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ORIGINAL ARTICLE

Evaluation of Phytochemical Content of White Tea Clone 100 and Changes the Expression of Tumor Suppressor Genes on Colorectal Cancer Cell Line HCT116

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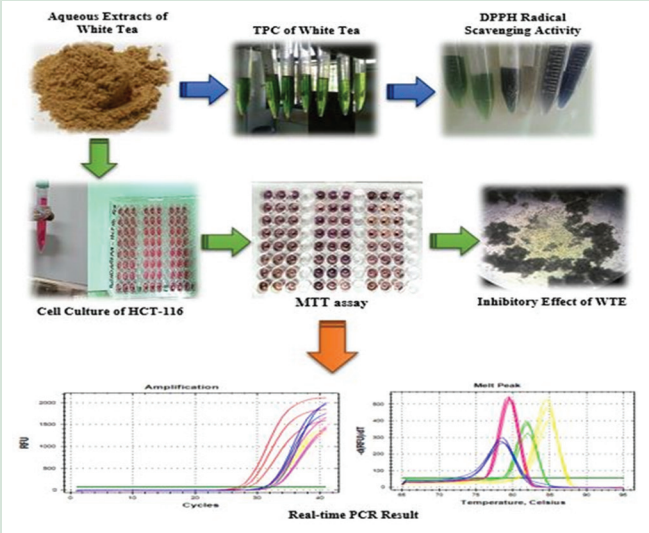
ABSTRACT

Aim/Background: Colorectal carcinoma cancer is one of the main types of cancer with high death rate of patients, according to their non-healthy lifestyle. In this research, we evaluated inhibitory of white tea clone 100 extract on colorectal cancer cell line HCT-116, and its effectiveness in expression level of three tumor suppressor genes. **Materials and Methods:** Total polyphenolic content in all samples were measured using the Folin–Ciocalteu method, and free radical adsorption investigated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the white tea extract inhibitory effect on HCT-116 cells. **Results:** Among different extraction methods, the white tea aqueous extracts produced the highest amount of polyphenols and DPPH radical scavenging activity (36.67 ± 0.54 mg gallic acid equivalent/g dry weight and 71.74% ± 0.42%, respectively). Cell survival analysis in the MTT assay indicated that aqueous white tea extract could reduce the viability of HCT-116 cells in 8, 16, and 24 h considerably, related to the concentration-dependent manner. The real-time polymerase chain reaction results indicated the significantly increased expression level ($P < 0.05$) of tumor suppressor genes *DCC*, *TGFBR2*, and *P53* in the concentration of 1000 µg/ml at 24 h. **Conclusion:** White tea aqueous extract because of having hydroxyl group in its structure at optimal concentration in all three times of experimental, could lead to a positive changes effect on gene expression in the tumor suppressor family.

Key words: 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, HCT-116 cell line, radical scavenging activity, tumor suppressor genes, white tea

SUMMARY

- White tea clone 100, is the inbreeding tea plant that specially growth in Iran.
- HCT-116 is one of the major colorectal cell line that incidence in Iran.
- This study aimed to investigate the best concentration of aqueous extract of white tea for treatment in colon cancer.
- Aqueous extract of white tea, could significantly scavenging free radicals.
- Aqueous extract of white tea had high potential for antiproliferation in colon cancer (cell line HCT-116)
- The results showed that aqueous extract of white tea, significantly control the regulation of gene expression in Tumor Suppressor families.
- The results indicated that, in future we can use super-beverage like white tea as pre-drug in appropriate doses for prevention and controlling colon cancer.



Abbreviations used: HCT-116: Homo sapiens colon colorectal carcinoma, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, GAE: Gallic acid equivalent, MTT: 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, DCC: Deleted in colorectal carcinoma, P53: Tumor protein, TGFBR2: Transforming growth factor-beta (TGF-β) receptor type 2, ISO: International Organization for Standardization, TPC: Total polyphenol content, HPLC: High performance liquid chromatography, DMEM: Dulbecco's modified eagle medium, WTE: White tea extract, RPMI1640: Roswell Park Memorial Institute, DMSO: Dimethyl sulfoxide, IC_{50}: The half-maximal inhibitory concentration.

