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Reprint of "Preclinical and clinical effects of *Coleus forskohlii*, *Salacia reticulata* and *Sesamum indicum* modifying pancreatic lipase inhibition *in vitro* and reducing total body fat"

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ABSTRACT

The herbal compositions of *Coleus forskohlii*, *Salacia reticulata*, and *Sesamum indicum*, standardized for forskolin, mangiferin, and sesamin, respectively, were shown, *in vitro*, to inhibit pancreatic lipase with differing degrees and dynamics. In the placebo controlled six weeks clinical study the daily intake of 1000 mg *C. forskohlii* stand-alone standardized for 10% forskolin, showed statistically significant lowering of total body fat vs. baseline and placebo group ($p < 0.05$). The computerized tomography showed decrease of total body fat and visceral fat in *C. forskohlii* group in comparison to the baseline. The potential of three herbal extracts preventing dietary fat absorption was emphasized by the *in vitro* synergy between *C. forskohlii* and *S. reticulata* inhibiting pancreatic lipase at higher rate than the fat-blocking activity generated by each component alone. The *in vitro* addition of *S. indicum* to the formula was found to synergistically assist inhibition of the pancreatic lipase in a lower dose range, while moderating the pancreatic lipase inhibition in a higher dose range. This dual mechanism of *S. indicum* was postulated as a safety mechanism preventing any potential side effects resulting from excessive inhibition of pancreatic lipase activity.

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Abbreviations: PLT, pancreatic triacylglycerol lipase; CCK, cholecystokinin; PYY, Peptide YY or Peptide tyrosine tyrosine; GLP-1, glucagon-like peptide 1

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1. Introduction

Digestion and absorption of dietary fat is regulated by the action of pancreatic triacylglycerol lipase (PTL), and colipase, a small protein cofactor, needed by pancreatic lipase for efficient dietary lipid hydrolysis. The nutrient-dense western type diet, super-sizing meals, overeating, snacking and sedentary life style are the risk-factors for developing overweight and obesity conditions. However, for as long as the digestive tract and its main metabolic organ liver are processing major groups of nutrients, i.e. proteins, fats and carbohydrates the population is safeguarded from overweight and obesity. Over time excess dietary fats and carbohydrates, which can be converted in the liver to fatty acids, pose major problem for metabolic health of the liver (Angulo & Lindor, 2002). The long standing excess dietary fats and carbohydrates contributes to accumulation of the liver fat content over 5%, impaired beta oxidation of fatty acids, decreased insulin sensitivity and potential comorbid conditions, i.e. obesity, pre-diabetes, diabetes, metabolic syndrome and chronic degenerative conditions (Vuppalanchi & Chalasani, 2009). Therefore, deterioration of metabolic functions of the liver due to excess fat content should be considered a direct cause or contributing factor to overweight and obesity.

The enzyme and cofactor are excreted from the pancreas to the duodenum, and PTL is the primary lipase that hydrolyzes dietary fat molecules in the human digestive tract, converting triacylglycerol substrates to monoacylglycerols and free fatty acids. The resulting molecules (2 free fatty acids and one 2-monoacylglycerol) are packed into micelle then absorbed into the lymphatic system by a specialized vessel called a lacteal. Colipase, an enzyme belonging to a family of pancreatic lipases, binds to the non-catalytic domain of lipase, and increases stability and hydrophobicity of the binding site of the enzyme. Colipase also prevents the inhibitory effect of various dietary factors on the lipase-catalyzed hydrolysis of dietary long-chain fatty acids of triacylglycerols (Kerfelec et al., 2008).

Blocking the mechanisms of dietary fat digestion and absorption has been utilized in the management of obesity (Wu et al., 2013). Inhibition of PTL activity with tetrahydrolipstatin (Orlistat/Xenical) and its diluted version, sold over-the-counter under brand name Alli, has been widely used in the pharmacotherapy of obesity (Chen, Wu, Liu, & Shen, 2014). While tetrahydrolipstatin based products have been clinically proven as safe and effective, there are several known and potential side effects of the therapy. The primary side effects of the drug are gastrointestinal tract-related, and include steatorrhea (oily, loose stools with excessive intestinal putrefaction and flatus due to unabsorbed fats reaching the large intestine), fecal incontinence, frequent bowel movements and urgency. The potential organ toxicity of tetrahydrolipstatin has been signaled by the U.S. Food and Drug Administration (FDA). On May 26, 2010, the FDA has approved a revised label for tetrahydrolipstatin based drugs to include new safety information about cases of liver injury that have been reported rarely with the use of this medication (FDA, 2010).

The use of tetrahydrolipstatin has been associated with isolated cases of acute kidney injury, possibly due to the fat malabsorption resulting from the excessive inhibition of pancreatic lipase, leading to the formation of soaps with calcium and resulting in increased free oxalate absorption and hyperoxaluria (Filippatos et al., 2008). Absorption of fat-soluble vitamins and other fat-soluble nutrients may be compromised with tetrahydrolipstatin, and to prevent the therapy associated vitamin deficiency, supplemental multivitamin containing vitamins A, D, E, K, and beta-carotene should be taken once a day while on tetrahydrolipstatin therapy. There are also recognized shortfalls of the weight loss mechanism of tetrahydrolipstatin, including increasing appetite and time dependent diminishing drug efficacy occurring after several weeks of treatment. In one study perception of satiety was significantly decreased in subjects on tetrahydrolipstatin coinciding with the significant decrease in circulating satiety hormones i.e. cholecystokinin (CCK), Peptide YY (PYY), and glucagon-like peptide 1 (GLP-1) in the study subjects (Ellrichmann et al., 2008).

