# Physiological Function of Phytopharma-ceuticals and its Utilisation XIII:

# Green Tea (Camellia sinensis)

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## Introduction

One of the functional materials recently getting much attention is green tea. Several effects of green tea on life-style related diseases have been reported, including antitumor activities (1), antioxidative activities (2), antiinflammatory activities (3).

Extracts containing concentrated compounds like catechins and theanine are used in most studies on green tea in Japan. In Europe, green tea is regarded as beneficial material for phytopharmaceuticals and green tea extracts are distributed in the European market as drugs and as supplementary food.

The 12th Food Development Fair 2001 was held on September 26-28, 2001. In the memorial seminar Mr. Marco Netsch from Emil Flachsmann AG hold a lecture titled “Aspects of Chemopreventive and Antiinflammatory Activities of Green Tea and its Preparations”. This article describes the pharmacological efficacy of EFLA®942 demonstrated in clinical trials and possible mechanisms evaluated in *in vitro* studies.

## 1. Use of green tea in Europe

Green tea had an official state of medicine in several European Pharmacopoeias, including Ph.Port. IV, Ph.Franç. VIII-X, BP Edition 1968 and Ph.Helv. V. Nowadays green tea is not listed in the Ph.Eur., which is the only legal pharmacopoeia for EC countries. Independently, in France green tea pharmaceuticals are approved as drugs on the basis of the Ph. Franç. and the French Gazette for Marketing Authorisation of Plant Medicines (1990). In contrast to the situation in France, no marketing authorisation for medicinal use is available in other EC countries, but there exist preparations on the base of green tea as supplementary food.

The French Gazette for Marketing Authorisation of Plant Medicines (1990) listed some traditional indications of green tea: For oral use, 1) diuresis, 2) mild diarrhoea, 3) recovery from fatigue, and 4) dietary supplement for weight reduction and for external use, 1) calm for itching of skin ailment and 2) treatment of cracks, grazes, and insect bites, etc. (4).

## 2. Diuretic effect and anti-diarrhoea effect

Regarding the diuretic effect of green tea there might exist two different mechanisms of action. On the one hand this effect could be exerted by the green tea component caffeine, which acts as a diuretic by increasing the plasma renin activity. On the other hand, diuresis could be influenced by the inhibition of the neutral endopeptidase (NEP). In an *in vitro* study EFLA®942 was shown to decrease NEP activity with an $IC_{50}$ of 40 mg/ml (5). The membrane ectoenzyme NEP, which is abundant especially in kidney, lung, brain and neutrophils, cleaves endogenous peptides such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and adrenomedullin (ADM). As ANP, BNP and ADM are hormones with a broad range of potent physiological effects including diuresis, it is suggested that one mechanism by which EFLA®942 promotes diuresis is the inhibition of NEP activity.

In two clinical pilot studies the effect of EFLA®942 on drug-associated diarrhoea was investigated. Subjects were HIV-patients with protease inhibitor-associated diarrhoea, pathogenic diarrhoea or unspecific diarrhoea who had more than 3 pultaceous-watery stools per day for more than 3 months simultaneously with an antibiotics resistance. Treatment adjustment started with 3 x 2 tablets containing 300 mg of EFLA®942 for 8 days with a following dosage reduction to 3 x 1 tablet over a time period of 6 months. The first pilot study including 15 patients demonstrated a normalisation of stool in all of the patients suffering on drug-associated diarrhoea at the beginning of the study (data not shown). Correspondingly, in the second pilot study the 29 patients suffering on drug-associated diarrhoea showed a normalisation of stool consistency (Fig.1) and stool frequency (Fig. 2) after treatment.

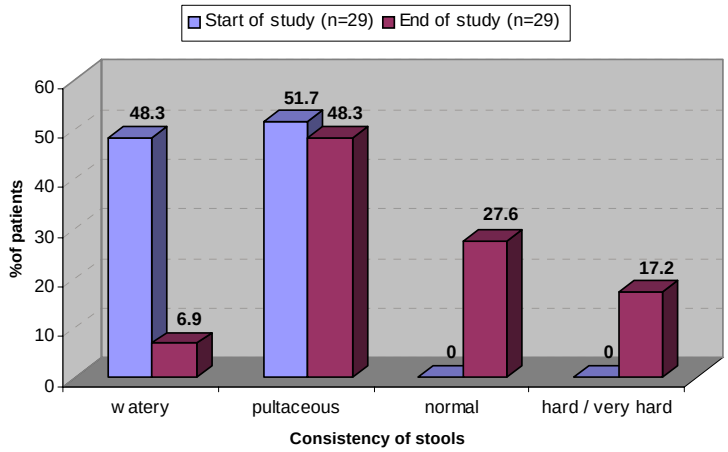


Fig. 1 : Consistency of stools with green tea extract in patients with drug-associated diarrhoea, without additional medication.

