53-upregulated modulator of apoptosis, Bax, Bcl-2 antagonist killer, and Bcl-2. By keeping a balance between pro- and antiapoptotic members, Bcl-2 proteins (located on the mitochondrial membrane) regulate apoptosis.^[37,38] In this study evaluation of apoptosis progress by annexin V-FITC, staining in HCT-116 cells treated with the desired concentrations of the kombucha beverages revealed a significant increase in early-phase apoptosis rate when doxorubicin administrated simultaneously with kombucha beverage in comparison with the doxorubicin individually.

Targeted induction of apoptosis in cancer cells is an important strategy for cancer prevention and therapy. The present study findings demonstrated that kombucha caused early apoptosis in HCT-116 human colorectal cancer cell line and enhanced the effect of doxorubicin as a chemotherapeutic agent. In addition, real-time PCR analysis showed a significant increase of Bax, p21, and p53 gene expression levels when doxorubicin administrated simultaneously with kombucha beverages versus doxorubicin alone. Hence, according to the finding of this study, kombucha beverage could be discussed as an assistor or booster of antitumor activity of doxorubicin as a chemotherapeutic agent.

Conclusion

As previous studies revealed that green tea had a great potential in cancer therapy, a recent study suggests that kombucha beverage inhibited antiapoptotic function of Bcl-2 and induced the cascade expression of Bax, p21, and p53 genes more strongly. Therefore, kombucha beverages prepared from green tea possess interesting antiproliferative properties associated with significant antiapoptotic activity in cellular and molecular scales. Furthermore, these findings declared a new potential of natural therapy alone or align to chemotherapy to elevate the efficiency of general therapeutic guidelines and decreased their side effects. On the other hand, this study reviled the impact of natural product manufacturing. As known, natural product therapy is one of the hopeful aims as therapeutic or therapeutic assistor in cancer medication. Use as nutrition or as drug in two ways had a unique goal in cancer therapy domain. As reported in the traditional documents the anticancer use of kombucha, this study demonstrated this biological activity against colorectal cancer in the *in vitro* model. In addition, this study reviled a promissful evidence for use of kombucha combined with chemotherapeutic guidelines to elevate the efficiency of general therapeutic medicines. Therefore, this study suggested that future researches will aimed to define new practical guidelines more efficient with less adverse effects assisted with natural products. Moreover, this study demonstrated that kombucha beverages cause boosted activity of doxorubicin antitumor agent in colorectal cancer treatment. Therefore, this study recommended the more focus on biological activity of kombucha beverage as a less known natural product in cancer therapy especially in the practical models.

Ethical considerations

The authors would like to thank the Iran University of Medical Sciences for funding this study as a PhD thesis. This study has been approved by Ethics Committee of IUMS (ethic code : IR.IUMS.FMD.REC.1396.92234775204).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Alemi A, Farrokhifar M, Zare-Zardini H, Karamallah MH. A comparison between the anticancer activities of free paclitaxel and paclitaxel-loaded niosome nanoparticles on human acute lymphoblastic leukemia cell line Nalm-6. *Iran J Pediatr Hematol Oncol* 2018;8:153-60.
2. Bishehsari F, Mahdavinia M, Vacca M, Malekzadeh R, Mariani-Costantini R. Epidemiological transition of colorectal cancer in developing countries: Environmental factors, molecular pathways, and opportunities for prevention. *World J Gastroenterol* 2014;20:6055-72.
3. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin* 2014;64:104-17.
4. Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. *CA Cancer J Clin* 2009;59:366-78.
5. Kolahdoozan S, Sadjadi A, Radmard AR, Khademi H. Five common cancers in Iran. *Arch Iran Med* 2010;13:143-6.
6. Moghimi-Dehkordi B, Safaee A, Zali MR. Prognostic factors in 1,138 Iranian colorectal cancer patients. *Int J Colorectal Dis* 2008;23:683-8.
7. Rafiemanesh H, Pakzad R, Abedi M, Kor Y, Moludi J, Towhidi F, et al. Colorectal cancer in Iran: Epidemiology and morphology trends. *EXCLI J* 2016;15:738-44.
8. Hosseini SV, Izadpanah A, Yarmohammadi H. Epidemiological changes in colorectal cancer in Shiraz, Iran: 1980-2000. *ANZ J Surg* 2004;74:547-9.
9. Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, et al. Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control* 2013;24:1207-22.
10. Sever R, Brugge JS. Signal transduction in cancer. *Cold Spring Harb Perspect Med* 2015;5:a006098.
11. Wang LH, Wu CF, Rajasekaran N, Shin YK. Loss of tumor suppressor gene function in human cancer: An overview. *Cell Physiol Biochem* 2018;51:2647-93.
12. Liu H, Lv L, Yang K. Chemotherapy targeting cancer stem cells. *Am J Cancer Res* 2015;5:880-93.
13. Alemi A, Reza JZ, Haghirsadat F, Jaliani HZ, Karamallah MH, Hosseini SA, et al. Paclitaxel and curcumin coadministration in novel cationic PEGylated niosomal formulations exhibit enhanced synergistic antitumor efficacy. *J Nanobiotechnol* 2018;16:28.
14. Wang BL, Shen YM, Zhang QW, Li YL, Luo M, Liu Z, et al. Codelivery of curcumin and doxorubicin by MPEG-PCL results in improved efficacy of systemically administered chemotherapy in mice with lung cancer. *Int J Nanomed* 2013;8:3521-31.
15. Hosseini S, Gorjian M, Rasouli L, Shirali S. A comparison between the effect of green tea and kombucha prepared from green tea on the weight of diabetic rat. *Biomed Pharmacol J* 2015;12:141-6.
16. Tehrani HG, Allahdadian M, Zarre F, Ranjbar H, Allahdadian F. Effect of green tea on metabolic and hormonal aspect of polycystic ovarian syndrome in overweight and obese women suffering from polycystic ovarian syndrome: A clinical trial. *J Educ Health Promot* 2017;6:36.
17. Rezaee E, Mirolohi M, Hassanzadeh A, Fallah A. Factors affecting tea consumption pattern in an urban society in Isfahan, Iran. *J Educ Health Promot* 2016;5:13.
18. Barati F, Javanbakht J, Adib-Hashemi F, Hosseini E, Safaie R, Rajabian M, et al. Histopathological and clinical evaluation of Kombucha tea and Nitrofurazone on cutaneous full-thickness

