Anti-proliferative activity of epigallocatechin-3-gallate and silibinin on soft tissue sarcoma cells

KAMRAN HARATI1, BJÖRN BEHR1, CHRISTOPH WALLNER1, ADRIEN DAIGELER1, TOBIAS HIRSCH1, FRANK JACOBSEN1, MARCUS RENNER2, ALI HARATI3, MARCUS LEHNHARDT1 and MUSTAFA BECERIKLI1

1Department of Plastic Surgery, Burn Center, Hand Center, Sarcoma Reference Center, BG-University Hospital Bergmannsheil, D-44789 Bochum; 2Institute of Pathology, University of Heidelberg, D-69120 Heidelberg; 3Department of Neurosurgery, Klinikum Dortmund, D-44145 Dortmund, Germany

Received July 24, 2016; Accepted October 26, 2016

DOI: 10.3892/mmr.2016.5969

Abstract. Disseminated soft tissue sarcomas (STS) present a therapeutic dilemma. The first-line cytotoxic doxorubicin demonstrates a response rate of 30% and is not suitable for elderly patients with underlying cardiac disease, due to its cardiotoxicity. Well-tolerated alternative treatment options, particularly in palliative situations, are rare. Therefore, the present study assessed the anti-proliferative effects of the natural compounds epigallocatechin-3-gallate (EGCG), silibinin and noscapine on STS cells. A total of eight different human STS cell lines were used in the study: Fibrosarcoma (HT1080), liposarcoma (SW872, T778 and MLS-402), synovial sarcoma (SW982, SYO1 and 1273) and pleomorphic sarcoma (U2197). Cell proliferation and viability were analysed by 5-bromo-2’-deoxyuridine and MTT assays and real-time cell analysis (RTCA). RTCA indicated that noscapine did not exhibit any inhibitory effects. By contrast, EGCG decreased proliferation and viability of all cell lines except for the 1273 synovial sarcoma cell line. Silibinin exhibited anti-proliferative effects on all synovial sarcoma, liposarcoma and fibrosarcoma cell lines. Liposarcoma cell lines responded particularly well to EGCG while synovial sarcoma cell lines were more sensitive to silibinin. In conclusion, the green tea polyphenol EGCG and the natural flavonoid silibinin from milk thistle suppressed the proliferation and viability of liposarcoma, synovial sarcoma and fibrosarcoma cells. These compounds are therefore potential candidates as mild therapeutic options for patients that are not suitable for doxorubicin-based chemotherapy and require palliative treatment. The findings from the present study provide evidence to support in vivo trials assessing the effect of these natural compounds on solid sarcomas.

Introduction

Soft tissue sarcomas (STS) are a heterogeneous group of solid tumours arising from transformed cells of mesenchymal origin. They may occur throughout the body and represent ~1% of all adult malignancies (1). In patients with primary diagnosed STS without distant metastasis, standard treatment involves surgical resection with negative margins, typically followed by adjuvant radiation to decrease the risk of recurrence (2,3). However, almost half of all patients with STS develop distant metastases, rendering them unsuitable for surgery (4,5). If metastasis has occurred, the median survival time regardless of chemotherapeutic treatment is <12 months (6,7). A limited number of chemotherapeutic agents, including doxorubicin and ifosfamide, are effective for the treatment of metastatic STS (2). However, the response rates of these agents are poor and often do not result in significant extension of survival (8). Doxorubicin is the predominant chemotherapeutic agent used for the treatment of metastatic STS, and has a response rate of ~30% (9,10). The combination of doxorubicin and ifosfamide exhibits greater response rates compared with doxorubicin alone; however, it is associated with severe short- and long-term adverse effects, including bone marrow suppression and cardiomyopathy (11-13).

A multicentre analysis by the European Organisation for Research and Treatment of Cancer (trial 62012) on 455 patients with advanced STS indicated that an intensified combination treatment with doxorubicin and ifosfamide is not suitable for treatment of locally advanced or metastatic STS as a result of the serious adverse effects, and should therefore only be used with a view to tumour shrinkage (13). Furthermore, the versatility of doxorubicin is limited by dose-associated and cumulative myocardial toxicity, particularly in older patients with a history of cardiac disease (14). However, the incidence of STS increases markedly >50 years of age, when the prevalence of cardiac diseases is also greater (15). Currently, there are no efficacious and safe agents for the palliative treatment of patients who may not undergo doxorubicin-based chemotherapy due to underlying cardiac disease. Therefore, the
development of novel therapeutic agents is required for the treatment of STS.