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INTRODUCTION

Camellia sinensis (L.) Kuntze is an extremely extended plant, which has a hereditary with the Theaceae family.[1] The white tea has a specific product for post-reap handling, containing a more valuable extent of sprouts, which are secured with a thin layer of silver-haired describing the tea shading.[2] Phenolic compounds are described as having at least one fragrant core-containing hydroxylated substituents and also its useful derivate; as flavonoids called catechins.[3,4] Essential monomeric catechins, which are available in the teas in high amount

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are as follows: (-)-epigallocatechin, (+)-catechin, (-)-epigallocatechin gallate, (-)-epicatechin, and (-)-epicatechin gallate.[5] The catechins and different flavonoids can catch responsive oxygen species, for example, superoxide radical (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH·), all considered remarkably harmful for lipids, proteins, and DNA.[6] Various genes have been identified in the colorectal carcinoma cancer, and out of them effects of nontoxic plant extracts in appropriate concentrations can cause an increase or decrease in their expression and also can play an important role in controlling the progress of cancer.[7] A tumor silencer gene, as *P53*, *TGFBR2*, and *DCC* are genes that shields a cell against the one stage on the way to disease. The *P53* is engaged with cell cycle hindrance, apoptosis, hereditary strength, and restraint of angiogenesis.[8] Transformations in the *P53* gene seem late in the adenoma-carcinoma succession and also are found in around 40%–50% of all colorectal cancer (CRC) cases.[9] By using specific tea polyphenols validated biomarkers, prospectively examined the specific tea catechins urinary concentrations, and their metabolites and the risk of developing CRC.[10] This study purpose was to evaluate the white tea extract anti-oxidant activities and its effect on the inhibition of proliferation in the CRC cell line (HCT-116), as well as its role for the tumor suppressor genes activities in colorectal carcinoma cancer.

MATERIALS AND METHODS

Plant material

In this research White tea clone 100 leaves, (*C. sinensis* (L.) O. kuntze), belonging to the Theaceae family were used. The sample was collected during the growing season, spring 2016, by Tea Research Institute of Iran (GPS location of 37° 14′ 30″ N latitude and 52° 3′ E longitude).

Extraction method

The method described by the International Organization for Standardization (ISO) 14502-1 (2004),[11] with some modifications was used to achieve the white tea extracts, and three solvents methods were applied for extraction.

Methanol extraction methods

In this method, 0.5 ± 0.001 g of white tea leaf sample (from clone 100 Iran) was ground in liquid nitrogen, and after that, it was put into extraction tubes. Then, 10 mL of 70% methanol was added at 70°C, and the mixture was heated at 70°C on bain-marie for 10 min. Afterward, the extract tubes were sonicated for about 5–7 min and were heated again at 70°C for 10 min. After cooling at room temperature, the extract was centrifuged at 3500 rpm for 10 min and also the supernatant was decanted into graduated tubes. One milliliter of extract was diluted with distilled water to 10 mL for using in the different chemical analysis.

Aqueous extraction method

In this method, 10 mL of boiling water was added to 0.5 ± 0.001 g of white tea leaf sample in the extraction tubes, and then, it followed the methanol method process as described above.

Aqueous – methanol extraction method

In this method, 5 mL of distilled water at 100°C, and 5 mL of 70% methanol at 70°C were added to 0.5 ± 0.001 g of white tea leaf sample in an extraction tube, and then, its process were as same as described for the methanol method.

