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HIGH BIOAVAILABILITY OF A STANDARDIZED GREEN TEA EXTRACT*
A Clinical study on anti-obesity activity

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SUMMARY
A recently developed oral formulation in the form of coated tablets (Monoselect Camellia®) (hereafter called MonCam) containing as a unique active highly bioavailable green tea extract (Greenselect® Phytosome®) has been clinically tested in obese (n=100) subjects of both sexes associated with a defined hypo-caloric diet. After 90 days of treatment, the product showed a clear weight loss effect along with an improvement in body mass index (BMI). Waistline was reduced only in male subjects.
Besides the anti-obesity effect of the product, almost all the biochemical parameters tested (total cholesterol, triglycerides, LDL, HDL, GH, IGF-1, insulin; cortisol) were found to be improved with respect to the hypo-caloric diet. Taking into consideration the high safety profile of the product and the total absence of adverse effects observed during and after the trial, MonCam could be proposed as a safe and useful tool for obesity.

Key words
Camellia sinensis L.
Green Tea
Obesity

INTRODUCTION
Many standardized botanical derivatives have recently been used in treatments to induce weight loss (e.g. *Garcinia cambogia, Gymnema silvestris, Cola nitida, Orthosiphon stamineus, Citrus aurantium*) claiming different mechanisms of action (citrate-lyase inhibition, amylase inhibition, phosphodiesterase inhibition, increased diuresis, etc) (**1-6**).
These extracts and their active principles (hydroxycitrate, flavons, methylxanthines, etc) have rarely demonstrated to be clinically effective (**7-10**).
Their lack of effectiveness can be partially explained by their low absorption resulting in plasma concentrations too low to exert a real biological effect (**11-12**).
Recent clinical studies have demonstrated that catechine derivatives - mainly in their gallate form - obtained by 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 270 mg of epigallocatechin gallate (EGCG). This thermogenic action has been thoroughly investigated by several authors (**13-15**) demonstrating a clear weight loss activity.

CAMELLIA SINENSIS L.
Composition and Activity
The active ingredients found in unfermented green tea leaves are polyphenolic structures belonging to the flavanol family. These flavanols, easily identified by HPLC-MS, are epigallocatechin, catechin, epigallocatechin-3-O-gallate

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(EGCG), gallocatechin-3-O-gallate, epigallo-3-O-methylgallate and epicatechin-3-O-gallate.
These compounds are commonly referred to as 'green tea catechins' (**16**).

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 (**16**). EGCG in its pure form is under investigation in oncology for its anti-angiogenetic and anti-metastatic properties, and is also being studied in virology (**17**).

EGCG is also known to be a strong antioxidant; it is an effective inhibitor of 5α-reductase; it seems to have an anti-bacterial action against *Helicobacter pylori* as well as against 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 (**18-34**).

Loss of Body Fat

The most common natural and synthetic treatments targeted to the loss of body fat use substances potentially able to reduce caloric intake (appetite suppressants, enzyme inhibitors, natural fibers, etc). The same effect can also be obtained by increasing daily energy expenditure (**35-38**).

An increase in energy expenditure can be achieved 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 (**40-43**).

By the end of the '90s on, substances such as catechin gallate were believed to increase caloric consumption by increasing thermogenesis (**39**).

In 1999, Dulloo *et al* (**13**) demonstrated that green tea increased daily calorie consumption by 4% in a controlled study. In addition, the oral administration of 270 mg of EGCG (as standardized green tea extract) produced the following effects over a 24 h period:

1 Energy expenditure (EE): 4% increase;
2 Respiratory quotient (RQ): 3.4% reduction;
3 Fat oxidation (FO): 35% increase;
4 Norepinephrine (NE) urinary concentration: 40% increase.

EE, measured as calories/*die*, is calculated by the equation:

$$3.9 \times Vo_2 + 1.1 \times Vco_2 \times 1.44$$

where Vo_2 = volume O_2 in ml/min and Vco_2 = volume Co_2 in ml/min

Oxygen consumption and Co_2 production are the only two variables in this equation.

An increase in Vo_2 and Vco_2 produces an increase in EE. EE expresses the 'slimming effect' of the extract while RQ, FO and NE indicate, theoretically, the mechanism of action.