A review of the literature reveals various potential well-tolerated and natural phytochemicals that exhibit anti-neoplastic effects on malignant cells, including the compounds epigallocatechin-3-gallate (EGCG), silibinin and noscapine. EGCG is the most abundant catechin in green tea and demonstrates anti-inflammatory, antioxidant and antineoplastic activities (16-18). Various *in vitro* studies have revealed that EGCG exhibits anticancer activity in lung (19), prostate (20), colon (21), gastric (22), breast (23) and cervical carcinoma cells (24). To date, EGCG has undergone various phase II trials and has been demonstrated to be well-tolerated following oral administration (25-29). The most frequent adverse reactions observed were gastrointestinal reactions, including nausea and vomiting. In rare cases, patients presented with elevated serum alanine aminotransferase levels following the administration of high doses of oral EGCG; however, liver function tests returned to baseline following discontinuation of EGCG (30). Therefore, EGCG is considered to be a safe and well-tolerated agent for the treatment of cancer patients (31,32).

Silibinin is the primary active constituent of silymarin, a standardized extract from the seeds of the milk thistle plant (*Silybum marianum*). Silibinin is available as a therapeutic agent in various European countries and is used for the treatment of toxic liver damage, particularly due to *Amanita phalloides* intoxication (33). It is well tolerated in cancer patients (34,35) and has demonstrated anti-neoplastic effects in various malignant cell lines including HT1080 fibrosarcoma cells (36-40).

Noscapine is a naturally occurring opium alkaloid and a widely used antitussive drug that is non-addictive and has a low toxicity profile (41). As a tubulin-binding agent, various preclinical studies have established its tumour-inhibitory effects in a wide range of malignancies (42-45). Currently, noscapine is undergoing phase II clinical trials for cancer chemotherapy (46).

Based on these results, the present study aimed to investigate the anti-proliferative activity of EGCG, silibinin and noscapine on eight different STS cell lines, including fibrosarcoma, liposarcoma, synovial sarcoma and pleomorphic sarcoma cells.

**Materials and methods**

**Cell lines.** Eight different human STS cell lines were used in the present study: HT1080 (fibrosarcoma), SW872 (liposarcoma), T778 (liposarcoma), MLS-402 (liposarcoma), SW982 (synovial sarcoma), SYO1 (synovial sarcoma), 1273 (synovial sarcoma) and U2197 (pleomorphic sarcoma/malignant fibrous histiocytoma). HT1080, SW872 and SW982 were purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany) and were cultured in Dulbecco's modified Eagle's medium (DMEM; PAN-Biotech GmbH, Aidenbach, Germany) supplemented with 10% foetal bovine serum (FBS; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 1% penicillin (100 U/ml) and 1% streptomycin (100 µg/ml; PAN-Biotech GmbH). The well-differentiated T778 liposarcoma cell line and the MLS-402 myxoid liposarcoma cell line were donated by Professor Pierre Áman (University of Gothenburg, Gothenburg, Sweden) and Professor Ola Myklebost (Oslo University Hospital, Oslo, Norway), respectively. T778 and MLS-402 cells were cultured in RPMI (PAN-Biotech GmbH) supplemented with 10% FBS and 1% penicillin/streptomycin as previously described (47,48). The SYO-1 and 1273 cell lines were donated by Dr Akira Kawai (National Cancer Center, Tokyo, Japan) and Professor Olle Larsson (Karolinska Institutet, Stockholm, Sweden) (49,50). The SYO-1 cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin and 0.5% sodium pyruvate. The 1273 cells were cultivated in Ham's F12 (PAN-Biotech GmbH) supplemented with 10% FBS and 1% penicillin/streptomycin. The U2197 cell line was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and was cultured in minimum essential medium (PAN-Biotech GmbH) supplemented with 20% FBS, 0.165% sodium bicarbonate and 1% penicillin/streptomycin (51). All cultures were maintained at 37°C in a humidified 5% CO2 atmosphere.

**Phytotherapeutic agents.** EGCG, silibinin and noscapine were obtained from Sigma-Aldrich; Merck Millipore (Darmstadt, Germany). The stock solution was dissolved in dimethyl sulfoxide (DMSO; Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and was further diluted in DMEM to concentrations of 50 µM (EGCG), 150 µM (silibinin) and 30 µM (noscapine) for all assays. These concentrations have been demonstrated to inhibit proliferation and induce apoptosis in various malignant cell lines (36,52,53).