Determination of total polyphenolic content

The total polyphenolic content (TPC) was measured spectrophotometrically, by using the Folin-Ciocalteu reagent, with gallic acid (GA) (99% purity, Sigma, Germany) as standard, and as same as described by the ISO 14502-1. Each of the diluted tea extract (1 mL) was taken (in triplicates) in separate tubes containing 5 mL of a 1:10 dilution of the Folin-Ciocalteu reagent (Merck chemicals, Germany) in water. Then, 4 mL of anhydrous sodium carbonate solution (7.5% w/v, 95% purity, Teb-azma Co., Iran) was added to it, and the tube contents were vortexed for 5 min, and after that allowed to stay at room temperature for 1 h. Sodium carbonate was omitted from the tubes in some references. In this survey, both methods were compared (in order to determine the differences between them). The amount of absorbance against distilled water was measured at 765 nm. The polyphenols concentrations in the samples were derived from a GA standard curve (Pearson's correlation coefficient $r^2 = 0.9877$), and the TPC was expressed as the mg gallic acid equivalents (GAE)/g dry weight.

Determination of 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity

The extracts 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities were examined by using the Blois modified method (1958),[12] A volume of 1 mL from each extract was mixed with 5 mL of 500 µM DPPH solution in absolute ethanol and 2.5 mL of 0.1 M Tris-HCI buffer, pH 7.4. This mixture kept at room temperature in darkness for 30 min. Then, the amount of absorbance was read at 517 nm in a spectrophotometer. The polyphenols free radical scavenging activity in the sample was derived from ascorbic acid standard curve (Sigma Co., Germany). Radical scavenging was calculated in terms of % inhibition as following:

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0\}100,$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the samples.

High-performance liquid chromatography analysis

The high-performance liquid chromatography (HPLC) system consisted of Shimadzu with a fixed wavelength UV-VIS detector (Model LC10AD VP), and a Rheodyne sample injector with a 20 µL sample loop. The chromatography column was C18 (300 mm × 3.9 mm ID × 5 µm). The mobile phase flow rate was 1 ml/min, and the detection was carried out by ultraviolet absorbance measurement at 280 nm. The mobile phase was composed of water, acetonitrile, methanol, ethyl acetate, and glacial acetic acid (89:6:3:1:1 v/v/v/v/v) (Merck Chemicals, Germany). A Star chromatography workstation, version 6.3 software (Varian 3800CP, www.varianinc.com) was used for the detector operation, and data processing static phase. Each samples Catechin concentration was determined as following:

$$C(u) = A(u) \times C(st)/A(st),$$

Where *C (u)* is the concentration of the unknown sample, *A (u)* is the peak area of the unknown sample, *C (st)* is the concentration of the standard, and *A (st)* is the area peak of standard.

Cell culture

Human colorectal carcinoma cells (HCT-116) were used to determine cell viability in 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. HCT-116 (ATCC_ HTB-38™) cells were cultured and grown in Dulbecco's Modified Eagle's Medium high glucose (Bio idea Co. Iran). The cells were supplemented with 10% fetal bovine serum, and 0.5% pen-strep (penicillin [10 U/ml], and streptomycin [10 µg/ml]) in a humidified incubator with 5% CO_2 as shown in Figure 1.

In vitro anti-proliferative effects of aqueous white tea extract

The white tea extract (WTE) inhibitory effect on the colorectal carcinoma cell line proliferation, HCT-116, was determined by using the MTT assay.

Briefly explain, cells were seeded in 96-well plates at 5000 cells/well, and allowed to attach overnight. Then, the media was changed, and the cells were treated with the extract various concentrations (0–1000 µg/mL) incubated for 8, 16, and 24 h. After that, 10 mL of MTT solution (100 mM of MTT bromide in RPMI-1640) was added to each well plate and incubated again for 4 h at 37°C. The supernatant was aspirated, and the MTT-formazan crystals formed by metabolically viable cells, which were dissolved in 50 ml of dimethyl sulfoxide. Eventually, each well-plates absorbance was observed in a microplate reader at a wavelength of 580 nm. Growth inhibition of the cells was calculated by using the formula (34) as below:

Inhibition (%) = $[(OD_{blank} - OD_{sample})/OD_{blank})] \times 100$

Determination of half-maximal inhibitory concentration

The extract concentration, which reduced the cells viability by 50% half-maximal inhibitory concentration (IC_{50}), was determined by plotting triplicate data points over a concentration range. The IC_{50} results were indicated as regression analysis by using GraphPad PRISM. Confidence limits and significance testing calculation were made at the level of $P = 0.05$.

