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EVALUATION OF ANTITUMOR EFFECT OF METHYLXANTHINE FRACTION ISOLATED FROM PU-ERH TEA

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ABSTRACT

In this study we investigated the effects of methylxanthine 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 used different concentrations of the methylxanthine fraction (2 – 1000 μg/ml) on the cultured cells and incubated them for 24 and 72 h. The cytotoxic effects were measured by the MTT dye reduction assay. The methylxanthine fraction extracted from Pu-erh tea has shown concentration-dependent cytotoxicity on colon and breast carcinoma cell lines. The combination of methylxanthine fraction and oxaliplatin did not result in synergism.

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

INTRODUCTION

The consumption of Pu-erh tea is associated with numerous health benefits, including weight-loss and reduce fat storage.[1] 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.[2]

Methylxanthines, natural products from plants, are part of the most consumed beverages, such as coffee, tea, and cacao. In these beverages, methylxanthines are represented mainly by caffeine, theophylline and theobromine (fig.1).

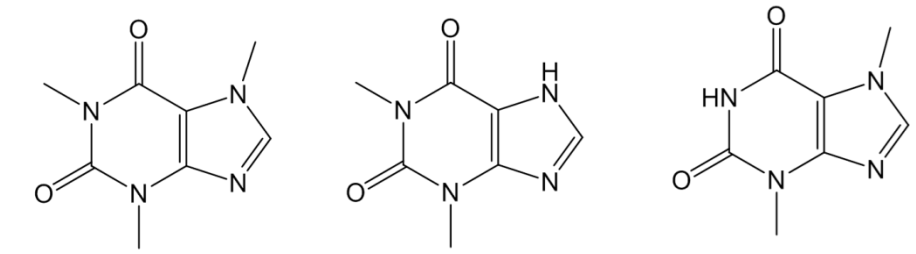

Figure 1. Chemical structures of methylxanthines

Classical pharmacological studies, mainly of caffeine, during the first half of XX century confirmed that the methylxathines have CNS (central nervous system) stimulant actions that elevate mood, decrease fatigue, and increase capacity for work. Further researches demonstrated that methylxanthines possess other important pharmacological properties as well.[3-5] The most likely mechanism of action of the methylxanthine is the antagonism at the level of adenosine receptors. The other proposed mechanisms like mobilization of intracellular calcium and inhibition of specific phosphodiesterases occur only at high non-physiological concentrations.[6]

The main objectives of the present study were: (A) to determine the potential cytotoxicity and antiproliferative effects of methylxanthine fraction isolated from Pu-erh tea on normal cell lines – BALB/3T3 and BJ; (B) to determine the antitumor effect of methylxanthine fraction isolated from Pu-erh tea on colon and breast carcinoma cell lines; and (C) to evaluate the combination of methylxanthine fraction isolated from Pu-erh tea together with oxaliplatin on carcinomas cell lines.

MATERIALS AND METHODS

Plant materials and chemicals

Dried Pu-erh tea leaves were purchased from a local market. All chemicals used in extraction were purchased from Sigma-Aldrich.

Extraction of methylxanthines from Pu-erh tea leaves

Accurately weighed amount of drug were boiled in water for 15 minutes. The combined aqueous extracts were acidified with sulfuric acid and concentrated. The solution was extracted with chloroform in separating funnel. Chloroform extract was washed with sodium hydroxide solution and then with water. After evaporation of chloroform a mixture of methylxanthines was obtained and its percentage was calculated.

Cell cultures

The BALB/3T3 clone A31 (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 x10^4 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 and statistical methods

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

By analogy with our previously published work about catechin fraction,[7] this time we investigated the potential antitumor effects of methylxanthine fraction isolated from Pu-erh tea. The yield of obtained methylxanthines from 10 g dried Pu-erh tea leaves were 0,121 g (or 1,21%). To investigate the potential cytotoxic and antiproliferative effects of the mehtylxanthine fraction extracted from Pu-erh tea, we used non-cancerous cell lines - BALB/3T3 and BJ. The results are shown on figure 2.