**Cell viability assay.** Metabolic activity was measured using an MTT assay. Cells were seeded in 96-well plates (Corning Incorporated, Corning, NY, USA) at 1x10^4 cells per well. The following day, the three agents were added in the aforementioned concentrations for 24 h. Subsequently, 50 µl 0.5 mg/ml MTT (Sigma-Aldrich; Merck Millipore) was added for 4 h. MTT is a yellow dye that is reduced to purple formazan in the mitochondria of vital cells. Cells were lysed following the addition of 200 µl DMSO and 25 µl glycine buffer (containing 0.1 M glycine and 0.1 M NaCl, adjusted to pH 10.5 with NaOH) per well. The quantity of integrated dye represented the level of metabolism and was measured at a wavelength of 562 nm using an ELx808 Ultra Microplate Reader (BioTek Instruments GmbH, Bad Friedrichshall, Germany).

**Proliferation assay.** To quantify the effects of EGCG, silibinin and noscapine on cell proliferation, a colorimetric cell proliferation 5-bromo-2'-deoxyuridine (BrDU)-ELISA assay (Roche Diagnostics GmbH, Mannheim, Germany) was performed according to the manufacturer's protocol. Briefly, cells were seeded at 1x10^4 cells/well in 96-well plates and cultured for 24 h. The phytotherapeutic agents were subsequently added in the appropriate concentrations for 24 h. The BrdU labelling solution was added and incubated for a further 24 h. BrdU, a pyrimidine analogue, integrates into the DNA of proliferating cells. The level of proliferation was quantified by the light emission detected via an Orion Microplate Luminometer (Berthold Detection Systems GmbH, Pforzheim, Germany). Cell proliferation was determined in quadruplicate. The results are expressed as a percentage of the proliferation of DMSO-treated control cells.
Real-time cell analysis (RTCA). Cells were seeded in two 8-well plates with an integrated microelectronic sensor array in 600 µl culture medium (iCELLigence Real Time Cell Analyser; ACEA Biosciences, San Diego, CA, USA). After 24 h, the therapeutic agents were added for a total volume of 50 µl. The cell proliferation and survival were monitored in real-time by measuring the cell-to-electrode responses of the seeded cells. In each individual E-well, the cell impedance was measured and converted to cell index (CI) values by the RTCA software version 1.2 (Roche Diagnostics GmbH) (54). The graphs were generated in real-time by the iCELLigence system. Untreated and DMSO-treated cells served as controls.

Statistical analysis. Data analyses were performed using the statistical program SPSS 16 (SPSS, Inc., Chicago, IL, USA). Data are expressed as the mean ± standard deviation. Comparisons between the experimental groups in BrdU and MTT assays were performed using one-way analysis of variance followed by post-hoc Tukey's test. P<0.05 was considered to indicate a statistically significant difference.

Results

EGCG significantly inhibits the proliferation and viability of STS cell lines. As indicated by the BrdU assay, the proliferation of all eight human STS cell lines was inhibited by EGCG (Fig. 1). By MTT analysis, EGCG decreased the viability of seven cell lines (Fig. 2). To evaluate the proliferation and viability of cells continuously over a longer time period, RTCA was performed. The viability, adhesion and proliferation of the cells were monitored prior to and during EGCG treatment in real time for 160 h (Figs. 3-5). EGCG markedly decreased the CI of all STS cell lines except the 1273 synovial sarcoma cell line. The administration of EGCG reduced the CI of the HT1080 fibrosarcoma cell line and the U2197 pleomorphic sarcoma cell line (Fig. 3). All three liposarcoma cell lines (SW872, T778 and MLS-402) exhibited a continuously decreased CI during EGCG treatment compared with untreated or DMSO-treated cells (Fig. 4), as did the remaining two synovial sarcoma cells lines (SW982 and SYO1; Fig. 5).

Table I. Summary of the cytostatic effects of EGCG, silibinin and noscapine, as assessed by MTT and BrdU assays and RTCA.