- wounds healing in rats: An experimental study. *Diagn Pathol* 2013;8:120.
19. Gaggia F, Baffoni L, Galiano M, Nielsen DS, Jakobsen RR, Castro-Mejia JL, et al. Kombucha beverage from green, black and rooibos teas: A comparative study looking at microbiology, chemistry and antioxidant activity. *Nutrients* 2018;11:1.
 20. Dufresne C, Farnworth E. Tea, kombucha, and health: A review. *Food Res Int* 2000;33:409-21.
 21. Kapp JM, Sumner W. Kombucha: A systematic review of the empirical evidence of human health benefit. *Ann Epidemiol* 2019;30:66-70.
 22. Aloulou A, Hamden K, Elloumi D, Ali MB, Hargafi K, Jaouadi B, et al. Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. *BMC Complement Altern Med* 2012;12:63.
 23. Dutta D, Gachhui R. Nitrogen-fixing and cellulose-producing *Gluconacetobacter kombuchae* sp. nov., isolated from kombucha tea. *Int J Syst Evol Microbiol* 2007;57:353-7.
 24. Jayabalan R, Marimuthu S, Thangaraj P, Sathishkumar M, Binupriya AR, Swaminathan K, et al. Preservation of kombucha tea-effect of temperature on tea components and free radical scavenging properties. *J Agric Food Chem* 2008;56:9064-71.
 25. Murugesan GS, Sathishkumar M, Jayabalan R, Binupriya AR, Swaminathan K, Yun SE. Hepatoprotective and curative properties of kombucha tea against carbon tetrachloride-induced toxicity. *J Microbiol Biotechnol* 2009;19:397-402.
 26. Abou-Taleb K, Ebeed N, Soheir S, El-Salam SS, Amin S. Antimicrobial and antiproliferative, pro-apoptotic actions of kombucha fermented solutions against colon and heptao cancer cell lines. *World Journal of Pharmaceutical and Life Sciences* 2017;3:120-32.
 27. Leal JM, Suárez LV, Jayabalan R, Oros JH, Escalante-Aburto A. A review on health benefits of kombucha nutritional compounds and metabolites. *CyTA – Journal of Food* 2018;16:390-9.
 28. Chen L, Zhang HY. Cancer preventive mechanisms of the green tea polyphenol (-)-epigallocatechin-3-gallate. *Molecules* 2007;12:946-57.
 29. Lecumberri E, Dupertuis YM, Mirahbell R, Pichard C. Green tea polyphenol epigallocatechin-3-gallate (EGCG) as adjuvant in cancer therapy. *Clin Nutr* 2013;32:894-903.
 30. Du GJ, Zhang Z, Wen XD, Yu C, Calway T, Yuan CS, et al. Epigallocatechin gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. *Nutrients* 2012;4:1679-91.
 31. Thangapazham RL, Singh AK, Sharma A, Warren J, Gaddipati JP, Maheshwari RK. Green tea polyphenols and its constituent epigallocatechin gallate inhibits proliferation of human breast cancer cells *in vitro* and *in vivo*. *Cancer Lett* 2007;245:232-41.
 32. Huang CY, Han Z, Li X, Xie HH, Zhu SS. Mechanism of EGCG promoting apoptosis of MCF-7 cell line in human breast cancer. *Oncol Lett* 2017;14:3623-7.
 33. Moradzadeh M, Hosseini A, Erfani S, Rezaei H. Epigallocatechin-3-gallate promotes apoptosis in human breast cancer T47D cells through down-regulation of PI3K/AKT and Telomerase. *Pharmacol Rep* 2017;69:924-8.
 34. Jayabalan R, Malbasa RV, Loncar ES, Vitas JS, Sathishkumar M. A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Compr Rev Food Sci Food Saf* 2014;13:538-50.
 35. Deghrigue MC, Battikh H, Abid K, Bakhrouf A. Antiproliferative and antimicrobial activities of kombucha tea. *Afr J Microbiol Res* 2013;7:3466-70.
 36. Beigmohammadi F, Karbasi A, Beigmohammadi Z. Production of high glucuronic acid level in kombucha beverage under the influence environmental condition. *Journal of Food Technology and Nutrition* 2010;7:30-38.
 37. Bai WK, Shen E, Hu B. The induction of the apoptosis of cancer cell by sonodynamic therapy: A review. *Chin J Cancer Res* 2012;24:368-73.
 38. Houghton JA. Apoptosis and drug response. *Curr Opin Oncol* 1999;11:475-81.