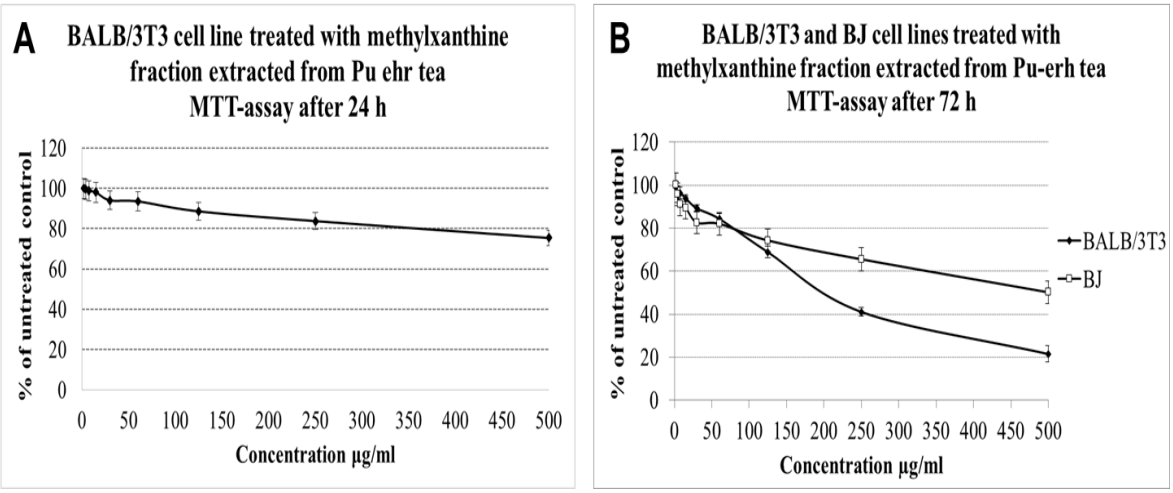

Figure 2.

A. Cytotoxic action of methylxanthine fraction extracted from Pu-erh tea on BALB/3T3 cell line measured by the MTT-assay.

B. Antiproliferative action of methylxanthine fraction extracted from Pu-erh tea on BALB/3T3 and BJ cell lines measured by the MTT-assay.

In the concentration range we used (2-500 µg/ml), methylxanthine fraction didn't exert direct cytotoxic effect on BALB/3T3 cell line. In contrast with catechin fraction, where we have shown that lower concentrations stimulate cell growth, methylxanthines doesn't have such

effect. The methylxanthine fraction extracted from Pu-erh tea leaves has shown concentration-dependent growth inhibitory activity on BALB/3T3 and BJ cell lines. The most sensitive cell line is BALB/3T3, which is shown by the calculated IC_{50} values (table 1).

Table 1: Table 1. IC_{50} values of methylxanthine fraction extracted from Pu-erh tea on BALB/3T3 and BJ cell lines.

Cell line	*IC_{50} values±SD (µg/ml)*
BALB/3T3	183,02±16,4
BJ	497,54±89,4

In the second set of our work, we tried to determine the antitumor effect of methylxanthine fraction isolated from Pu-erh tea with the same concentrations we used previously on colon and breast carcinoma cell lines – HT-29 and MDA-MB-231 respectively. After incubation of 72 hours with methylxanthine fraction, we obtained the following results shown on figure 3. As can be seen on the figure, methylxanthine fraction exhibit concentration-dependent growth inhibition on the both cancerous cell lines. The both cell lines are nearly equal sensitive to the action of methylxanthines and display similar curves moves.

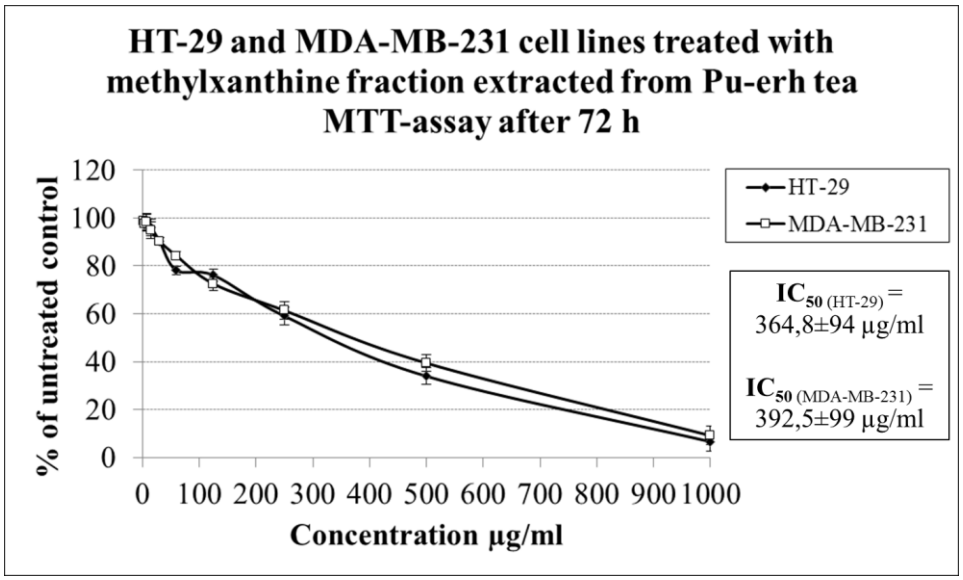

Figure 3. Inhibition of cell proliferation of the methylxanthine fraction extracted from Pu-erh tea on HT-29 and MDA-MB-231 cell lines. IC_{50} values are included in the legend as well.

The efficacy of combination treatment with methylxanthine fraction and oxaliplatin was tested on colon carcinoma cell line – HT-29 and breast cancer cell line – MDA-MB-231. The results are illustrated on figure 4.