Treatment by aqueous white tea extract

Each concentrate of IC_{50} for aqueous WTE (0, 150, 500, and 1000 µg/mL) was calculated and treated to cells. The medium was expelled from the monolayer cancer cells at 8, 16, and 24 h after treatment.

RNA extraction and c-DNA synthesis

Total RNA was extracted with Fermentas RNA isolation kit, and 0, 5 µg RNA was transformed to cDNA by using a first-strand cDNA synthesis kit (Pars Toos Co., Iran (during the real-time polymerase chain reaction (RT-PCR) process. RNA was determined by measuring the optical density at 260 and 280 nm by using nanodrop (NanoDrop-ND-1000), after purification and quantification.

Real-time polymerase chain reaction

RT-PCR test was used in order to determine the white tea extract effect on the level of gene expression, the *P53* primer set 5'-TTCACCGTACTAACCAGGGAAG-3' (forward) and 5'-CTCTGTGAGGTAGGTGCAAATG-3' (reverse), the *TGFBR2* primer set 5'-GAGGTCTATAAGGCCAAGCTGA-3' (forward) and 5'-TCCTTCTCTGTCTTCCAAGAGG-3' (reverse), The *DCC* primer set 5'-CAAGCCAGATGAGGGACTTTAC-3' (forward) and 5'-GAACC TCAGTGGTCCTGCTACT-3' (reverse) and the *ACTB* primer set as housekeeping gene 5'-GATCAGCAAGCAGGAGTATGAC-3' (forward) and 5'-CCAATCTCATCTTGTTTTCTGC-3' (reverse) from a RT-PCR with Bio-Rad CFX manager RT-PCR Detection System (Bio-Rad, USA). *Beta-actin* was used as a housekeeping gene. Each reaction was performed in triplicate by using the following procedure: 95°C for 10 min for 1 cycle, 50 cycles of 95°C for 15 s, and 59°C for 1 min. The gene expression relative quantification was attained by the comparative CT Method $2^{-\Delta\Delta Ct}$.

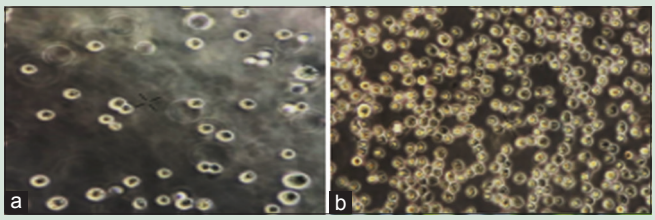


Figure 1: (a) First day of culture of cell line HCT-116, (b) third day of cell culture

Statistical analysis

Data are presented as a mean ± standard deviation, and all measurements and analysis were carried out in triplicate ($n = 3$). Excel 2013 and SPSS Version 22.0 (Armonk, New York, United States) statistical packages were used for the statistical and graphical evaluations. Absorbency value of each group was divided to absorbency value of control group in order to calculate viability percentage. Graphpad Prism 5 (5.04 Version) program was utilized to evaluate MTT results. In addition, Microsoft Excel (Office 2013) program was used to give the raw data meaning and converting the statistical data into computer graphics. Finally, variance's one-way analysis (one-way ANOVA) and Duncan's test were used to determine the differences between the experimental groups, apart from statistical analyses. In case ($P < 0.05$ = *, $P < 0.01$ = **), our results were accepted statistically meaningful.

