Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2019;44(1):66-71
DOI: 10.17826/cumj.435450

ARAŞTIRMA / RESEARCH

Effects of boric-acid-applied green tea on the expressions of heat shock proteins in MCF-7 cells

MCF-7 hücrelerinde borik asit uygulanan yeşil çayın ısı şok proteinlerinin ekspresyonlarına etkileri

Melike Ersöz[1], Ayse Karatuğ Kaçar[2], Işıl Sezekler[3], Zeynep Mine Coşkun[1]

[1]Istanbul Bilim University Faculty of Science and Art, Department of Molecular Biology and Genetics, Istanbul, Turkey
[2]Istanbul University, Faculty of Science, Department of Biology, Istanbul, Turkey
[3]Marmara University, Faculty of Science and Art, Department of Biology, Istanbul, Turkey

Cukurova Medical Journal 2019;44(1):66-71

Abstract

Purpose: The study aims to examine the effects of boric acid at different concentrations applied to green tea on heat shock protein (HSP) 70 and HSP 90 in MCF-7 cells.

Materials and Methods: Boric acid in sodium tetraborate buffer of 0 mg/m^2 (group 1), 100 mg/m^2 (group 2), 300 mg/m^2 (group 3), and 500 mg/m^2 (group 4) were applied to soil where *Camelia sinensis* (green tea) grown. The collected green tea leaves were dried and brewed in water at 80°C for 40 minutes. Cytotoxicity was analyzed by MTT method. The prepared green tea extracts (250 µg/ml) were applied to MCF-7 cells. The mRNA expressions of HSP 70 and HSP 90 were determined by qRT-PCR. The HSP 70 and HSP 90 protein levels were measured by Western blotting method.

Results: 100 and 500 mg/m^2 boric-acid-applied green tea extracts increased HSP 90 mRNA expressions in MCF-7 cells compared to green tea extract without boric acid . HSP 70 and HSP 90 protein levels did not show any change between MCF-7 cells with boric-acid-applied green tea extract and green tea extract without boric acid.

Conclusion: According to our results, the protein expressions of HSP 70 and HSP 90 in MCF-7 cells changed by neither boric-acid-applied green tea extracts nor green tea extracts without any boric-acid-applied.

Keywords: : MCF-7 cells, heat shock protein, green tea, boric acid, cytotoxicity

Öz

Amaç: Çalışmanın amacı, farklı konsantrasyonlarda borik asit uygulanan yeşil çayın MCF-7 hücrelerinde ısı şoku proteini (HSP) 70 ve HSP 90 üzerine etkilerini incelemektir.

Gereç ve Yöntem: *Camelia sinensis* (yeşil çay)'in yetiştirildiği toprağa 0 mg/m^2 (grup 1), 100 mg/m^2 (grup 2), 300 mg/m^2 (grup 3) ve 500 mg/m^2 (grup 4) borik asit sodyum tetraborat tamponu içerisinde uygulandı. Toplanan yeşil çay yaprakları kurutuldu ve 40 dakika boyunca 80° C'de suda demlendi. Sitotoksisite MTT yöntemi ile analiz edildi. Hazırlanan yeşil çay ekstraktları (250 µg/ml) MCF-7 hücrelerine uygulandı. HSP 70 ve HSP 90'ın mRNA ekspresyonları qRT-PZR ile HSP 70 ve HSP 90 protein seviyeleri Western blotting yöntemi ile belirlendi.

Bulgular: 100 ve 500 mg/m^2 borik asit uygulanan yeşil çay ekstraktları MCF-7 hücrelerinde HSP 90 mRNA ekspresyonunu borik asit içermeyen yeşil çay ekstraktına göre arttırdı. Borik asit uygulanmış yeşil çay ekstraktı ve borik asit içermeyen yeşil çay ekstraktının MCF-7 hücrelerindeki HSP 70 ve HSP 90 protein seviyeleri arasında herhangi bir değişiklik saptanmadı.

Sonuç: Bulgularımıza göre, MCF-7 hücrelerinde HSP 70 ve HSP 90'ın protein ekspresyonları borik asit uygulanmış ve uygulanmamış yeşil çay ekstraktları ile değişmedi.