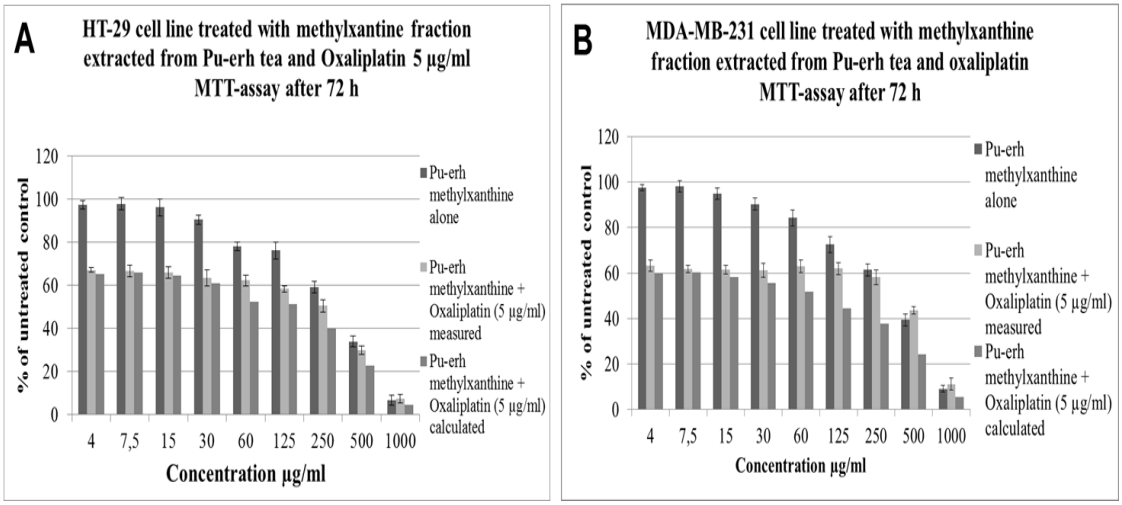

Figure 4.

A. Combination treatment of methylxanthine fraction and oxaliplatin on HT-29 cell line.

B. Combination treatment of methylxanthine fraction and oxaliplatin on MDA-MB-231 cell line.

Oxaliplatin, used in fixed concentration of 5 µg/ml, induces approximately 40% and 30% inhibition of cell proliferation on MDA-MB-231 and HT-29 cell lines, respectively. Mehtylxanthine fraction was in the concentration range of 4-1000 µg/ml. On the both cell lines, the combination did not show the expected synergistic, but rather antagonistic effects.

CONCLUSION

Anticancerogenic activity of green tea (*Camellia sinensis* L.) is well defined. These effects are primarily due to catechin components, including epigalocatechin-3-gallate (EGCG), but other components, such as methylxanthines, cannot be excluded. Due to its special fermented processing and the incorporation of microbes, Pu-erh tea might be different from green tea in terms of its tumor cell growth inhibition activity, as well as its molecular targets. Also, the fermentation by microbes might increases the bioavailability of Pu-erh tea components. In this study, we demonstrated that methylxanthine fraction alone exerts concentration-dependent growth inhibition on HT-29 and MDA-MB-231 cell lines with IC_{50} values of 364,8 and 392,5 µg/ml respectively. These values are significantly higher than those produced by the treatment with catechin fraction (87,1 and 96,01 µg/ml on HT-29 and MDA-MB-231 respectively). However, our opinion is, that methylxanthine fraction contributes to the antitumor activity of the consuming Pu-erh tea. The combination with oxaliplatin does not lead to the expected synergistic, but rather to antagonistic effects.

REFERENCES

1. Ding Y, Zou X, Jiang X, Wu J, Zhang Y, Chen D, Liang B. Pu-erh tea down-regulates sterol regulatory element-binding protein and stearyol-CoA desaturase to reduce fat storage in Caenorhaditis elegans. PLoS One., 2015 Feb 6; 10(2): e0113815.
2. Zhao L, Jia S, Tang W, Sheng J, Luo Y.Pu-erh tea inhibits tumor cell growth by down-regulating mutant p53.Int J Mol Sci., 2011; 12(11): 7581-93.
3. Fredholm BB. Cardiovascular and renal actions of methylxanthines. Prog Clin Biol Res., 1984; 158: 303-30.
4. Sawynok J. Pharmacological rationale for the clinical use of caffeine. Drugs., 1995 Jan; 49(1): 37-50.
5. Chou TM, Benowitz NL. Caffeine and coffee: effects on health and cardiovascular disease. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol., 1994 Oct; 109(2): 173-89.
6. Nehlig A, Daval JL, Debry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev., 1992 May-Aug; 17(2): 139-70.
7. Georgiev K, Iliev I, Jelev I. Antitumor effects of Pu-erh tea catechins in human cancer cell lines and evaluation of combination effects with oxaliplatin. WJPR., 2015; 4(6): 438-444.