RESULTS AND DISCUSSION

Total phenolic content estimation by Folin-ciocalteu's assay

According to Table 1, the polyphenol content amount of aqueous, methanol, and white tea extracts aqueous methanol were measured by using Folin-ciocalteu's and Folin-ciocalteu's with sodium carbonate methods. The results indicated that the sodium carbonate presence in the media increased the polyphenol detection efficiency. With comparison of both methods with each other, the white tea aqueous methanol extract significantly had the highest amount of polyphenol (36.67 ± 0.54, 23.08 ± 0.76 GAE/g DW), and aqueous extract considerably had the more polyphenol compound in compare to the methanol extract (27.08 ± 0.59, 17.91 ± 0.33 GAE/g DW). This study results of TPC for white tea stated that aqueous and aqueous-methanol extraction were effective methods for polyphenols and catechins detection. Earlier research reported that the best solvent combination was distilled water and methanol for polyphenol extraction (70%).[13,14]

Estimation catechins content by high-performance liquid chromatography method

HPLC profile results of aqueous, methanol, and white tea aqueous methanol extracts, showed 6 main chromatograms, indicating the phytochemical catechins existence in the tea. Due to the compounds retention time, GA, (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC), and (-)-epicatechin gallate (ECG) were identified. Meanwhile, the white tea aqueous and aqueous methanol extracts had the highest concentrations of different catechins in their biochemical structures [Table 2]. It was

Table 1: Total phenolic content in white tea clone 100, by Folin-Ciocalteu method

Extraction type/tea sample	TPC via Folin-Ciocalteu's	TPC via Folin-Ciocalteu's and sodium carbonate
Aqueous/white tea	17.91±0.33*	27.08±0.59*
Methanol (70%)/white tea	13.68±0.26	21.52±0.81
Aqueous - methanol (70%)/white tea	23.08±0.76**	36.67±0.54**

Expressed as gallic acid equivalent, GAE/g DW. Results are expressed as mean±SD of triplicate measurements. Statistically significant at (*P<0.05, **P<0.01). SD: Standard deviation; TPC: Total polyphenolic content

Table 2: Amount of catechins content in white tea samples extracted by methanol (70%), aqueous and aqueous - methanol (70%) methods

Sample	GA	EGC	C	EC	EGCG	ECG
Methanol (70%)/white tea	2.44[d]	5.63[d]	0.68[b,c]	1.62[d]	6.91[b,c]	1.08[c]
Aqueous/white tea	2.56[b,c]	6.02[b,c]	0.74[b]	1.71[b,c]	6.95[b,c]	1.14[a,b]
Aqueous - methanol (70%)/white tea	2.66[a]	6.14[a]	0.90[a]	1.83[a]	7.03[a]	1.19[a]

Data are expressed as Duncan. [a,b]variance comparison (n=3). GA: Gallic acid, EGC: (-)-epigallocatechin, C: (+)-catechin; EGCG: (-)-epigallocatechin gallate, EC: (-)-epicatechin; ECG: (-)-epicatechin gallate

observed that EGCG presented in all of extracts and according to the solvents that used for extraction, can be represent more amounts in dry mass. The detection of these compounds is in good agreement with the tea chemical composition as it was described elsewhere extensively.[15] A study reported that the most efficient mobile phase for catechin separation (EGCG and ECG) was a mixture of acetonitrile, methanol, and acetic acid.[16]

Evaluation of 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity

The antioxidant activity results in white tea extracts indicated that amongst aqueous, methanol, and aqueous methanol extracts, aqueous methanol extracts with 71.74 ± 0.42 μg/ml, and aqueous extract with 66.61 ± 0.27 μg/ml had the highest inhibition of free radicals' activity percentage compared with the standard sample of ascorbic acid [Table 3]. Tea polyphenols are one of those compounds, which have high anti-oxidant properties naturally. This study DPPH result found that more catechins in white tea extract could directly affect antioxidant activity significantly in compare with black and green tea. This study DPPH result found that more catechins in white tea extract could directly affect anti-oxidant activity significantly in compare with black and green tea. Tea extracts also inhibited the formation of reactive oxygen species, and induced cytochromes P450 1A1, 1A2 and 2B1 and glucuronosyl transferase, leading to glucuronide, which is one of the important mechanisms in biological detoxification system.[6] Phenolic structures in different tea extracts inhibit the tea radical scavenging activity. The polyphenols usually decrease the oxygen and nitrogen species reaction.

