WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 438-444. Research Article ISSN 2277– 7105

ANTITUMOR EFFECTS OF PU-ERH TEA CATECHINS IN HUMAN CANCER CELL LINES AND EVALUATION OF COMBINATION EFFECTS WITH OXALIPLATIN

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Article Received on
02 April 2015,

Revised on 24 April 2015,
Accepted on 18 May 2015

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ABSTRACT

In this study we investigated the effects of catechin fraction extracted from the Pu-erh tea leaves against human colon carcinoma cell line HT-29, human breast carcinoma cell line MDA-MB-231 and healthy cell lines – BALB/3T3 and BJ. We added different concentrations of the catechin fraction (2 – 1000 μg/ml) to the cultured cells and incubated them for 24 and 72 h. To detect cytotoxic and antiproliferative effects, we used MTT assay. The catechin fraction extracted from Pu-erh tea slightly exerted a direct cytotoxic effect; while the anti-proliferative action was concentration-dependent on forth used cell lines. Combination of Pu-erh catechins with oxaliplatin did not result in synergistic effects.

KEYWORDS: *Camellia sinensis*, Pu-erh tea, green tea, anticancer, catechins.

INTRODUCTION

Tea, a commonly consumed beverage, is most often derived from the dried leaves of *Camellia sinensis* plant. This plant has been studied extensively for its health benefits, including cancer prevention as protecting the cells from carcinogen-induced DNA damage, or cancer treatment as promoting apoptosis of tumor cells and inhibiting angiogenesis.[1-3] The

beneficial effects of green tea are mainly related to its polyphenol compounds, particularly flavonoids that are commonly known as catechins. The major catechins (a group of flavan-3-ols) in green tea are (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG) and (–)-epicatechin (EC) (fig. 1) which constitute around 90% of the total catechin fraction,[4] and (+)-catechin (C) and (+)-gallocatechin (GC) which present about 6% of the fraction. Thereare some minor catechins that constitute less than 2% of the total catechins. The catechins that are water-soluble, colorless compound contribute to astringency and bitterness in green tea. EGCG is the major phenolic constituent found in green tea and is associated with the health benefits of the green tea consumption – numerous scientific studies suggests that EGCG (and other catechins) is responsible.[5]

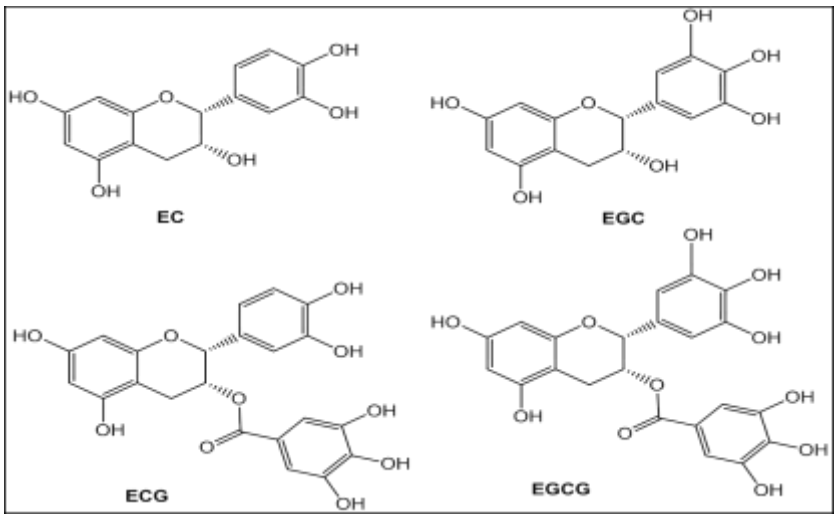

Figure 1. The major catechins in green tea.

The production of Pu-erh tea is carried out in the Yunnan province of China.The key process of Pu-erh preparation includes the step of fermentation, in which microorganisms play a very important role in producing the taste, color, fragrances, as well as the functional components. This special preparation process makes Pu-erh tea unique in terms of its shelf life, as well as its bioactive ingredients.[6] There is very limited data about the bioactive components of Pu-erh tea extracts. There has been a speculation about the content of total catechins in Pu-erh tea, as some studies suggest that the content of total catechins in Pu-erh tea is one tenth of that in green tea.[7]

The main objectives of the present study are to evaluate the potential anticancerogenic effect of the catechins derived from Pu-erh tea leaves on non-carcinomas and carcinomas cell lines, as well as evaluation of combination effects with oxaliplatin.

MATERIALS AND METHODS

Plant materials and chemicals

Dried Pu-erh tea leaves (*Camellia sinensis L.*) were purchased from a local market. All chemicals used in extraction were purchased from Sigma-Aldrich.