| Subtype            | Cell line | EGCG  | Silibinin | Noscapine |
|--------------------|-----------|-------|-----------|-----------|
|                    |           | MTT   | BrdU      | RTCA      | MTT   | BrdU      | RTCA      | MTT   | BrdU      | RTCA      |
| Fibrosarcoma       | HT1080    | +     | +         | +         | +     | +         | +         | +     | +         | -          |
| Liposarcoma        | SW872     | +     | +         | +         | +     | +         | +         | +     | +         | -          |
|                    | T778      | +     | +         | +         | +     | +         | +         | +     | +         | -          |
|                    | MLS-402   | +     | +         | +         | +     | +         | +         | +     | +         | -          |
| Synovial sarcoma   | SW982     | -     | +         | +         | +     | +         | +         | +     | +         | -          |
|                    | SYO1      | +     | -         | +         | +     | +         | +         | +     | +         | -          |
|                    | 1273      | +     | -         | +         | +     | +         | +         | +     | +         | -          |
| Pleomorphic sarcoma| U2197     | +     | +         | +         | +     | +         | -         | +     | +         | -          |

+, cytostatic effect; -, no cytostatic effect. EGCG, epigallocatechin-3-gallate; BrdU, 5-bromo-2’-deoxyuridine; RTCA, real-time cell analysis.
Silibinin significantly decreases the proliferative activity and viability of STS cell lines. Treatment with silibinin significantly reduced the proliferation of seven STS cell lines (Fig. 1), and significantly decreased the cell viability of all eight assessed STS cell lines, as analysed by MTT assay (Fig. 2). By RTCA, silibinin was the only compound that exhibited a strong inhibitory effect on all three synovial sarcoma cells (Fig. 5). In addition, silibinin reduced the CI of all liposarcoma cell lines; however, not to the extent of EGCG. Only the U2197 pleomorphic sarcoma cell line did not respond to silibinin treatment.

By RTCA, STS cell lines are unaffected by noscapine treatment. Noscapine exhibited cytostatic effects on STS cells, as assessed using BrdU (Fig. 1) and MTT (Fig. 2) assays at 24 h. However, these effects could not be validated by RTCA over a longer time period. The proliferation inhibition resulting from noscapine treatment in six cell lines at 24 h did not result in a continual decrease of the CI. In all cell lines, the CI of noscapine-treated cells increased steadily and was comparable to the CI of DMSO-treated or untreated control cells during the 160 h of real-time analysis (Figs. 3-5).

Discussion

STS are a heterogeneous group of rare mesenchymal malignancies. To date, systemic treatment options are limited following metastasis. Patients with distant metastases have a median survival time of less than one year despite systemic chemotherapy (6,7). Due to the infrequent and heterogeneous nature of STS the development of novel systemic therapeutic agents is challenging and novel chemotherapy strategies are lacking. Therefore, the development of well-tolerated and effective chemotherapeutic agents for the treatment of STS is required.

The present study assessed the cytostatic effects of the naturally occurring compounds noscapine, silibinin and EGCG on eight STS cell lines. By RTCA, noscapine did not exhibit any relevant anti-proliferative effects (Table I). In contrast, silibinin and EGCG exerted cytostatic effects in almost all examined STS cell lines, as assessed by BrdU, MTT and RTCA. Administration of EGCG decreased proliferation and viability of all liposarcoma cell lines and two synovial sarcoma cell lines for more than five days. In addition, it
inhibited HT1080 fibrosarcoma and U2197 pleomorphic sarcoma cells. Of the three analysed compounds, EGCG exerted the greatest anti-proliferative activity in the three assessed liposarcoma cell lines, rendering it a potential agent of interest. Liposarcomas represent the most frequent somatic STS subtype and respond poorly to anthracycline-based chemotherapy, with well-differentiated and de-differentiated tumours exhibiting response rates of only 12 and 13%, respectively (55). Pleomorphic liposarcomas are the least responsive to chemotherapy, with a response rate of 5%, whereas myxoid liposarcomas have been revealed to be the most sensitive to chemotherapy, exhibiting response rates of 44-48% (56-58). In the present study, EGCG exhibited a distinct inhibitory effect on T778 cells from a well-differentiated liposarcoma, SW872 cells from a pleomorphic liposarcoma and MLS-402 cells from a myxoid liposarcoma. Although these findings were in vitro, they suggested a potential anti-proliferative activity of EGCG on liposarcoma cells that should be further investigated in vivo.

In comparison with EGCG, the inhibitory effect of silibinin was reduced in liposarcoma cells, but greater in synovial sarcoma cells. Silibinin significantly decreased proliferation and viability in all three synovial sarcoma cell lines. Although synovial sarcomas have typically been considered relatively chemosensitive, the European Organisation for Research and Treatment of Cancer recently reported a chemotherapy response rate of only 28% for patients with advanced synovial sarcoma (59). Therefore, there remains a requirement for

Figure 4. Real-time cell analysis of liposarcoma cell lines. (A) SW872, (B) T778 and (C) MLS-402 liposarcoma cells were seeded in 8-well plates with an integrated microelectronic sensor array. The CI reflecting the number of viable cells was monitored continuously in real-time. The CI curve of SW872 cells was slightly decreased by silibinin. T778 and MLS-402 cells exhibited a moderate response to silibinin, and a strong response to EGCG. EGCG, epigallocatechin-3-gallate; CI, cell index; DMSO, dimethyl sulfoxide.

