

# Highly Bioavailable Green Tea (\*)

## Clinical study on obese subjects

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## INTRODUCTION

Recently, many standardized botanical derivatives have been used in treatments to induce weight loss (*Garcinia cambogia*, *Gymnema silvestris*, *Cola nitida*, *Orthosiphon stamineus*, *Citrus aurantium* to mention a few) claiming different mechanisms of action (respectively citrate-lyases inhibition, amylases inhibition, phosphodiesterases inhibition, increased diuresis and other).

These extracts and their active principles (hydroxycitrate, flavons, methylxanthines, etc.) have rarely demonstrated to be clinically effective. Their lack of effectiveness can be partially explained by their low absorption: their plasmatic concentration is too low to exert a real biological effect (1-10).

Recent clinical studies have demonstrated that catechin derivatives – mainly in their gallate form – obtained extracting the aerial, unfermented parts of *Camellia sinensis*, L. (Green Tea) increase the basal energy expenditure by 4% after oral administration of the extract containing at least 270mg of epigallocatechin gallate (EGCG). This thermogenetic action has been thoroughly investigated by many Authors demonstrating a clear weight loss activity.

(\*) **Monoselect Camellia** developed by Velleja Research (Pontenure, PC), produced by SIIT (Trezzano S/N, MI), commercialized by Omeopiacenza (Pontenure, PC) containing Greenselect® Phytosome® (Indena, MI)

## **CAMELLIA SINENSIS, L.: COMPOSITION AND ACTIVITY**

The active ingredients found in unfermented green tea leaves are polyphenolic structures that belong to the flavanol family. These flavanols are easily identified by HPLC-MS and are epigallocatechin, catechin, epigallocatechin-3-O-gallate, galocatechin-3-O-gallate, epigallo-3-O-methylgallate and epicatechin-3-O-gallate.

These fractions are commonly defined as "green tea catechins". From a pharmacological standpoint, EGCG is the most interesting molecule: very often the activity of a product can be defined by its content in EGCG (**14**). EGCG, in its pure form, is under investigation in oncology due to its anti-angiogenetic and anti-metastatic properties. Its properties are also being studied in virology.

Furthermore, EGCG is known to be one of the most powerful antioxidants; an effective inhibitor of 5- $\alpha$ -reductase and it probably has an anti-bacterial action against *Helicobacter pylori* and bacteria responsible for tooth-decay mechanisms.

These biological activities are only a few of those historically linked to green tea catechins and nowadays green tea catechins are the subject of many other studies (**15-32**).

## **CAMELLIA SINENSIS, L. AND LOSS OF BODY FAT**

The most common natural and synthetic treatments targeted to the loss of body fat use substances potentially able to reduce the caloric intake (appetite suppressants, enzyme inhibitors, natural fibers to name a few). The same effect can also be obtained by increasing daily energy expenditure.

An increase in energy expenditure can be obtained by simply increasing physical activity through exercise. In theory, there are other ways to increase energy expenditure. Thermogenesis, the production of body heat, is linked to oxidation of body fat and is controlled mainly by the sympathetic nervous system. The sympathetic nervous system uses biogenic amines -such as norepinephrine- and self-modulates by activating enzymes such as monoamine oxidase (MAO) or catechol-O-methyl transferase (COMT). These enzymes inactivate the neurotransmitters and are involved in the reduction of the thermogenetic function.

Already at the end of the '90s, substances such as catechin gallate obtained from unfermented green tea were believed to increase caloric consumption by increasing thermogenesis.

In 1999, *Dulloo et al* (11) demonstrated that green tea increases daily calorie consumption by 4%. More specifically, the controlled study indicated that oral administration of 270mg of EGCG (as standardized green tea extract) produces the following effects over a 24 hour period:

1. Energy Expenditure (EE): 4% increase
2. Respiratory Quotient (RQ): 3.4% reduction
3. Fat Oxidation: 35% increase
4. Norepinephrine urinary concentration: 40% increase

The EE (energy expenditure), measured as calories/die, is calculated from the ratio:

$$(3.9 \times \text{Vol O}_2 \text{ as mL/min} + 1.1 \times \text{Vol CO}_2 \text{ as mL/min}) \times 1.44$$

Oxygen consumption and CO<sub>2</sub> production are the only two variables in this equation. An increase in these two values produces an increase in the EE value. The EE value increases by 4% after a single dose of the extract. The EE parameter expresses the "slimming" effect of the extract.

The other 3 parameters indicate, theoretically, the mechanism of action.