Anahtar kelimeler: MCF-7 hücreleri; ısı şok protein; yeşil çay; borik asit, sitotoksisite

Yazışma Adresi/Address for Correspondence: Dr. Zeynep Mine Coskun, [1]Istanbul Bilim University Faculty of Science and Art, Department of Molecular Biology and Genetics, Istanbul, Turkey E-mail: zeynepminecoskun@gmail.com
Geliş tarihi/Received: 21.06.2018 Kabul tarihi/Accepted: 20.08.2018 Çevrimiçi yayın/Published online: 13.10.2018

INTRODUCTION

Cancer is a serious disease in worldwide. Nowadays, different types of cancers represent major challenges. For women, breast cancer is the one of the 3 most common cancer types. Only breast cancer constitutes 30% of all new cancer diagnoses in women. According to statistics, it is estimated that approximately 40.610 women and 460 men died from breast cancer in 2017. Numerous risk factors including genetic, hormonal, environmental, sociobiological and physiological factors may influence the development of breast cancer [1-3].

It is reported that some selected bioactive compounds and extracts from traditional herbs have anti-inflammatory, anti-carcinogenic, and anti-oxidant effects. One of these is *Camellia sinensis* (green tea) which is commonly used as a medical plant. Its phytocompounds have anticancer properties, and provide protection against environmental factors, such as free radicals [4,5]. The studies showed that the consumption of green tea may provide a protection against various diseases thanks to its anti-viral, anti-oxidant, anti-proliferative and apoptosis effects [4-6]. The green tea and its components have significant anti-cancer properties [7].

Boron is an essential element for the growth and development of plants. Boron has two forms as boric acid and borates. The element is indirectly significant for animal life [8]. Barronco et al. [9] suggested that boric acid reduced the risk of prostate cancer and showed anti-proliferative effects. It is expected that boric acid may lead to the development of potent therapeutic agents [10]. According to the study of Yazici et al. [11], boric acid can modulate oxidative stress and enhance the antioxidant defense mechanism in rodent model. Boric acid also may inhibit the proinflammatory cytokines, such as TNF-α and IL-6.

Heat shock proteins (HSPs) were found in the cells of all studied organisms that act as molecular chaperones. HSPs play crucial role in process of proteins folding and, have anti-apoptotic properties. It is also known that these proteins are highly conserved molecules that are expressed by cells in response to stress conditions. High expression of these proteins is associated with cancer progression, such as breast, prostate, colorectal and, lung cancer [12, 13]. HSP 70 and HSP 90 are the members of HSPs family. There is a positive correlation between the expression of HSP70 and HSP90 [14].

Boric acid is dissolved in soil in extreme rain water, which causes boric acid deficiency in plants. *C. sinensis* grows in areas with abundant rainfall and consequently boric acid deficiency may occur in the growth of tea plants. In the absence of boric acid, there is a decrease in the antioxidant effect in plant roots and leaves. Therefore, boric acid application to *C. sinensis* was carried out under controlled land conditions. Thus, the present study investigated the effects of boric acid at different concentrations applied to green tea on the expressions of HSP 70 and HSP 90 in MCF-7 cells.

MATERIALS AND METHODS

The application of boric acid and green tea sample preparation

Green tea was grown up in Cayeli, Rize. The land was divided into four pieces. Five different regions were formed from each piece. The first piece was accepted control region (group 1) and there was no boric acid application to soil. A single dose boric acid in sodium tetraborate buffer of 100 (group 2), 300 (group 3), and 500 (group 4) mg/m^2 was applied the other three pieces, respectively. The collected leaves were dried at room temperature. Dried leaves (1 g) were brewed in water (50 ml) at 80°C for 40 minutes.