Figure 5. Real-time cell analysis of synovial sarcoma cell lines. (A) SW982, (B) SYO1 and (C) 1273 synovial sarcoma cells were seeded in 8-well plates with an integrated microelectronic sensor array. The CI reflecting the number of viable cells was monitored continuously in real-time. The CI curve gradient of SW982 and SYO1 cells was markedly decreased by silibinin. EGCG reduced the CI gradient of SW982 and SYO1 cells, but had no effect on 1273 cells. Silibinin markedly decreased the viability of all three synovial sarcoma cell lines. EGCG, epigallocatechin-3-gallate; CI, cell index; DMSO, dimethyl sulfoxide.
alternative cytostatic agents for the treatment for synovial sarcomas, and the in vitro effects of silibinin demonstrated in the present study should be further examined in vivo.

A literature review revealed that the green tea polyphenol EGCG has further notable properties. Various in vivo studies have confirmed that EGCG mitigates doxorubicin-induced cardiotoxicity by suppressing oxidative stress (60-63). The oxygen free radical scavenging ability of EGCG has been demonstrated to protect cardiomyocytes from doxorubicin-mediated cardiotoxicity according to histopathological analysis (64). Furthermore, EGCG has been revealed to synergistically enhance the anticancer activity of doxorubicin in various in vivo studies on prostate and liver cancer (65-67). Notably, similar chemosensitizing and chemopreventive activities have been described for silibinin; in vivo studies revealed that silibinin synergistically enhances the apoptosis-inducing activity of doxorubicin and ameliorates doxorubicin-induced cardiotoxicity (68-73). Therefore, EGCG and silibinin may additionally function as chemopreventives and chemosensitizers for doxorubicin, which remains the first-line cytostatic for the systemic treatment of disseminated STS.

In conclusion, the present in vitro study demonstrated that EGCG and silibinin inhibit the proliferation and viability of liposarcoma, synovial sarcoma, fibrosarcoma and pleomorphic sarcoma cells. Liposarcoma cell lines responded particularly well to EGCG while synovial sarcoma cell lines were more sensitive to silibinin. To the best of our knowledge, this is the first study to assess the effects of EGCG and silibinin on such a wide range of STS cell lines, including liposarcoma, synovial sarcoma, fibrosarcoma and pleomorphic sarcoma cells. EGCG and silibinin are not intended to supplant doxorubicin for the treatment of patients with disseminated STS; however, they may be a potential therapeutic option for patients who require palliative treatment but are considered unsuitable for chemotherapy. The present study provides evidence to support in vivo trials to examine the effects of these natural compounds on STS.

Acknowledgements

The present study was supported by a FoRUM grant from the Ruhr-University Bochum (Bochum, Germany; grant no. K090-15).