RQ is calculated from the ratio:

$$Co_2 \text{ produced}/O_2 \text{ consumed}$$

Carbohydrates (basic formula $C_6H_{12}O_6$) have an RQ of 1 since the values for carbon and oxygen are identical. Fats have RQ of 0.7 since the molar value of carbon is higher than the molar value of oxygen). Under conditions of rest and moderate workload, protein catabolism is practically absent, therefore, the 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 is 0.82. Any stimulation that increases fat catabolism lowers the global RQ: it increases the percentage 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 270 mg of EGCG increases FO (catabolism) by 35%. This translates into a 3.4% decrease in the global RQ value. These variations in EE, RQ and FO are linked to, or coexist with, an increased basal thermogenesis as demonstrated by the increased urinary excretion of NE. The action of NE 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 NE's thermogenic role. MAO and COMT inhibition *in vivo* can be studied by determining the urinary excretion of NE: inhibiting MAO and COMT increases the urinary concentration of NE (**40-43**).

After oral administration of 270 mg of EGCG as standardized extract, the urinary concentration of NE increases by 40% in comparison with the basal value. This is probably due to the fact that EGCG inhibits the enzymes responsible for NE's catabolism. This is supported by the recent study in human (**36**) showing the COMT inhibition by EGCG. COMT is indeed responsible for NE's catabolism, and such inhibition determines the 40% increase in terms of urinary concentration of NE.
All gallate-catechins of green tea possess COMT-inhibitory activity; this activity is highest for EGCG and is evident at nM concentrations: EGCG is a non-competitive inhibitor with an IC_{50} of 70 nM (**44-45**).

Use of a highly bioavailable form of green tea extract

Despite the large amount of pharmacological, and, to some extent, clinical data on the effect of naturally occurring polyphenols on animal and human health, these molecules are nonetheless documented to be poorly absorbed by the oral route.
In fact, after the oral assumption of purified polyphenol plant extracts showing low oral bioavailability, the concentration of polyphenols in the blood is only a small percentage of the amount administered (1-20% depending on the derivative).

Complexation with phospholipids - whose polar head reacts well with the polyphenol's hydroxyl (OH) groups in an aprotic solvent - leads to the formation of stable complexes (named Phytosomes®) that show increased bioavailability of the polyphenolic fraction after oral administration. Even considering the lack of a general rule, Phytosome is often 3 to 5 times more bioavailable (AUC value) than the free form (**46-47**).

Green tea presents the same property: after oral administration to healthy subjects of Greenselect® (a Green tea extract standardized to contain not less than 60% polyphenols with 40% of EGCG), EGCG reaches a C_{max} of 0.8 µg/mL after 2 h (average value of 12 subjects). After oral assumption of an equal dose of the same Green tea extract complexed with phospholipids, (Greenselect Phytosome), the C_{max} was 1.9 µg/mL (n=12) after 2 h. The AUC value for the complexed form was 3 times higher than the one for the free form. Furthermore, following the administration of Greenselect, EGCG cannot be traced in plasma 4 h after oral administration.
On the contrary, after the administration of Greenselect Phytosome, the EGCG plasma values after 4 h are superior to the C_{max} at t=2 h relative to the free form (C_{max}=0.8 µg/mL) (**48**).

MATERIALS AND METHODS

MonCam is a nutritional supplement submitted to the Italian Health Authorities in 2007.
It is manufactured in the form of coated tablets by SIIT (Trezzano S/N, Milan, Italy) and contains, as its sole active ingredient, 150 mg/tablet of Greenselect Phytosome.

MonCam was studied on overweight subjects (20-40% over their ideal weight) in a multicenter clinical trial, on 100 subjects (44 women and 56 men) between the ages of 25 and 60 randomly subdivided in 2 groups (A and B) composed of 50 subjects each. The subjects were enrolled by the Clinic of Allergology and Clinical Immunology (Rome, Italy) the Centro Polispecialistico di Ricerca (Rome; Italy) and the Terme di Fontecchio, Citta' di Castello (Perugia, Italy) 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 MonCam

Group A (23 women, 27 men) at t=0 presented an average weight of 95.1kg (Standard Deviation ±16.38). Group B (21 women, 29 men) presented an average weight of 96.1 kg (SD ±18.01). The high SD is partially due to both groups being composed of male and female subpopulations: the two subpopulations have considerably different average body weights. At enrollment, the male population showed a weight range of 80-120 kg, the range of the female population was 60-100 kg. It may be noticed that the weight variation was ample even within the same subpopulation: this contributed to the relatively high SD.

Another factor that contributed to the high SD was that the initial weight was not considered a selection criterion (only subjects whose weight was considerably different from their optimal weight were selected); this clinical design allowed for more realistic data collection, truly representing a Gaussian distribution of weights in an obese population.

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

The protocol was meant to establish if, and in what amount, the treatment with MonCam induced a weight loss higher than the one obtained by the low caloric diet alone.