In vitro antiproliferative effect of white tea extract on HCT-116 cell lines

Aqueous WTE, in concentration of 1000 μg/mL, had strong anti-proliferative activity on HCT-116 cells in all experiments times (8, 16, and 24 h). According to Figure 2, the result indicated that the best inhibitory doses of WTE for inhibitory cell growth at 8 h was (1000 μg/ml with 41.21% ± 0.58%), in 16 h was (1000 μg/ml with 55.80% ± 0.92%), and in 24 h was (1000 μg/ml with 75.87% ± 0.29%). The aqueous WTE best concentration was 1000 μg/ml, because of nonsignificant differences between 150, 500, and 1000 μg/ml concentration. All three aqueous WTE concentrations were used for gene expression analysis in order to investigate the transcription behavior. Polyphenol compounds can also be referred as an important compound in the pathway for the colon cancer genes expression. Many researches have described that the anti-cancer effect of tea focused on catechins mostly.[17] However, some studies paid much attention to evaluate the anti-cancer effects of EGCG. Many researches have described that the anti-cancer effect of tea focused on catechins mostly. However, some studies paid much attention to evaluate the anti-cancer effects of EGCG.[18] The white tea, which was studied in this research, seems to have a higher anti-cancer effect than other teas, because of the presence of EGCG in its structure and due to different fermentation process. Therefore,[19] showed that one of the anti-carcinogenetic mechanisms of tea might be involved in catalytic activities regulation of the P450 enzymes and glucuronosyltransferase.

Table 3: 2, 2-diphenyl-1-picrylhydrazyl scavenging activities in white tea clone 100 extracted with different solvents

Extraction type/tea sample	DPPH radical scavenging activity (%)	IC_{50} (μg/ml)
Aqueous/white tea	66.61±0.27**	143.25
Aqueous - methanol (70%)/white tea	71.74±0.42**	355.09
methanol (70%)/white tea	47.90±0.31*	116.38
Ascorbic acid	3.002±0.45	54.67

Results are expressed as means±SD of triplicate measurements. Statistically significant at (*P<0.05, **P<0.01). DPPH: 2, 2-diphenyl-1-picrylhydrazyl; SD: Standard deviation

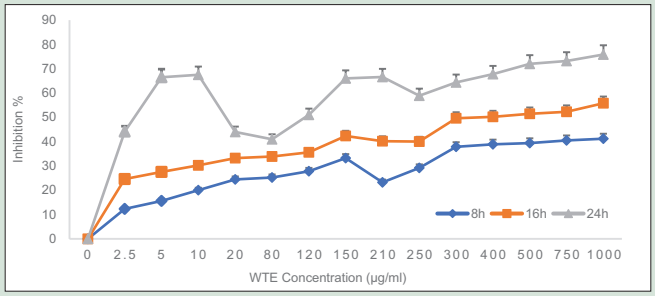
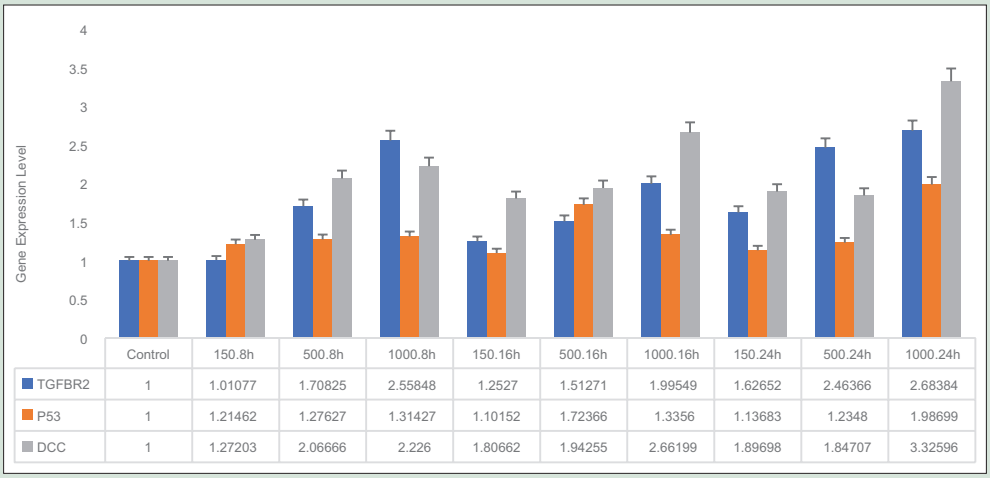