References

1. Hoos A, Lewis JJ and Brennan MF: Weichgewebssarkome-prognostische Faktoren und multimodale Therapie. Der Chirurg 71: pp87-794, 2000.
2. Patrikiou A, Domont J, Cioffi A and Le Cesne A: Treating soft tissue sarcomas with adjuvant chemotherapy. Curr Treat Options Oncol 12: 21-31, 2011.
3. Kaushal A and Citrin D: The role of radiation therapy in the management of sarcomas. Surg Clin North Am 88: 629-646, viii, 2008.
4. O’Brien GC, Cahill RA, Bouchier-Hayes DJ and Redmond HP: EGCG inhibits recepteur d’origine nantais expression in tumour cells. J Agric Food Chem 58: 10016‑10019, 2010.
5. Kalaiselvi P, Rajashree K, Bharathi Priya L and Padma VV: Epigallocatechin-3-gallate (EGCG) inhibits the invasion of highly invasive CL1-5 lung cancer cells through suppressing MMP-2 expression via JNK signaling and induces G2/M arrest. J Agric Food Chem 59: 13318‑13327, 2011.
6. Karavasilis V, Seddon BM, Ashley S, Al-Muderis O, Fisher C and Judson I: Significant clinical benefit of first-line palliative chemotherapy in advanced soft-tissue sarcoma: Retrospective analysis and identification of prognostic factors in 488 patients. Cancer 112: 1585-1591, 2008.
7. Billingsley KG, Lewis JJ, Leung DH, Casper ES, Woodruff JM and Brennan MF: Multifactorial analysis of the survival of patients with distant metastasis arising from primary extremity sarcoma. Cancer 85: 383-395, 1999.
8. Pezzi CM, Pollock RE, Evans HL, Lorigan JG, Pezzi TA, Benjamin RS and Romsdahl MM: Preoperative chemotherapy for soft-tissue sarcomas of the extremities. Ann Surg 216: 476-481, 1990.
9. Donato Di Paola E and Nielsen OS: EORTC Soft Tissue and Bone Sarcoma Group: The EORTC soft tissue and bone sarcoma group. European Organisation for Research and Treatment of Cancer. Eur J Cancer 38: (Suppl 4) S138-S141, 2002.
10. Nedea EA and DeLaney TF: Sarcoma and skin radiation oncology. Hematol Oncol Clin North Am 20: 401-429, 2006.
11. Brodowicz T, Schwameis E, Widder J, Amann G, Wiltschke C, Dominkus M, Windhager R, Ritschl P, Pötter R, Kozt R and Zielinski CC. Intensified adjuvant IFADIChem therapy for adult soft tissue sarcoma: A prospective randomized feasibility trial. Sarcoma 4: 151-160, 2000.
12. Frustaci S, Gherlinzoni F, Paoletti P, Bonetti M, Azzarelli A, Comandone A, Olmi P, Buonadonna A, Pignatti G, Barbieri E, et al: Adjuvant chemotherapy for adult soft tissue sarcomas of the extremities and girdles: Results of the Italian randomized cooperative trial. J Clin Oncol 19: 1238-1247, 2001.
13. Lee YH, Lang SA, Stoeltzing O and Brennan MF: Multifactorial analysis of the survival of patients with distant metastasis arising from primary extremity sarcoma. Cancer 85: 383-395, 1999.
14. Swain SM, Whaley FS and Ewer MS: Congestive heart failure in patients treated with doxorubicin: A retrospective analysis of three trials. Cancer 97: 2869-2879, 2003.
15. Buringham Z, Hashibe M, Spector L and Schiffman JD: The epidemiology of sarcoma. Clin Sarcoma Res 2: 14, 2012.
16. Jiang L, Tao C, He A and He X: Overexpression of miR-126 sensitizes osteosarcoma cells to apoptosis induced by epigallocatechin-3-gallate. World J Surg Oncol 12: 383, 2014.
17. Lambert JD, Sang S, Hong J and Yang CS: Anticancer and anti-inflammatory effects of catechins and their metabolites in regulating HIF-1alpha expression in HT-29 cells. Food Chem Toxicol 56: 110-118, 2013.
18. Deng YT and Lin JK: EGCG inhibits the invasion of highly invasive CL1-5 lung cancer cells through suppressing MMP-2 expression via JNK signaling and induces G2/M arrest. J Agric Food Chem 59: 13318‑13327, 2011.
19. Kobalka AJ, Keck RW and Jankun J: Synergistic anticancer activity of biologicals from green and black tea on DU 145 human prostate cancer cells. Cent Eur J Immunol 40: 1-4, 2015.
20. Park JJ, Lee YK, Hwang JY, Hu J and Park OF: Green tea catechin controls apoptosis in colon cancer cells by attenuation of H2O2-c-stimulated COX-2 expression via the AMPK signaling pathway at low-dose H2O2. Ann N Y Acad Sci 1171: 538-544, 2009.
21. Park JS, Choi PN, Joo YE, Lee YH, Lang SA, Stoeltzing O and Jung YD: EGCG inhibits receptor d’origine nantais expression by suppressing Egfr-1 in gastric cancer cells. Int J Oncol 42: 1120-1126, 2013.
22. Braicu C, Gherman CD, Irimie A and Berindan-Neagoe I: Epigallocatechin-3-Gallate (EGCG) inhibits cell proliferation and migratory behaviour of triple negative breast cancer cells. Nanosci Nanotechnol 13: 632-637, 2013.
23. Zhou C, Liu H, Feugang JM, Hao Z, Chow HH and Garcia-F: Green tea compound in chemoprevention of cervical cancer. J Agric Food Chem 59: 13318‑13327, 2011.
26. de la Torre R, de Sola S, Hernandez G, Farré M, Pujol J, Rodriguez J, Espadaler JM, Langohr K, Cuenca-Royo A, Principe A, et al.: Safety and efficacy of cognitive training plus epilobiolea in young adults with Down's syndrome (TSEDSAD): A double-blind, randomised, placebo-controlled, phase 2 trial. Lancet Neurol 15: 801–810, 2016.