Cell lines and culture conditions

The human breast cancer (MCF-7, ATCC, Manassas, VA, USA) cells were maintained in the recommended Dulbecco's modified Eagle's nutrient F-12 Ham (DMEM-F12) medium (D6421, Sigma-Aldrich, Oslo, Norway) supplemented with 10% fetal bovine serum (FBS) (S0115, Seromed, Turkey), L-glutamine (03020-1C, Biological Industries, Israel), penicillin (50 units/mL) and streptomycin (0.05 mg/mL) (03031-1C, Biological Industries, Israel). The cells at 25 cm^2 flasks were cultured in a humidified atmosphere with 5% CO_2 at 37°C. The growth medium of cells was replaced by fresh growth medium in 2 to 3 times per week.

Cytotoxicity assay

Culture medium was removed after the cells became 80-100% confluent. The cell layer was rinsed with trypsin solution and removed, and then 2 mL trypsin solution was added to flask for 5 minutes. The fresh growth medium (5 mL) was added to flask and, medium and cells were transferred to the centrifuge

tube. The cell suspension was centrifuged at approximately 1000 rpm for 5 minutes. The supernatant was discarded and the cell pellet was suspended in fresh growth medium. $1x10^4$ cells/well were plated on 96-well plates. The cells were treated with different concentrations (0-4 mg/mL) of the green tea extracts for 24 h. After the treatment, medium was carefully discarded. MTT was carried out according to the manufacturer's instructions of CellTiter 96® AQueous One Solution Cell proliferation Assay (G3580, Promega Corporation, Madison, WI, USA). The cells were incubated with MTT solution at 37°C, 5% CO_2 environment for 2 h. The optic density at 490 nm was measured by ELISA reader (BioTek, Synergy H1 Hybrid Multi-Mode Microplate Reader, Winooski, VT, USA). The percentage of relative cell viability was calculated using the following formula: % cell viability = (OD490 treated cells/OD490 control) x 100.

The mRNA expression of HSP 70 and HSP 90 in MCF-7 cells

$1x10^6$ cells/well were seeded in 6 well plates and incubated in a humidified atmosphere with 5% CO_2 at 37°C until the cells proliferated. After that green tea extracts (250 μg/ml) were applied to the cells for 24 h. The cells were washed with cold PBS. The Trizol protocol was applied to cells. The cell pellets in micro tube were homogenized into the trizol and, chloroform (200 μL) was added to tube. The samples were centrifuged at 12,000 g for 15 minutes at 4 °C. The aqueous phase was transferred to new tube and then isopropanol (500 μL) was added. Following centrifugation, RNA pellets were washed with 80 % ethanol. The pellet was dissolved in DEPC water. The obtained 1 μg RNA was turned into cDNA by using the cDNA synthesis kit (SuperScript III First-Strand, 18080400, Invitrogen, Carlsbad, CA, USA). A mix was prepared with RNA, random hexamer primer, annealing buffer and RNase/DNase-free water were incubated in a thermal cycler at 65°C for 5 minutes. 2X First-Strand Reaction Mix and SuperScript™ III/RNaseOUT™ Enzyme Mix were added to the tube on ice and then the samples were incubated in the thermal cycler for 5–10 minutes at 25°C, followed by another 50 minutes at 50°C. The PCR reaction mixture was made with TaqMan Gene Expression Master Mix and PCR primers. PCR products were obtained by using the 7500 Fast Instrument Real-Time PCR System (Applied Biosystems, California, USA). The amounts of reaction products of the target genes were calculated in relation to Beta-actin expression with Pfaffl method [15]. The primers of HSP 70, HSP 90 and Beta actin were procured from Applied Biosystems.

Protein extraction and western blotting

The culture cells ($1x10^6$ cells/well) were seeded in 6 well plates and incubated in a humidified atmosphere with 5% CO_2 at 37°C until the cells proliferated. After that, green tea extracts (250 μg/ml) were applied to the cells for 24 h. Cell lysis buffer (9803, Cell Signaling Technology, Boston, MA, USA) and sonication were used for lysed to the cells. Protein concentrations were analyzed with the protein kit using Bradford method (Millipore, Billerica, MA, USA). SDS-PAGE (7.5% gel) was used for separation of proteins.