The RQ (respiratory quotient) value indicates what type of catabolism -and if any- is increased after administration of the active. The respiratory quotient is calculated from the ratio:

$$\text{CO}_2\text{produced}/\text{O}_2\text{consumed}$$

Carbohydrates (basic formula C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) have a RQ=1 since the two values relative to carbon and oxygen are identical. Fats have RQ=0.7 since the molar values of carbon and oxygen are not equal (the molar value of carbon is higher than the oxygen). Under conditions of rest and moderate workload, proteic catabolism is practically absent, therefore proteic RQ (proteic RQ=0.82) does not influence the final RQ calculation: the final value is solely determined by catabolism of fats and carbohydrates.

In humans, 60% of the total RQ is influenced by the RQ of fats and 40% by the RQ of carbohydrates. In resting condition, without any catabolic stimulation, the global RQ=0.82. Any stimulation that increases fat catabolism, lowers the global RQ: it increases the % value of RQ relative to fats that contributes to the final RQ value (for example from the 60% basal value to 70%). The extract, after oral administration, increases fatty acids demolition.

Oral assumption of 270mg of EGCG increases fat oxidation (catabolism) by 35%.

This translates in a 3.4% decrease of the global RQ value. These variations in EE, RQ and fat oxidation are linked to -or coexist with- an increased basal thermogenesis as demonstrated by the increased urinary excretion of norepinephrine. Norepinephrine's mechanism of action is normally blocked by a sympathetic response involving enzymes such as MAO and/or COMT. These enzymes inhibit the functions of the biogenic amine. On the contrary, their inhibition favors norepinephrine's thermogenetic role. MAO and COMT inhibition can be studied by evaluating the urinary excretion of norepinephrine: inhibiting MAO and COMT, the urinary concentration of norepinephrine increases.

After oral administration of 270mg of EGCG as standardized extract, the urinary concentration of norepinephrine increases by 40% in comparison with the basal value. This demonstrates that EGCG directly inhibits the enzymes responsible for norepinephrine's catabolism.

According to a recently published study (34) regarding EGCG's mechanism of action, the 40% increase in urinary concentration of norepinephrine is due to EGCG's strong inhibitory activity on human COMT.

All green tea gallate-catechins are endowed with the same COMT-inhibitory activity, but this is especially high for EGCG and is evident at nano-molar concentrations: EGCG is a non- competitive inhibitor with  $IC_{50}=70nM$  (33-43).

## **ORAL ACTIVITY AND BIOAVAILABILITY**

### ***Comparison between free and complexed form***

Oral assumption of purified polyphenols obtained through extraction process shows low bioavailability: the concentration reaching blood circulation is only a small percentage of what was administered (1-20% depending on the derivative).

Complexation with phospholipids -whose polar groups react well with the polyphenol's oxydrilic groups in an aprotic solvent- leads to the formation of stable complexes (Phytosomes®) that show increased bioavallability of the polyphenolic fraction after oral administration.

Even considering the lack of a general rule, the Phytosome® is generally 3 to 5 times more bioavallable (AUC value) than the free form (44-45).

Green tea presents the same evidence: after oral administration in healthy subjects, EGCG reaches a  $C_{max}=0.8\mu\text{g/mL}$  at  $t=2\text{hrs}$  (average value on 12 subjects).

After oral assumption of an equal dose of EGCG complexed with phospholipids (Phytosome®), after 2 hrs. the  $C_{max}=1.9\mu\text{g/mL}$  ( $n=12$ ). The AUC value for the complexed form is 3 times higher than the one for the free form. Furthermore, EGCG in the non-complexed form cannot be traced in plasma 4hrs after oral administration. When the Phytosome® is analyzed, the plasmatic values after 4hrs are superior to the  $C_{max}$  at  $t=2\text{hrs}$  relative to the free form ( $C_{max}=0.8\mu\text{g/mL}$ ) **(46)**.

## **CLINICAL STUDY ON GREEN TEA PHYTOSOME® AND OBESE SUBJECTS**

In order to evaluate the clinical activity of a green tea preparation, its Phytosome® form was used (based on pharmacokinetic data). In particular, the product used was Greenselect® Phytosome® (Indena S.p.A, Milan), whose chemical, kinetic and toxicological characteristics are well known (60% total catechins; 40% EGCG;  $DL_{50} < 4000 \text{mg/kg/os}$ ). The preparation (\*) containing Greenselect® Phytosome® as the sole active ingredient -150mg/tablet- was clinically studied on overweight subjects (20-40% over their ideal weight). In a multicenter clinical trial, 100 subjects - 44 women and 56 men- between the ages of 25 and 60- were randomly subdivided in 2 groups composed by 50 subjects each. The subjects were enrolled by the Clinic of Allergology and Clinical Immunology (Rome), the Centro Polispecialistico di Ricerca (Rome) and the Terme di Fontecchio, Citta' di Castello (PG) between June 2007 and February 2008.