Figure 2: Inhibitory effect of aqueous extracts of white tea on the growth of HCT-116 line in 8, 16 and 24 h

Result of gene expression

For investigating and selecting the effect of aqueous white tea extract different concentration (150, 500, and 1000 μg/ml), we used MTT assay and calculated 50% inhibitory of extract doses. In this research, changes in the gene expression level in tumor suppressor families *(P53, TGFBR2* and *DCC*), were compared in treated cells with aqueous WTE against and control sample. The effect of aqueous WTE on genes expression levels, were investigated by using the RT-PCR method in 3 times (8, 16, and 24 h) [Figure 3], which was obtained according to the MTT assay results. The specific effects of the desired gene segments, the lack of primer pairing, and the nonreplicating of nonspecific parts for each genes were performed by using melting curve. The CT values of genes were normalizes against the mRNA level of *Beta actin* as a housekeeping gene and the relative expression was calculated for each treated group. Expression of *P53* gene as one of the main members of tumor suppressor family and superior gene in most cancer studies showed that, after treated with aqueous WTE different concentrations (150, 500 and 1000 μg/ml), at 8 h, the expression level was changed nonsignificantly. On the contrary, after 16 h treatment in 500 μg/ml of WTE, the expression level of *P53* was considerably increased (1.72 unit, $P < 0.05$). On the other hand, the expression level of *P53* between 150 and 1000 μg/ml of WTE treatments were not significant after 16 h. After 24 h, as well as 16 h the same concentration of aqueous WTE (1000 μg/ml), increased expression level of *P53* significantly (1.98 unit ($P < 0.05$). In general, in case of *P53* changes transcription level, by passing the time from 8 to



	Control	150.8h	500.8h	1000.8h	150.16h	500.16h	1000.16h	150.24h	500.24h	1000.24h
TGFBR2	1	1.01077	1.70825	2.55848	1.2527	1.51271	1.99549	1.62652	2.46366	2.68384
P53	1	1.21462	1.27627	1.31427	1.10152	1.72366	1.3356	1.13683	1.2348	1.98699
DCC	1	1.27203	2.06666	2.226	1.80662	1.94255	2.66199	1.89698	1.84707	3.32596

Figure 3: Expression of *P53*, *DCC* and *TGFBR2* genes in human colorectal cancer cell line, HCT-116 after treated by aqueous extracts of white tea. 150.8 h: Treated with 150 (μg/ml) aqueous extracts of white tea in 8 h; 500.8 h: Treated with 500 (μg/ml) aqueous extracts of white tea in 8 h; 1000.8 h: Treated with 1000 (μg/ml) aqueous extracts of white tea in 8 h; 150.16 h: Treated with 150 (μg/ml) aqueous extracts of white tea in 16 h; 500.16 h: Treated with 500 (μg/ml) aqueous extracts of white tea in 16 h; 100.16 h: Treated with 1000 (μg/ml) aqueous extracts of white tea in 16 h; 150.24 h: Treated with 150 (μg/ml) aqueous extracts of white tea in 24 h; 500.24 h: Treated with 500 (μg/ml) aqueous extracts of white tea in 24 h; 1000.24 h: Treated with 1000 (μg/ml) aqueous extracts of white tea in 24 h