The separated proteins were transferred to nitrocellulose membranes. 5% nonfat dried milk was used for blocking of the membrane and then with the primary antibody (for HSP 70; sc-1060, for HSP 90; sc-101494, for Beta actin; sc-47778, Santa Cruz, Oregon, USA) at 4°C, overnight. After that, the secondary antibody was applied for 1 hour (anti-goat sc-2350, anti-mouse sc-3589, Santa Cruz, Oregon, USA). The bands were made visible with luminol reagent (sc-2048, Santa Cruz, Oregon, USA) using Gel Logic 1500 Imaging System Device (Eastmen Kodak Company, Rochester, NY, USA). The protein band densities were done with Kodak Carestream MI analysis program (Eastmen Kodak Company, Rochester, NY, USA) for data analysis. The levels of protein were normalized with Beta actin.

Statistical analysis

Statistical analysis was done using GraphPad Prism 5 computer program. The results were expressed as mean ± SEM. Statistical analysis of the differences in the measured properties of the groups was performed with one-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* test. There was a statistically significant as $p < 0.05$.

RESULTS

Cell viability in MCF-7 cells treated with boric-acid-applied green tea extracts

The concentrations producing 50 % growth inhibition (IC50) of the extracts from boric-acid-applied green tea and green tea without boric acid on the MCF-7 cells for 24 h were determined. The IC50 values of 0, 100, 300, and 500 mg/m² boric-acid-

applied green tea extracts on MCF-7 cells were 3.012, 2.908, 2.731, and 2.625 mg/mL, respectively.

HSP 70 and HSP 90 mRNA expressions in MCF-7 cells

HSP 70 mRNA expressions in MCF-7 cells were not observed by qRT-PCR for 40 cycles (data not shown). However, HSP 90 mRNA expression was detected. HSP 90 mRNA expressions in MCF-7 cells were elevated by 100 and 500 mg/m^2 boric-acid-applied green tea extract compared to MCF-7 with green tea extract without boric acid ($p<0.05$ and $p<0.01$, respectively). Interestingly, the levels of HSP 90 mRNA expression in MCF-7 cells treated with 300 mg/m^2 boric-acid-applied green tea extract were lower than MCF-7 cells treated with 100 and 500 mg/m^2 boric-acid-applied green tea extract ($p<0.05$ and $p<0.01$, respectively; Figure 1).

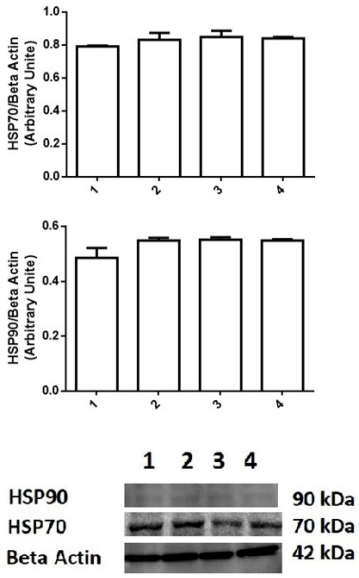

Figure 1. HSP 90 mRNA expression in MCF-7 cells. 1: green tea without boric acid; 2: 100 mg/m2 boric-acid-applied green tea; 3: 300 mg/m2 boric-acid-applied green tea; and 4: 500 mg/m2 boric-acid-applied green tea. *$p<0.05$ and **$p<0.01$.

Western blot analysis of HSP 70 and HSP 90 protein in MCF-7 cells

The levels of HSP 70 protein did not show any changes between MCF-7 cells with boric-acid-applied green tea extract and with green tea extract without boric acid. Similarly, HSP 90 protein levels were the same among groups (Figure 2).

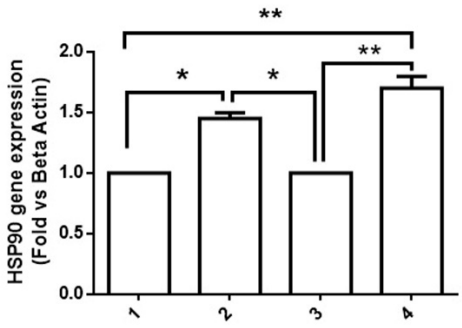

Figure 2. HSP 70 and HSP 90 protein levels in MCF-7 cells. 1: green tea without boric acid; 2: 100 mg/m2 boric-acid-applied green tea; 3: 300 mg/m2 boric-acid-applied green tea; and 4: 500 mg/m2 boric-acid-applied green tea.