All analysis and measurement have been done on consenting subjects. At t=0 and after 45 and 90 days of treatment, the variation in weight was measured on all 100 subjects. Other parameters were measured only at t=0 and t=90 days, i.e. body mass index (BMI; 30 subjects/per group), waistline (WL; 50 subjects/group), total cholesterol (TC, 30 subjects/group; basal glycemia (BG, 30 subjects/group; total triglycerides (TT, 30 subjects/group). At t=0 and t=90, 10 subjects/group [between 90 and 105 kg, (selected to avoid excessive SD on the basis of their body weight), and with a the BMI between 28.00 and 36.00] were analyzed for the following parameters: LDL, HDL, GH, IGF-1, insulin and cortisol.

RESULTS

Group A, that at t=0 presented an average body weight of 95.086 kg, at t=45 had an average weight of 93.138 kg.
Group B, that at t=0 had an average weight of 96.142Kg, showed an average weight of 90.128 kg at t=45 (***Table 1***).

The statistical analysis (*unpaired t test*) of the data did not indicate any statistically significant difference between the two groups at t=0 and t=45, but did show a highly significant decrease (p=0.0089) in the body weight of Group B (82.298 kg) vs Group A (90.490 kg) at t=90.
Tables 2A and ***B*** show that BMI and WL were not

Table 1 *Weight variation (average±SD) in overweight subjects after 45 and 90 days of treatment with: A, a hypo-caloric diet; B, a hypo-caloric diet and MonCam 150 mg, twice dail*

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

Table 2A *BMI variation (%) after 90 days of treatment with: A, a hypo-caloric diet; B,a hypo-caloric diet and MonCam, 150 mg twice daily*

N°	GROUP	**BMI** (%)
30[a]	A	-5
30[a]	B	-12*

*p<0.001 *vs.* Group A

[a], 15 male and 15 female subjects per group

significantly altered in Group A at t = 90 vs t = 0. On the other hand, BMI was significantly reduced by 12% in Group B; there was also a decrease in WL of 10% in the treated group although this was not statistically significant.

The analysis of data relative to the waistlines of male sub-population (WLa; ***Table 2B***) indicated a statistically significant decrease of 14% in Group B *vs* Group A.
All the statistical tests comparing percentage values were done by the Mann-Whitney U test.
The difference between WLa (all subjects) and

Table 2B *Waistline variation (%* vs. *t=0) after 90 days of treatment with: A,a hypo-caloric diet; B, a hypo-caloric diet and MonCam, 150 mg twice daily.* (WLa, a=all subjects; WLm, m=male subjects)

Group	**N°**	**WLa** (%)	**n**	**WLm** (%)
A	50	-5	22	-7
B	50	-10	29	-14*

*p<0.001 *vs.* Group A

WLm (male subjects) 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.
Measurements of total cholesterol, basal glycemia and total triglycerides (***Table 3***) were made on 30 subjects per group, equally subdivided between men and women: TC, BG and TT were found to be decreased by 10%, 8% and 20%, respectively in Group A and by 25%, 10% and 33% in Group B. The decrease in TC and TT of Group B was statistically significant *vs.* Group A.

Table 3 *Plasma TC, BG, TT (%) at 0 days and 90 days of treatment with: A, hypo-caloric diet; B, hypo-caloric diet and* MonCam, *150mg twice daily.*

N°	**Group**	**TC** (%)	**BG** (%)	**TT** (%)
30[a]	A	-10	-8	-20
30[a]	B	-25*	-10	-33*

*p<0.001 vs. Group A BG: basal glycemia
TC: total cholesterol TT: total triglycerides

[a], 15 male and 15 female subjects per group

At day 0 and day 90, , 10 subjects of Group A and 10 subjects of group B were selected on the basis of the body weight (between 90 and 105 kg) and the BMI (between 28.0 and 36.0) and their plasma was analyzed for LDL (low density lipoproteins), HDL (high density lipoproteins), GH (growth hormone), IGF-1 (insulin growth factor-1), I (insulin), and C (cortisol).
As shown in ***Tables 4*** and ***5,*** in terms of percentage values, Group A (hypo-caloric diet) demonstrated a reduction in LDL (from 130 ± 21 to 118 ± 22 mg/L), an increase in HDL (from 40 ± 5 to 44 ± 7 mg/L), an increase in GH (from 4.0 ± 2.2 to 4.8 ± 3.1 µg/L), an increase in IGF-1 (from 142 ± 28 to 163 ± 35 µg/L), a decrease in insulin (from 18 ± 2 to 15 ± 3 mU/L) and a decrease in cortisol (from 150 ± 30 to 130 ± 15 µg/L).
As shown in ***Tables 4*** and ***5*** in terms of percentage values, the Group B (hypo-caloric diet plus MonCam) demonstrated a reduction in LDL (from 132 ± 25 to 105 ± 15 mg/L), an increase in HDL (from 42 ± 6 to 51 ± 7 mg/L), an increase in GH (from 3,8 ± 1,5 to 12,2 ± 4,1 µg/L), an increase in IGF-1 (from 140 ± 25 to 174 ± 28 µg/L), a decrease in insulin (from 19 ± 3 to 12 ± 5 mU/L) and a decrease cortisol (from 152 ± 37 to 116 ± 25 ug/L).
These results show a clear trend relative to all the parameters, but cannot be considered statistically significant due to the low number of subjects tested. Only the increase in GH was found to be statistically significant (Group B versus Group A).