24 h, increasing in aqueous WTE concentration (from 150 increases to 1000 μg/ml), directly up-regulate the expression level considerably. The expression of *DCC* gene was increased in all treatments. The results indicated that after 8 h, the expression level of *DCC*, in 500 and 1000 (μg/ml) was significantly increased in compared to control sample (2.06 and 2.22 unit, $P < 0.05$ respectively). Furthermore, by passing 16 and 24 h, the expression level of *DCC* in concentration 1000 (μg/ml) was also increased significantly (2.66 unit and 3.32 unit, $P < 0.05$, respectively). According to the time (8 and 16 h), the results indicated that in concentration 150 (μg/ml), the expression level decreased after treated with aqueous WTE (from 2.6 to 1.84 unit ($P < 0.05$)), on the contrary, treated with 1000 (μg/ml), obtained that the ratio of expression level of *DCC* increased significantly from 2.22 to 3.32 unit ($P < 0.05$). The gene third member, which was studied, is known as the *TGFBR2*. The expression level of third studied gene *TGFBR2* were significantly increased in 1000 (μg/ml) of WTE after 8 h (2.55 unit, $P < 0.05$). The *TGFBR2* expression level treated with 150 (μg/ml) after 8 h in compare with control group was nonsignificantly changed. The expression level of *TGFBR2* after 16 h, belonged to the samples that treated with 1000 (μg/ml) aqueous WTE, was increased significantly (1.99 unit, $P < 0.05$). After 24 h, both concentrations 500 and 1000 (μg/ml), were increased the level of expression significantly (2.46 and 2.68 unit, $P < 0.05$ respectively). In general, results indicated that both 500 and 1000 (μg/ml) doses of aqueous WTE in all experiments times had the adequate factor for increasing *TGFBR2* level expression. White tea extract exhibits a higher anti-inflammatory activity than green tea and also black tea extracts.[20] In this research, interaction of MTT and DPPH results showed that increasing the WTE concentration from 150 to 1000 (μg/ml) during the different time of estimation, could increase and up regulated the expression level in tumor suppressor genes. As Hajiaghaalipour *et al.* has been reported about the role of EGCG in the gene expression pathway in colon cancer.[21] In addition, the EGCG treatment in HT29 cells significantly up regulated *caspase 9*, and also down regulated *MMP2* expression in 24 and 48 h.[22] Yen *et al*. indicated that the greater anti-mutagenic effect was found in white tea than in the green tea or black tea, and some anti-mutagenic substances might be formed during the tea manufacturing processes.[20] Recent studies by Tewari *et al*., presented that methanol and aqueous extracts of black, green, and white tea could be affected by the high polyphenols presence in the liver cancers gene expression.[23] Kim *et al*., reported that nearly about half of all cancers have inactivated *P53*, which has numerous instruments of anticancer capacity and assumes a part in apoptosis, genomic dependability, and restraint of angiogenesis.[24] Furthermore, polyphenols compound and EGCG reduced Akt levels, therefore decreasing cell duplication and starting apoptosis *DCC* and *APC* quality in mice.[25] Changes in the *TGF-beta* compose II receptor (*TGFBR2*) are estimated to occur in around of 30% of colorectal carcinomas.[26-28] On the other hand, this increase is important, when the tea extract can reduce some genes group expression levels like tumor suppressor genes.

CONCLUSION

In summary, this research results are declare that, the white tea extract pro-apoptotic properties and the effectiveness of this supplement can play a significant role against the CRC cell line HCT-116 in the *in vitro* condition.

Naturally, low cost and public access to this product, is one of the advantages for considering, and according to the findings, it seems that taking white tea extract as a herbal supplement in optimal dosage could change the regulation of tumor suppressor genes pathway under *in vitro* condition effectively.

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Conflict of interest

There are no conflicts of interest.

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