DISCUSSION

Breast cancer is a complex and heterogeneous disease with a very high death rate. It is known that there is a reasonable number of breast cancer cell lines available to reflect the molecular characteristics of its sub-types. MCF-7 is one of these cell line types and there is a suitable model cell line for breast cancer investigations worldwide[16, 17]. We preferred MCF-7 cell line in the present study.

Recently, the use of herbal products has shown significantly increase. Although the studies on human and animals reported a protective role of green tea against various cancer types, the definitive evidence has not been provided for beneficial or deleterious effects of green or black tea consumption on cancer [18]. Ogunleye et al. [19] suggested that green tea consumption may be associated with a decrease in risk of breast cancer recurrence. According to the study of Zhang et al. [20], green tea extract cause cell death and inhibit cell proliferation in vitro and in vivo. Similarly, Liu et al. [21] mentioned that green tea extract induced inhibition of cell proliferation in human oral squamous carcinoma cell lines.

It is known that boric acid was utilized as an anti-septic for food conservation, as well as soil sterility [22]. Furthermore, boric acid was used as a therapeutic agent. Sogut et al. [23] suggested that boric acid showed apoptotic and antioxidant effects against

alcohol-induced hepatoxicity in rats. In the current study, we tried to increase the effectiveness of green tea by applying boric acid because it is basic element for plant and has therapeutic properties. Thus, boric acid was applied to *Camellia sinensis* at different doses. The tea leaf samples used in the research were collected from the Eastern Black Sea Region considering the size, distribution, climatic conditions of tea farming areas and the quality of tea farming areas in the region [24]. So, we did not prefer using a controlled laboratory that was not suitable for the natural conditions to grow tea.

HSP 70 and HSP 90 stabilize pro-survival client proteins and so cause tumor growth [25]. Khan et al. [26] reported that HSP 70 may increase in the sensitivity of breast cancer cells. In breast cancer patient, the over-expression of HSP70-2, a member of HSP 70 family, was shown and caused progression of breast cancer. It is suggested that HSP70-2 might be potential molecule for breast cancer treatment [27]. Similar to HSP 70, HSP 90 promise for the treatment of cancer disease. The anti-proliferative effect was observed via the inhibition of HSP 90 as client protein degradation [28]. Moses et al. [29] showed that (-)-Epigallocatechin gallate, a major tea polyphenol, can prevent human prostate cancer progression by inhibiting HSP 90. Likely, the study of Li et al. [30] on pancreatic cancer cells observed that (-)-epigallocatechin gallate caused degradation of HSP 90 and showed anti-proliferative effect. Boric acid supplementation increased the expression of HSP 70 and decreased apoptosis dependent on concentration in the spleen of African ostrich. Thus, HSP 70 played a role anti-apoptotic function [31]. In our finding, we did not observe any change the mRNA and protein expressions of HSP 70 between MCF-7 cells treated with boric-acid-applied green tea extract and green tea extract without boric acid. The studies were reported that there is a poor correlation between mRNA and protein levels in the cells [32, 33]. We evaluated that HSP 90 mRNA levels in MCF-7 cells increased by boric-acid-applied green tea extract compared to non-treated cells, but HSP 90 protein levels did not change. It may be that the proteins differ substantially in their half-lives in vivo, and/or post-transcriptional and post-translational parameters may be the cause of this difference.

Different cell types in the human body have various shapes and functions, even though they have the same genome and the same organelles. The cells are characterized by genetic material and the other components, but also by the genes they express. The differentially expressed genes lead to the production of proteins with different morphology, shape or function within the cell. The limitations of this study are that animal models are not utilized for the systemic investigation of HSP proteins. The animal studies are needed so that the level of the circulating and tissue HSP proteins can be compared with that at the cellular level.