Extraction of catechins from Pu-erh tea leaves

Dried Pu-erh tea leaves were extracted with 100 ml of methanol, and evaporated on a water bath. To the dried extract were added 10 ml of water, sodium chloride and phosphoric acid to pH 3,5. The mixture was placed in a separating funnel with 10 ml of ethyl acetate and the combined ethyl acetate extracts were washed twice with water until neutral reaction. After evaporating of the solvent a mixture of catechins were obtained and its percentage was calculated.

Cell cultures

The BALB/3T3 clone A31 (standard mouse embryonic fibroblast cell line), BJ (human skin fibroblast cell line), HT-29 (human colon cancer cell line) and MDA-MB-231 (breast cancer cell line) cells were cultured in Dulbecco Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Austria), 100 U/ml penicillin and 0.1 mg/ml streptomycin (Lonza, Belgium) under 5% CO_2 atmosphere at 37^{o}C. Plastic flasks supplied by Greiner, Germany, were used to grow the cells. For experiments the cells in exponential phase of growth after treatment with trypsin-EDTA (FlowLab, Australia) were seeded into 96-well plates (Greiner, Germany) in a concentration $2x10^4$ cells/well.

Cell viability assay

24-hours post seeding, the cultivated cells were treated with Pu-erh tea catechins in a wide concentration range (2-1000 µg/ml, double increasing manner). Untreated cells were used as controls. Cytotoxicity was measured by colorimetric assay based on tetrazolium salt MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma Chemical Co.). The assay was performed 24- and 72-hours after treatment with the catechin fraction extracted from Pu-erh tea leaves as described elsewhere.[12] ELISA plate reader (TECAN, Sunrise TM, Grodig/Sazburg, Austria) was used for reading the results. Optical density was determined at a wavelength of 540 nm and a reference wavelength of 620 nm. Cell cytotoxicity determined by MTT assay was expressed as per cent of untreated control.

Evaluation of combination effects

Predicted theoretical values were calculated according to the equation.

C = a.b/100, where **a** and **b** are cell survival values with single agents, presented as a percent of untreated control. For each concentration applied theoretical values were calculated and compared with the real value of the combination: for $C_{measured} = C_{calculated}$ the combination effect is additive; for $C_{measured} < C_{calculated}$ the combination effect is synergistic; and for $C_{measured} > C_{calculated}$ the combination effect is antagonistic.

Statistical Analysis

Results are expressed as arithmetic means ± standard deviation (SD) of the means of three separate experiments (each experiment was done with three parallels). The statistical evaluation was performed using parametric unpaired t-test. A difference at $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Based on previous published results, investigating different varieties of tea (*Camellia sinensis L* – green, black, white) extracts[8-10] and how they affect tumor growth and their mechanism of anticancer action, we wanted to explore the popular in the recent years in Bulgaria for its anti-obesity action Pu-erh tea for tumor suppression action. The yield of obtained catechins from 10 g dried Pu-erh tea leaves were 0,319 g (or 3,19%). To investigate the direct cytotoxic effect of the catechin fraction extracted from Pu-erh tea, we used non-cancerous cell line BALB/3T3. The results are shown on figure 2. In the low concentration range 2-250 µg/ml we observed a slight stimulation of cell growth, while at high concentrations – 500-1000 µg/ml, it was detected a less pronounced cytotoxic effect.

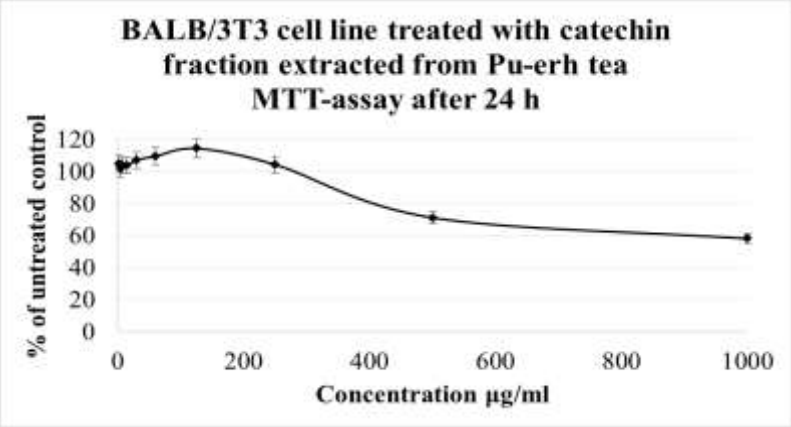

Figure 2. Cytotoxic action of catechin fraction extracted from Pu-erh tea on BALB/3T3 cell line.

To explore cell growth inhibition, we use four cell lines – two non-cancerous BALB/3T3 and BJ and two cancerous HT-29 (colon cancer) and MDA-MB-231 (breast cancer) cell lines. Inhibition of cell proliferation was observed following treatments with different concentrations of catechin extraction from Pu-erh tea leaves (Figure 3).