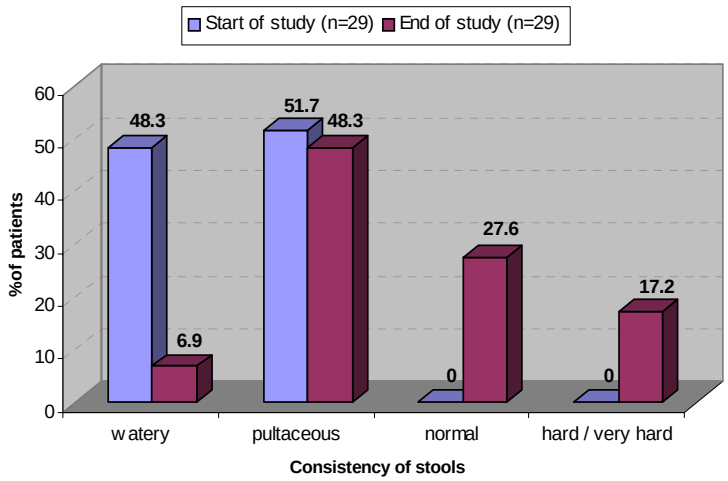


Fig. 2 : Stool frequency per day with green tea extract in patients with drug-associated diarrhoea, without additional medication.

## 3. Pharmacology

To study the mechanism of the anti-diarrhoea effect observed in the pilot studies, necrosis suppression and anti-inflammatory effects using EFLA®942 were examined.

### a) Necrosis

In many cases diarrhoea is caused by necrosis. Therefore, in this study multicellular spheroids of human WiDr colon carcinoma cells that characteristically develop a central necrosis were used (Fig.3). In comparison to conventional monolayer cell models they resemble far more to the histogenic cells and the similarity is clear in many respects including structure, cellular functions of metabolism, growth, differentiation and micromilieu of the cells (6). The treatment of

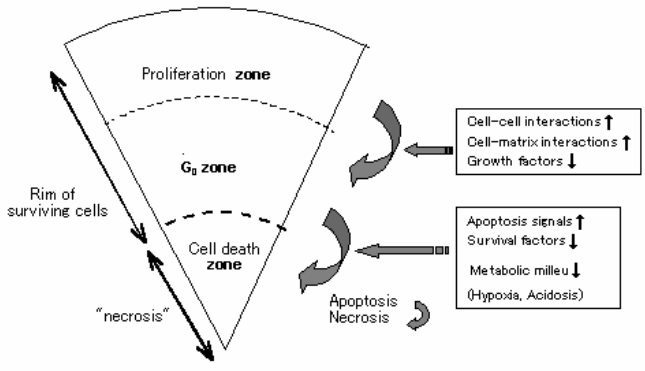


**Fig.3 :**
Complexity of cell death in multicellular spheroids.

multicellular spheroids with 100mg/ml EFLA®942 confirmed the effects of EFLA®942 described below:

(1) Inhibition of hypertrophy(enlargement of volume and diameter) of multicellular spheroids (Fig.4)

(2) Enlargement in thickness of the proliferative zone in multicellular spheroids (Fig.5)

(3) Suppression or delay of necrosis development in multicellular spheroids (Fig.6)