Table 4 *Plasma LDL, HDL, GH variation percentage from t=0 to t=90 after treatment with: A, a hypo-caloric diet; B, a hypo-caloric diet and MonCam , 150 mg twice daily*

N°	**Group**	**LDL** (%)	**HDL** (%)	**GH** (%)
10	A	-9.33	+10	+20
10	B	-20.45	+21.43	+321*

*p<0.001 vs. Group A HDL: high density lipoproteins
LDL: low density lipoproteins GH: growth hormon

Table 5 *Plasma IGF-1, insulin, cortisol variation percentage from t=0 to t=90 after treatment with: A, a hypo-caloric diet; B, a hypo-caloric diet and MonCam, 150 mg twice daily.*

N°	Group	IGF-1 (%)	I (%)	C (%)
10	A	+14.79	-16.77	-13.33
10	B	+24.29*	-36.84*	-23.68*

*p<0.05 vs. Group A I: insulin
IGF-1: insulin growth factor-1 C: cortisol

CONCLUSIONS

The aim of the study was to clinically evaluate the anti-obesity activity of MonCam, a preparation, containing, as a unique active ingredient, a green tea standardized extract whose oral bioavailability was significantly increased by complexation with phospholipids having a carrier function (*Greenselect Phytosome*).

The results show that the oral assumption twice a day of 150 mg of formulate, associated with a hypo-caloric, normo-proteic diet leads to a significant weight loss after 90 days of treatment. As a matter of fact, the formulation associated with the hypo-caloric diet induces an average weight loss of approximately 14 kg *vs.* about 5 kg lost following the hypo-caloric diet.

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

Treatment with MonCam led to a significant weight loss, more specifically to a reduction in fat mass. The subjects in Group B showed a better lean mass/fat mass ratio (measured as BMI). The BMI was decreased by 5% in the subjects following only the hypo-caloric diet while it was decreased by 12% in the treated subjects. Furthermore, the male subjects treated with MonCam showed a 14% waistline reduction vs. the 7% reduction showed by the non-treated subjects.

This latter result was obtained by splitting the male and female subpopulations (Group A: -3%; Group B: -6%). The reduction in waistline was not statistically significant unless the two subpopulations were differentiated due to a different fat mass distribution in male and female subjects. In males, the fat mass is mainly distributed in the abdominal region and the waistline is highly influenced by weight loss. In females, the fat mass is often localized in the lower segment of the body; therefore weight loss is not strictly related to a reduction in waistline.

The treatment also positively influenced the subjects' lipid profile, as shown by the plasma values of cholesterol and triglycerides. According with the anti-obesity effect shown by the product and the lipid profile results, relevant results were also obtained when comparing LDL, HDL, GH, IGF-1, insulin and cortisol levels. Especially the GH value, where Group B demonstrated more than a 3-fold increase, while in Group A the increase was much less visible. Taken altogether, the results describe for the product (hypo-caloric diet associated) a relevant weight loss along with an improved ratio lean/fat.

According to these results, the biochemical parameters considered show a reduction in terms of total cholesterol, LDL, triglycerides, insulin and cortisol (this being released by fat).

On the contrary, HDL, IGF-1 and GH are clearly increased. Most of these results seem to be directly related to the occurred weight loss due to the hypo-caloric diet, but an additive effect due to the treatment with MonCam is also evident. Considering both the very high safety profile (DL_{50}>4000mg/kg/*os*; no toxicity in sub-chronic and chronic toxicological investigations) of the active (*Greenselect Phytosome*) used in the formulation of MonCam and the total absence of side effects and unwanted effects in treated subjects, the product could be considered a safe, suitable and useful tool aimed to prevent and treat obesity and obesity-like disorders like diabetes.

Additional studies need to be performed to better understand if, and in what extent, the product can show an anti-obesity effect without the hypo-caloric diet. Moreover it needs to be understood more precisely how the formulation affects the lipid profile (especially LDL *vs.* oxidized LDL) on a larger number of subjects and other biochemical markers more clearly linked to obesity, for example leptin.

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