Group A followed a hypo-caloric diet while Group B followed the same hypo-caloric diet associated with 2 tablets/day of Camellia Monoselect (300mg/day of Greenselect® Phytosome®).

Group A (23 women, 27 men [\*\*]) at  $t=0$  presented an average weight of 95.086kg (SD: 16.377). Group B (21 women, 29 men) presented an average weight of 96.142kg (SD: 18.012). The high standard deviation (SD) is partially due to both groups being composed by a male and female subpopulation: the 2 subpopulations have sensitively different average weights. At the enrollment the male population showed a weight of 80-120kg, the weight for the female population was 60-100kg. It can be observed that the weight variation was ample even within the same subpopulation: this contributes to the relatively high standard deviation.

Other 2 factors compute for a high standard deviation value: the initial weight value was not considered a selection criterion (only subjects whose weight was sensitively different from their optimal weight were selected); also, the male and female populations were studied together. These 2 decisions allowed for a more realistic data, truly representing a Gaussian distribution of weights in an obese population.

The subjects were not administered any additional treatment other than the one established by the protocol. Their hypo-caloric diet was normo-proteic and corresponded to 1850Kcal/die for the male subjects and 1350Kcal/die for the female subjects.

The protocol evaluated the impact of a *Camellia sinensis*, L.-based treatment on a hypo-caloric diet. Essentially the protocol was meant to establish if and in what amount the treatment induced a weight loss higher than the one obtained by the low calories diet alone.

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After 45 days and 90 days of treatment, the variation in weight was measured on all 100 subjects. Other parameters were measured exclusively at t=0 and t=90, but only on subjects that had agreed to: Body Mass Index (BMI), Waistline (WL), Total Cholesterol (TC), Basal Glycemia (BG), Total Triglycerides (TT).

### RESULTS OF THE STUDY

The Group A, that at t=0 presented an average weight of 95.086kg, at t=45 had an average weight of 93.138kg.

The Group B, that at t=0 had an average weight of 96.142Kg, showed an average weight of 90.128kg at t=45 (**table 1**).

<b>Table 1: Weight variation (average±SD) in overweight subjects after 45 and 90 days of treatment with: A, hypo-caloric diet; B, hypo-caloric diet and Camellia Monoselect (Greenselect® Phytosome®), 150mg twice daily</b>				
n	Group	Weight (kg)		
		t=0	t=45	t=90
50	A	95.086±16.377	93.138±15.977	90.490±15.388
50	B	96.142±18.012	90.128±16.651	82.298±15.326*
*p<0.001 vs. Group A at t=90				

The statistical analysis (*unpaired t test*) of collected data did not indicate any statistically relevant difference between the 2 groups at t=0 and t=45. It indicated a highly relevant difference (p=0.0089) at t=90: the Group A had an average weight of 90.490kg and the Group B had an average weight of 82.298kg. The analysis of the non-plasmatic parameters (BMI and WL) performed on the 2 groups (**table 2A and 2B**) was evaluated at t=90 vs. t=0 and showed a 5% BMI reduction and a 5% WL reduction. The Group B showed a statistically significant 12% BMI reduction and a 10% WL reduction, high value, but not statistically significant.

The analysis of data relative to the male sub-population (**table 2B**) indicated a statistically significant result: the Group A showed a 7% WL reduction; for the Group B the WL reduction totaled 14%.

**Table 2A: BMI variation expressed as % (vs. t=0) after 90 days of treatment with: A, hypo-caloric diet; B, hypo-caloric diet and Camellia Monoselect (Greenselect® Phytosome®), 150mg twice daily**

n°	Group	BMI (%)
30	A	-5
30	B	-12*

\* p<0.001 vs. Group A  
° 15 male and 15 female subjects per group

**Table 2B: Waistline variation expressed as % (vs. t=0) after 90 days of treatment with: A, hypo-caloric diet; B, hypo-caloric diet and Camellia Monoselect (Greenselect® Phytosome®), 150mg twice daily. (WL<sub>a</sub>, a=all subjects; WL<sub>m</sub>, m=male subjects)**

Group	n	WL <sub>a</sub> (%)	n	WL <sub>m</sub> (%)
A	50	-5	22	-7
B	50	-10	29	-14*

\*p<0.001 vs. Group A

The difference between WL<sub>a</sub> (Waistline all) and WL<sub>m</sub> (Waistline men) can be attributed to the different anatomical distribution of the fat mass: in obese male subjects, more than in obese female subjects, the fat mass is predominantly localized in the abdominal area.