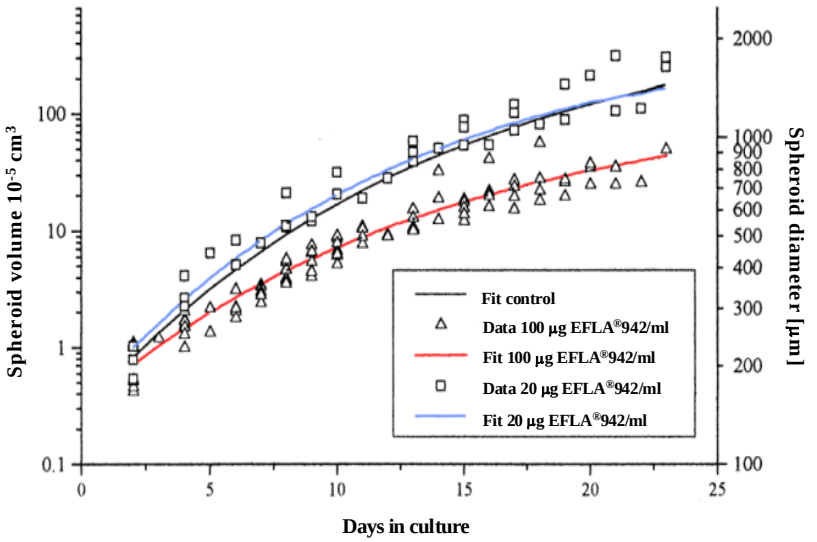


**Fig.4 :** Volume growth curves of multicellular WiDr spheroids.

| | Control | 100 µg EFLA®942/ml |
|---|---|---|
| Cell diameter | 16.3 ± 8.00 µm | 17.0 ± 8.17 µm |
| Thickness of proliferative zone | 108.0 ±9.00 µm | 271.0 ±35.00 µm |

**Fig.5 :**
Effect of EFLA®942 on cell diameter and thickness of the proliferative zone in multicellular WiDr spheroids ($p<0.01$).

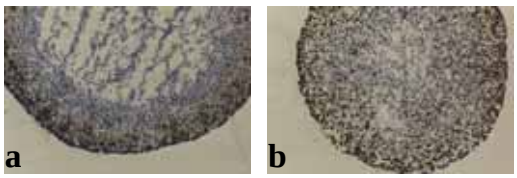


**Fig.6 :** Frozen sections of multicellular WiDr spheroids at day 16: a. Control, ∅ 1070.0 mm/ b. 100 mg EFLA®942/ml, ∅ 821.1 mm

(4) Reduction of multiplicity and increase of cell volume (Fig.7)

The delayed start of necrosis development as well as further suppression of necrosis by suppression of hypertrophy and by increase of the diameter of the proliferative zone might contribute to the results of the pilot studies.

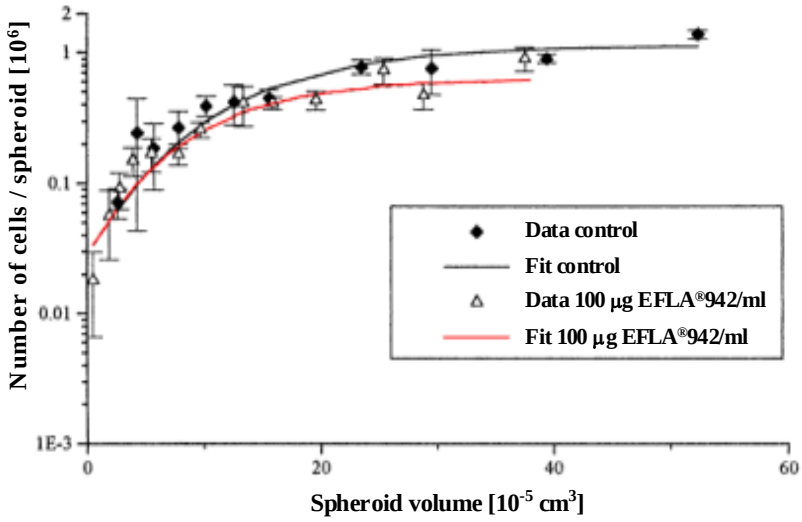


**Fig.7 :** Multiplicity of multicellular WiDr spheroids.

### b) Necrosis and glutathione

In cancer research, a consequence frequently associated with free radicals is the development of tumors. Cell division is a critical factor in mutagenesis, where non-repaired DNA damages can be the origin of mutations in tumor suppressor genes or proto-oncogenes (7). The fact that oxidants are ranked to the class of cell division stimulating agents (8) underlines the important role of antioxidants.

The antioxidative defence systems of the cell can be subdivided into enzymatic (e.g. superoxide dismutase) and non-enzymatic defence systems (e.g. glutathione (GSH), vitamin A, C and E). The tripeptide GSH is localised in liver, kidney, lung and digestive tract. In addition to the antioxidative activity GSH has a broad influence on the cellular metabolism including DNA synthesis and repair and metabolism of toxins and carcinogens. Since EFLA®942 suppressed necrosis, the influence of EFLA®942 on the cellular GSH concentration was examined. After treatment of human colon carcinoma spheroids with EFLA®942 a significant increase of the cellular GSH concentration by 15% was observed (Fig.8). It is not clear of this effect is due to the increase in GSH production or the stabilisation of reduced GSH by the antioxidative activity of green tea polyphenols. As green tea is generally known for its radical scavenging potential, it appears to exert a dual antioxidative effect including the increase of GSH concentration. If necrosis is caused by oxidative stress and excess of free radicals in multicellular spheroids, EFLA®942 may enhance cellular viability in spheroids by delay of GSH exhaustion and scavenging free radicals.