27. Zhao H, Xie P, Li X, Zhu W, Sun X, Sun X, Chen X, Xing L and Yu J: A prospective phase II trial of EGCG in treatment of acute radiation-induced esophagitis for stage III lung cancer. Radiat Oncol 11: 351-356, 2016.

28. Trudel D, Labbé DP, Araya-Farias M, Doyen A, Bazine L, Duchesne T, Plante M, Grégoire J, Renaud MC, Bachvarov D, et al.: A two-stage, single-arm, phase II study of EGCG-enriched green tea drink as a maintenance therapy in women with advanced colorectal cancer. Gynecol Oncol OncoL 137: 367-371, 2013.

29. Dostal AM, Samavat H, Bedell S, Torkelson C, Wang R, Swenson K, Le C, Wu AH, Ursin G, Yuan JM and Kurzer MS: The safety of green tea extract supplementation in postmenopausal women at risk for breast cancer: Results of the Minnesota Green Tea Trial. Food Chem Toxicol 83: 26-35, 2015.

30. Garcia FA, Cornelison T, Nuño T, Greenspan DL, Byron JW, Hsu CH, Alberts DS and Chow HH: Results of a phase II randomized, double-blind, placebo-controlled trial of Polyphenol E in women with persistent high-risk HPV infection and low-grade cervical intraepithelial neoplasia. Gynecol Oncol OncoL 137: 372-376, 2014.

31. Singh BN, Shankar S and Srivastava RK: Green tea catechin, epigallocatechin-3-gallate (EGCC): Mechanisms, perspectives and clinical applications. Biochem Pharmacol 82: 1807-2011, 2011.

32. Shanafelt TD, Call TG, Zent CS, LaPlant B, Bowen DA, Roos M, Secord CA, Gates AL, Khatib BF, et al.: Phase I trial of daily oral Polyphenol E in patients with asymptomatic Rai stage 0 to II chronic lymphocytic leukemia. J Clin Oncol 27: 3808-3814, 2009.

33. Mengs U, Pohl KT and Mitchell T: Legalan® SIL: The anitode of choice in patients with acute hepatotoxicity from amatoxin poisoning. Curr Pharm Biotechnol 13: 1964-1970, 2012.

34. Flagg TW, Glodé M, Gustafson D, van Bokhoven A, Tao Y, Wilson S, Li J, Li Y, Harrison G, Aggarwal R, et al.: A study of high-dose oral silybin-phytosome followed by prostatectomy in patients with localized prostate cancer. Prostate 70: 848-855, 2010.

35. Flagg TW, Gustafson DL, Su LJ, Zirrolli JA, Crighton F, Harrison G, Aggarwal R, and Glodé LM: Silibinin suppresses growth and induces apoptotic death of human colorectal carcinoma LoVo cells in culture and tumor xenograft. Mol Cancer Ther 8: 2366-2374, 2009.

36. Singh RP and Aggarwal R: Prostate cancer chemoprevention by silybin: Bench to bedside. Mol Carcinog 45: 436-442, 2006.

37. Leet CC, Leet CC, Di Virgilio AM, Adler L, Williams PA, Bollati-Fogolín M and Etcheverry SB: Noscapinoids as microtubule-targeted cancer therapeutics. Mol Med Rep 63: 627-633, 2016.

38. Yu X, Skytting B, Nilsson G, Gasbarri A, Haslam K, Bartolazzi A, Brodin B, Mandahl N and Larsson O: Silybin-SSX is critical for cyclin D1 expression in synovial sarcoma cells: A gain of function of the t(12;16)(q13;p11) translocation. Cancer Res 62: 3861-3867, 2002.

39. Bollati-Fogolín M and Etcheverry SB: Green tea catechin, epigallocatechin-3-gallate in young adults with Down’s syndrome: A mechanistic study. Biochem Pharmacol 89: 288-297, 2014.

40. Wilson S, Su LJ, Li Y, Harrison G, Aggarwal R and Glodé LM: High-dose oral silybin-phytosome followed by prostatectomy in patients with localized prostate cancer. Prostate 70: 848-855, 2010.