Taken together, our study indicated that green tea extract without boric acid and boric-acid-applied green tea supplementations were not effective the protein expressions of HSP 70 and HSP 90 in MCF-7 cells. Future work is needed to better ascertain the role of green tea extract with boric acid in cancer cells.

Yazar Katkıları: Çalışma konsepti/Tasarımı: ME, ZMC, AKK, IS; Veri toplama: ME, AKK, ZMC; Veri analizi ve yorumlama: ME, ZMC; Yazı taslağı: ZMC; İçeriğin eleştirel incelenmesi: ME; Son onay ve sorumluluk: ME, AKK, IS, ZMC; Teknik ve malzeme desteği: IS, ME; Süpervizyon: ME, ZMC; Fon sağlama (mevcut ise): yok.
Bilgilendirilmiş Onam: Katılımcılardan yazılı onam alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.

Author Contributions: Concept/Design: ME, ZMC, AKK, IS; Data acquisition: ME, AKK, ZMC; Data analysis and interpretation: ME, ZMC; Drafting manuscript: ZMC; Critical revision of manuscript: ME; Final approval and accountability: ME, AKK, IS, ZMC; Technical or material support: IS, ME; Supervision: ME, ZMC; Securing funding (if available): n/a.
Informed Consent: Written consent was obtained from the participants.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

REFERENCES

1. World Health Organization (WHO). http://www.who.int (accessed Feb 2018).
2. American Cancer Society. https://www.cancer.org (accessed Feb 2018).
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68:7-30.
4. Lee YH, Jang YH, Kim YS, Kim J, Seong BL. Evaluation of green tea extract as a safe personal hygiene against viral infections. J Biol Eng. 2018;12:1.
5. Forester SC, Lambert JD. Antioxidant effects of green tea. Mol Nutr Food Res. 2011;55:844–54.
6. Han DH, Jeong JH, Kim JH. Anti-proliferative and apoptosis induction activity of green tea polyphenols on human promyelocytic leukemia HL-60 cells. Anticancer Res. 2009;29:1417-21.
7. Bimonte S, Cascella M, Leongito M, Palaia R, Caliendo D, Izzo F et al. An overview of pre-clinical studies on the effects of (-)-epigallocatechin-3-gallate, a catechin found in green tea, in treatment of