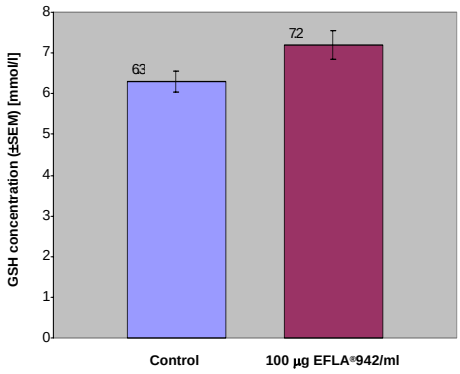


**Fig.8 :**
Glutathione concentration of multicellular WiDr spheroids in the diameter range of 300-800 mm.

### c) Antiinflammatory activity and elastase inhibition

Inflammation is an important symptom of various diseases including rheumatoid arthritis (9), atherosclerosis (10), gastric mucosal injury (11) and tumor metastasis (12). Recently, green tea was demonstrated to suppress the effect of proinflammatory mediators (13). However, this article reports on the effect of EFLA®942 on the human leukocyte elastase (HLE = NE: neutrophil elastase), which is one of the most destructive proteases frequently involved in inflammatory events.

HLE, a serine protease secreted by neutrophils from azurophilic granules, is an important mediator in the initiation of an inflammation. It is involved in inflammatory diseases such as gastric mucosal injury (11) and rheumatoid arthritis (14).

EFLA®942 strongly inhibited HLE activity with an $IC_{50}$ of 2.5 mg/ml. Compared to epigallocatechin gallate (EGCG) exhibited the strongest inhibition of HLE among single polyphenolic green tea compounds, but at 1/6 in comparison to EFLA®942. This indicates synergistic effects of multiple components including EGCG. As HLE is involved in stress-induced gastric mucosal injury by decreasing gastric production of prostacyclin ($PGI_2$) (11), its inhibition by EFLA®942 might contribute to the effect of EFLA®942 on drug-associated diarrhoea.

## 4. Synergy

Recent research focused on EGCG as an active ingredient of green tea. Nevertheless, many results have demonstrated a superiority of the multicomponent system green tea extract to single compounds or combinations thereof. In addition to the inhibitory effect on HLE described above, some reports supporting this phenomenon are described below.

a) EFLA®942 more efficiently reduced the relative metabolic cell activity in human WiDr colon carcinoma cells than single compounds or combinations thereof (Fig.9).

b) Regarding the inhibitory effect on the expression of a cytochrome responsible for the metabolism of many carcinogens, green tea extract was more efficient than single polyphenols (15).

c) Addition of epicatechin significantly increased the induction of apoptosis, the tumor growth reduction and the inhibition of tumor necrosis factor a release by other single polyphenolic green tea compounds in human lung tumor cells (16).

d) Oral administration of green tea was markedly more effective than decaffeinated green tea at inhibiting the tumorigenic effect of UVB light in SKH-1 mice (17, 18).

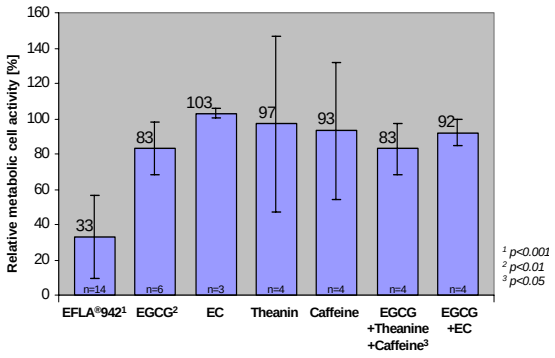


**Fig.9 :**

Relative metabolic cell activity of human WiDr colon carcinoma cells treated with 100 mg EFLA®942/ml or green tea compounds or combinations thereof corresponding to 100 mg green tea extract/ml.

## 5. Conclusion

The various kinds of efficacy demonstrated for green tea appears to result from synergistic effects of a variety of compounds and from complex mechanisms. The green tea extract EFLA®942 described in this article is developed on the basis of the multicomponent system. EFLA®942 is manufactured in accordance to the Good Manufacturing Practice (GMP) and has a standardised reproducible composition with regard to the content of polyphenols, xanthines, and theanine. It is the extract that addresses the most importance on phytoequivalence with the “green tea beverage” proved for the safety and efficacy through the long history and food culture.

The development of EFLA®942 has made feasible the research to prove scientifically many benefits known for green tea beverages. Presently, research on the efficacy of green tea is conducted with EFLA®942 in Europe, Japan and the USA.

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