Biochemical evaluations (**table 3**) were performed on 30 subjects per group, equally subdivided between men and women: total cholesterol, basal glycemia and total triglycerides decreased respectively by 10%, 8% and 20% in Group A and by 25%, 10% and 33% for Group B. The decrease in TC and TT was statistically significant vs. Group A.

**Table 3: TC, BG, TT variation expressed as % (vs. t=0) after 90 days of treatment with: A, hypo-caloric diet; B, hypo-caloric diet and Camellia Monoselect (Greenselect® Phytosome®), 150mg twice daily.**

n°	Group	TC (%)	BG (%)	TT (%)
30	A	-10	-8	-20
30	B	-25*	-10	-33*

\*p<0.001 vs. Group A      BG: basal glycemia  
TC: total cholesterol      TT: total triglycerides  
°15 male and 15 female subjects per group

## CONCLUSION

The object of this study was the clinical evaluation of the anti-obesity activity of a single component preparation (*Camellia Monoselect*), a green tea standardized extract whose oral bioavailability was significantly increased by complexation with phospholipids with a carrier function (*Greenselect® Phytosome®*).

With this procedure, an extract extremely rich in polyphenolic fractions can be complexed with a highly purified phospholipidic matrix in an aprotic solvent. The complex that may be formed is called Phytosome® and is characterized by elevated bioavailability of the polyphenolic fraction. After oral assumption of the complexed form, the polyphenolic matrix reached plasmatic values (AUC) 3 to 5 times higher than those obtained by administration of an equal dosage of the non-complexed form.

The active ingredient chosen and tested, given its high content in catechinic-gallate fractions and its Phytosome® form, can be considered an innovative ingredient used in weight loss (fat mass) therapies.

The results show that the oral assumption twice a day of 150mg of formulate, associated to a hypo-caloric, normo-proteic diet (based on the initial weight and sex of the subject) leads to a significant weight loss after 90 days of treatment. The formulation, associated to the hypo-caloric diet, induces an average weight loss of approximately 14kg vs. the 5kg lost following only the hypo-caloric diet.

It must be considered that, since the subjects were not hospitalized, those given a treatment clearly targeted to enhance the slimming effect obtained with the diet, may have followed the diet more strictly. All subjects were asked to keep daily notes on how accurately they followed the diet criteria: 90% of the subjects complied with this request. These notes show an equally strict observance of the criteria for both groups.

The treatment with *Camellia Monoselect* leads to a sensitive weight loss, more specifically to a reduction in the subject's fat mass. The treated subjects (Group B) show a better lean mass/fat mass ratio (measured as BMI). The BMI decreases by 5% in the subjects following only the hypo-caloric diet while it decreases by 12% in the treated subjects (Group B). Furthermore, the male subjects treated with *Camellia Monoselect* showed a 14% waistline reduction vs. the 7% reduction showed by the non-treated subjects.

This last result is obtained separating the male and female subpopulations (Group A: -3%; Group B: -6%). The reduction in waistline is not statistically significant unless we differentiate the 2 subpopulations, due to a different fat mass distribution in male and female subjects. In male subjects the fat mass is mainly distributed in the abdominal region and the waistline (WL) parameter is highly influenced by weight loss. In female subjects the fat mass is often localized in the lower segment of the body, therefore weight loss is not strictly related to a reduction in WL.

The treatment leads to better biochemical parameters, positively influencing cholesterol and triglycerides. The formulation can be considered a safe and effective treatment used in weight loss (fat mass) treatments given:

- Its biological and clinical activity on weight loss and reduction of BMI, WL and lipids
- Its pharmacokinetic and pharmacodynamic properties (high  $C_{max}$  and AUC)
- Its safety profile ( $DL_{50} < 4000 \text{mg/kg/os}$ )
- The well known biochemical evidence supporting its mechanism of action (EGCG shows COMT inhibition with  $IC_{50} = 70 \text{nM}$ ; 4% increase in EE; 3.4% decrease in RQ; 35% increase in fat catabolism; 40% increase in urinary excretion of norepinephrine)

Additional evaluations need to be performed to better understand the lipidic profile (HDL, LDL, oxidized LDL, IDL, etc.), as well as other biochemical markers linked to obesity (leptin).

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