pancreatic cancer. Recenti Prog Med. 2017;108:282-7.
8. Herera-Rodrigez M, González-Fontes A, Rexach J, Camacho-Cristobal JJ, Maldonado JM, Narrova-Gochicoa MT. Role of boron in vascular plants and respononse mechanisms to boron stresses. Plant Stress. 2010;4:115-22.
9. Barranco WT, Hudak PF, Eckhert CD. Evaluation of ecological and in vitro effects of boron on prostate cancer risk (United States). Cancer Causes Control. 2007;18:71-7.
10. Das BC, Thapa P, Karki R, Schinke C, Das S, Kambhampati S et al. Boron chemicals in diagnosis and therapeutics. Future Med Chem. 2013;5:653-76.
11. Yazıcı S, Akşit H, Korkut O, Sunay B, Çelik T. Effects of boric acid and 2-aminoethoxydiphenyl borate on necrotizing enterocolitis. J Pediatr Gastroenterol Nutr. 2014;58:61-7.
12. Lianos GD, Alexiou GA, Mangano A, Mangano A, Rausei S, Boni L et al. The role of heat shock proteins in cancer. Cancer Lett. 2015;360:114-8.
13. Saini J, Sharma PK. Clinical, prognostic and therapeutic significance of heat shock proteins in cancer. Curr Drug Targets. 2017;doi:10.2174/1389450118666170823121248 (in press).
14. Alexiou GA, Vartholomatos G, Stefanaki K, Patereli A, Dova L, Karamoutsios A et al. Expression of heat shock proteins in medulloblastoma, J Neurosurg Pediatr. 2013;12:452-7.
15. Pfaffl MW. A new mathematical model for relative quantification in real-time RT - PCR. Nucleic Acids Res. 2001;29:e45.
16. Comsa Ş, Cîmpean AM, Raica M. The story of MCF-7 breast cancer cell line: 40 years of experience in research. Anticancer Res. 2015;35:3147-54.
17. Holliday DL, Speirs V. Choosing the right cell line for breast cancer research. Breast Cancer Res. 2011;13:215.
18. Yuan JM, Sun C, Butler LM. Tea and cancer prevention: epidemiological studies. Pharmacol Res. 2011;64:123-35.
19. Ogunleye AA, Xue F, Michels KB. Green tea consumption and breast cancer risk or recurrence: a meta-analysis. Breast Cancer Res Treat. 2010;119:477-84.
20. Zhang D, Al-Hendy M, Richard-Davis G, Montgomery-Rice V, Sharan C, Rajaratnam V et al. Green tea extract inhibits proliferation of uterine leiomyoma cells in vitro and in nude mice. Am J Obstet Gynecol. 2010;202:1-9.
21. Liu XL, Liu HQ, Li J, Yang L, Zhao X. Experimental study on anti-tumor effect and mechanism of green tea extract. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2014;34:1369-73.
22. Becker L, Scheffczyl A, Förster B, Oehlmann J, Princz J, Römbke J et al. Effects of boric acid on various microbes, plants, and soil invertebrates. J Soils and Sediments. 2011;11:238-48.
23. Sogut I, Paltun SO, Tuncdemir M, Ersoz M, Hurdag C. The antioxidant and antiapoptotic effect of boric acid on hepatoxicity in chronic alcohol-fed rats. Can J Physiol Pharmacol. 2018;96:404-11.
24. Turan MA, Balcı M, Taşkın MB, Kalcıoğlu Z, Soba MR, Müezzinoğlu N et al. Doğu Karadeniz Bölgesi'nde yetiştirilen çay bitkisi (Camellia sinensis L.) yaşlı yapraklarının su ekstraktı, toplam kül, toplam polifenol, kafein ve ham selüloz içerikleri. Toprak Su Dergisi. 2016;5:52-8.
25. Cesa LC, Shao H, Srinivasan SR, Tse E, Jain C, Zuiderweg ERP, et al. X-linked inhibitor of apoptosis protein (XIAP) is a client of heat shock protein 70 (Hsp70) and a biomarker of its inhibition. J Biol Chem. 2018;293:2370-80.
26. Khan GN, Kim EJ, Shin TS, Lee SH. Azacytidine-induced chemosensitivity to doxorubicin in human breast cancer MCF7 Cells. Anticancer Res. 2017;37:2355-64.
27. Jagadish N, Agarwal S, Gupta N, Fatima R, Devi S, Kumar V et al. Heat shock protein 70-2 (HSP70-2) overexpression in breast cancer. J Exp Clin Cancer Res. 2016;35:150.
28. Forsberg LK, Liu W, Holzbeierlein J, Blagg BSJ. Modified biphenyl Hsp90 C-terminal inhibitors for the treatment of cancer. Bioorg Med Chem Lett. 2017;27:4514-9.
29. Moses MA, Henry EC, Ricke WA, Gasiewicz TA. The heat shock protein 90 inhibitor, (-)-epigallocatechin gallate, has anticancer activity in a novel human prostate cancer progression model. Cancer Prev Res (Phila). 2015;8:249-57.
30. Li Y, Zhang T, Jiang Y, Lee HF, Schwartz SJ, Sun D. (-)-Epigallocatechin-3-gallate inhibits Hsp90 function by impairing Hsp90 association with cochaperones in pancreatic cancer cell line Mia Paca-2. Mol Pharm. 2009;6:1152-9.
31. Haseeb K, Wang J, Xiao K, Yang KL, Sun PP, Wu XT et al. Effects of boron supplementation on expression of Hsp70 in the spleen of African Ostrich. Biol Trace Elem Res. 2017;182:317-27.
32. Maier T, Güell M, Serrano. Correlation of mRNA and protein in complex biological samples. FEBS Letters. 2009;583:3966-73.
33. Koussounadis A, Langdon SP, Um IH, Harrison DJ, Smith1 VA. Relationship between differentially expressed mRNA and mRNA protein correlations in a xenograft model system. Sci Rep. 2015;5:10775.