41. Flagg TW, Gustafson DL, Su LJ, Zirrolli JA, Crighton F, Harrison G, Aggarwal R, and Glodé LM: A phase I and pharmacokinetic study of silybin-phytosome in prostate cancer patients. Invest New Drugs 25: 139-146, 2007.

42. Kaur M, Velmurugan B, Tyagi A, Deep G, Katyar S, Agarwal C and Agarwal R: Silybin suppresses growth and induces apoptotic death of human breast cancer cells. Int J Oncol 48: 2666-2674, 2016.

43. Lopus M and Naik PK: Taking aim at a dynamic target: Noscapinoids as microtubule-targeted cancer therapeutics. Pharmacol Rep 67: 56-62, 2015.

44. Shen J, Amin S, Cao G, Chen Y, Li X, Su LJ, Yu J, Wang G, ten R brows R, et al.: Characterization of liposarcoma cell lines for preclinical and biological studies. Sarcoma 2012: 148614, 2012.

45. Aman P, Ron D, Mandahl N, Fioretos T, Heim S, Arheden K, Willén H, Rydholm A and Mitelman F: Rearrangement of the transcription factor gene CHOP in myxoid liposarcomas with t(12;16)(q13;p11). Genes, chromosomes cancer 5: 278-285, 1992.

46. Bollati-Fogolín M and Etcheverry SB: Noscapine recirculates enterohepatically and induces the death of Ewing tumor cells. Mol Cancer Ther 11: 345-351, 2012.

47. Rutgers-Valenzuela EO and Calaf GM: Apoptotic effect of noscapine in breast cancer cell lines. Int J Oncol 48: 2666-2674, 2016.
64. Cheng T, Liu J, Ren J, Huang F, Ou H, Ding Y, Zhang Y, Ma R, An Y, Liu J and Shi L: Green tea catechin-based complex micelles combined with doxorubicin to overcome cardiotoxicity and multidrug resistance. Theranostics 6: 1277-1292, 2016.

65. Stearns ME, Amatangelo MD, Varma D, Sell C and Goodyear SM: Combination therapy with epigallocatechin-3-gallate and doxorubicin in human prostate tumor modeling studies: Inhibition of metastatic tumor growth in severe combined immunodeficiency mice. Am J Pathol 177: 3169-3179, 2010.

66. Chen L, Ye HL, Zhang G, Yao WM, Chen XZ, Zhang FC and Liang G: Autophagy inhibition contributes to the synergistic interaction between EGCG and doxorubicin to kill the hepatoma Hep3B cells. PloS One 9: e85771, 2014.

67. Liang G, Tang A, Lin X, Li L, Zhang S, Huang Z, Tang H and Li QQ: Green tea catechins augment the antitumor activity of doxorubicin in an in vivo mouse model for chemoresistant liver cancer. Int J Oncol 37: 111-123, 2010.

68. Singh RP, Mallikarjuna GU, Sharma G, Dhanalakshmi S, Tyagi AK, Chan DC, Agarwal C and Agarwal R: Oral silibinin inhibits lung tumor growth in athymic nude mice and forms a novel chemocombination with doxorubicin targeting nuclear factor kappaB-mediated inducible chemoresistance. Clin Cancer Res 10: 8641-8647, 2004.

69. Tyagi AK, Agarwal C, Chan DC and Agarwal R: Synergistic anti-cancer effects of silibinin with conventional cytotoxic agents doxorubicin, cisplatin and carboplatin against human breast carcinoma MCF-7 and MDA-MB468 cells. Oncol Rep 11: 493-499, 2004.

70. Tyagi AK, Singh RP, Agarwal C, Chan DC and Agarwal R: Silibinin strongly synergizes human prostate carcinoma DU145 cells to doxorubicin-induced growth inhibition, G2-M arrest, and apoptosis. Clin Cancer Res 8: 3512-3519, 2002.

71. Rašković A, Stilinović N, Kolarović J, Vasović V, Vukmirović S and Mikov M: The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. Molecules 16: 8601-8613, 2011.

72. Chloupčíková S, Psotová J, Miketová P and Simánek V: Chemoprotective effect of plant phenolics against anthracycline-induced toxicity on rat cardiomyocytes. Part I. Silymarin and its flavonolignans. Phytother Res: 18: 107-110, 2004.

73. Psotová J, Chloupčíková S, Grambal F, Simánek V and Ulrichová J: Influence of silymarin and its flavonolignans on doxorubicin-iron induced lipid peroxidation in rat heart microsomes and mitochondria in comparison with quercetin. Phytother Res 16: (Suppl 1) S63-S67, 2002.