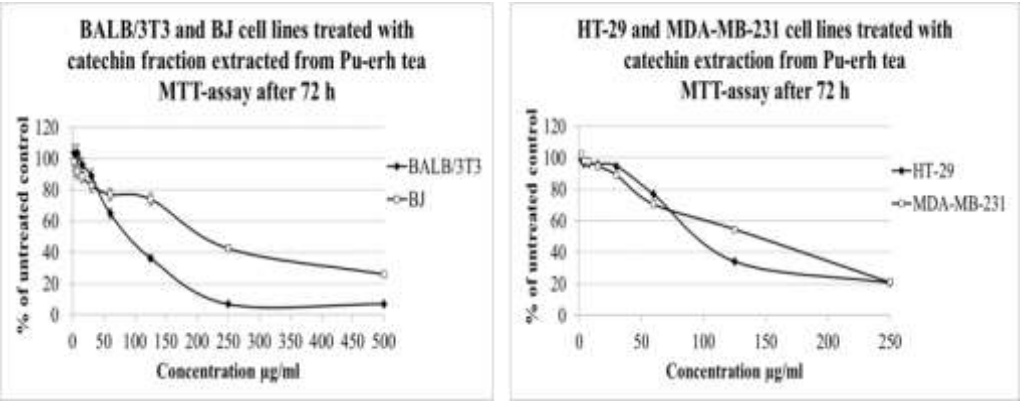

Figure 3. Inhibition of cell proliferation of the catechin fraction extracted from Pu-erh tea on BALB/3T3, BJ, HT-29 and MDA-MB-231 cell lines.

The catechin fraction extracted from Pu-erh tea leaves has shown concentration-dependent inhibitory activity on forth tested cell lines. The determined IC_{50} values are shown in table 1.

Table 1: IC_{50} values of catechin fraction extracted from Pu-erh tea on forth tested cell lines.

Cell lines	***IC_{50} values of catechin fraction (µg/ml)***
BALB/3T3	78,98±6,46
BJ	188,09±49,06
HT-29	87,10±2,73
MDA-MB-231	96,01±12,87

The both cancerous cell lines – HT-29 and MDA-MB-231, are very sensitive to the action of catechin fraction, as shown in table 1.

In order to evaluate the possible interactions of Pu-erh catechins and one of the antineoplastic drugs, oxaliplatin – standard treatment of colon cancer, we used them in combination on HT-29 cell line. Oxaliplatin is the only platinum compound to show clinical activity in colorectal cancer. It is used as adjuvant treatment for colon cancer in combination with 5-Fluorouracil and leucovorin – FOLFOX regimen.[11] We used oxaliplatin fixed concentration – 5 µg/ml, while Pu-erh catechins were in concentration range of 4-1000 µg/ml. The results are shown on figure 4.

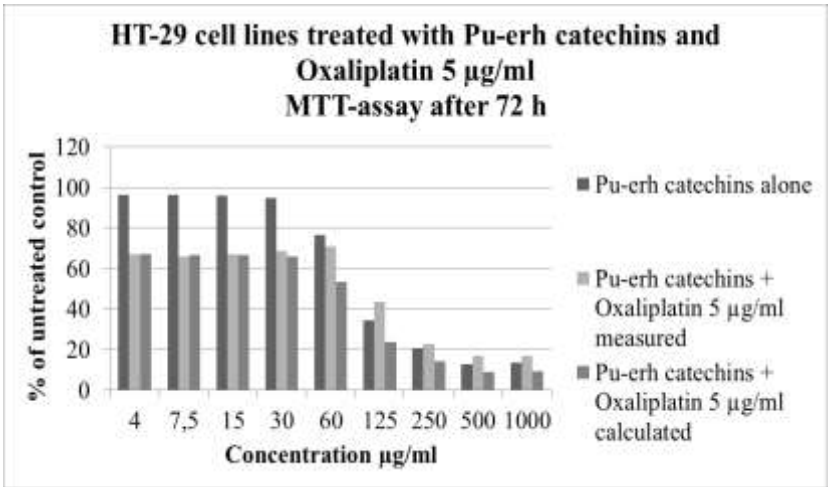

Figure 4. Combination treatment of HT-29 cell line with Pu-erh catechins and oxaliplatin.

When using fixed concentration – 5 µg/ml, oxaliplatin induced approximately 30% inhibition of tumor cell growth. The combination with different concentrations of Pu-erh catechins did not result in synergistic effects. In low concentrations (4-15 µg/ml) of Pu-erh catechins we observed additive effects. At concentrations greater than 30 µg/ml, it was observed mostly antagonistic effects.

CONCLUSION

Our study found that Pu-erh tea catechin extract could inhibit tumor cell growth. At this stage the mechanism by which Pu-erh tea catechins achieve their effects remains elusive. Combination of Pu-erh catechins with oxaliplatin did not result in synergistic effects. However, we will work in the direction to clarify these mechanisms in our future developments, not only in the total catechin extract, but also in its additional separated fractions.

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