There is continuous search for products, especially nutritional products which would possess dietary fat blocking abilities (Sung et al., 2014). Several plants have been identified as potential fat blockers. One of those, *Salacia reticulata* root extract comes from a climbing woody plant native to India, which has played a role in traditional Ayurvedic medicine as an anti-diabetic herb (Shivaprasad et al., 2013). *S. reticulata* containing polyphenols and thiosulgars has the ability to inhibit pancreatic triacylglycerol lipase and alpha glucosidase, reducing the rate of dietary fat and sugar absorption after a meal. Similarly *Sesamum indicum* seed extract is a pancreatic triacylglycerol lipase inhibitor. It is also a rich source of protein, minerals and lignans believed to support healthy circulatory, immune and metabolic functions (Mirmiran, Bahadoran, Golzarand, Rajab, & Azizi, 2013).

Best known for supporting weight management is *Coleus forskohlii*. This plant, which comes from the mountains of Asia, has been used for centuries in tradition of India cuisine (pickles) and more recently in formulation of Ayurveda medicines (Badmaev, Majeed, Conte, & Parker, 2002; Godard, Johnson, & Richmond, 2005). *C. forskohlii* supports fat loss through a cascade of chemical reactions – especially its ability to facilitate the action of hormones via hormonal second messenger cyclic AMP and also preventing triacylglycerol lipase mechanism. Balanced hormonal play corresponds well with healthy metabolism and helps the body prevent storage of excess fat. In addition, research studies have shown that *C. forskohlii* reduces the percentage of body fat and decreases waist circumference, visceral fat, cholesterol, blood triacylglycerols, and caloric intake (Badmaev et al., 2002; Godard et al., 2005). However, when used alone, *C. forskohlii* may not work equally effectively in men and women, and its effectiveness may decrease with continued use and as people age (Badmaev, 2011 personal communication). This contribution discusses preclinical and clinical research in support of a formula that involves *C. forskohlii*, *Salacia reticulate* and *S. indicum* and their complementary mechanisms in controlling excess of dietary fat absorption and effective and safe body weight management.

2. Materials and methods

2.1. *Assay for pancreatic lipase inhibition* in vitro

2.1.1. *Reagents and chemicals*

Porcine pancreatic lipase, 3-(*N*-morpholino)propansulfonic acid (MOPS), disodium salt of ethylenediaminetetraacetic acid ($Na_2EDTA•2H_2O$), Trizma base, calcium chloride dihydrate ($CaCl_2•2H_2O$), *N*,*N*-dimethylformamide and 4-nitrophenyl butyrate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Hydrochloric acid was obtained from Wako Pure Chemical Industries (Osaka, Japan). Orlistat (tetrahydrolipstatin) was obtained from Cayman Chemical Company (Ann Arbor, MI, USA).

2.1.2. *Preparation of test articles*

2.1.2.1. *Test articles*. Obtained from Japan Bio Actives Inc. (Tokyo, Japan) and included *C. forskohlii* extract (98%), *C. forskohlii* extract (40%), *S. reticulata* extract, *S. indicum* extract (70%) and stored at room temperature. Each of the test articles were dissolved in the solution for test article preparation so that the concentration would be 1000 μg/mL. The stock solutions were then diluted by factors of 10 successively to prepare 100, 10, and 1 μg/mL solutions. The resulting solutions were used as samples in this study. For controls, only the solution for test article preparation was added.

2.1.3. *Pancreatic lipase assay*

The reagents and measurements of pancreatic lipase were prepared and performed according to Lee et al., 2010 and Lunder, Bratkovic, Kreft, and Strukelj (2005). The reagents were: 1. Tris buffer. A total of 100 mmol/L Tris–HCl (pH: 6.8) was prepared to prepare 5 mmol/L $CaCl_2$ and 1 mmol/L Na_2EDTA; 2. Porcine pancreatic lipase solution. A total of 10 mmol/L of MOPS solution was added to porcine pancreatic lipase to form a suspension so that the concentration will be approximately 1000 unit/mL; 3. Enzyme solution. To each 30 μL of porcine pancreatic lipase solution, 850 μL of Tris buffer was added; 4. Substrate solution. The 4-nitrophenyl butyrate was dissolved by adding *N*,*N*-dimethylformamide so that the concentration would be 40 mmol/L. 5. Solution for test article preparation. The *N*,*N*-dimethylformamide and Tris buffer were mixed at the ratio of 1:4 and used as the solution for test article preparation. A total of 100 μL of the sample (n = 2) and 880 μL of enzyme solution was added to the microplate, and then incubated for 15 min at 37 °C. After incubation was complete, 20 μL of the substrate solution were added to commence the enzyme reaction at 37 °C. After the enzyme reaction commenced, the absorbance was measured every minute at 405 nm for 15 min continually. The results obtained were processed using MikroWin Lite 2000 Advance II Version 4.41 (Mikrotek Laborsysteme GmbH, Overath, Germany), Microsoft Excel 2003 or 2007 (Microsoft Corporation, Redmond, WA, USA).

The inhibition rate of each test article on the pancreatic lipase activity was calculated for each concentration using the following formula:

$$\text{Inhibition rate} = (\%)\,(1 - \text{Mean absorbance after 15 min of enzyme reaction with the addition of each test article Mean absorbance after 15 min of enzyme reaction for control}) \times 100.$$

2.2. *Weight management study with* C. forskohlii

The study protocol was approved by the IRB of Miyawaki Orthopedics Clinic at Miyawaki Seika Hospital, 3-1-6 Ariake-cho, Eniwa-shi, Hokkaido, Japan.

2.2.1. *Selection of subjects*

Male and female healthy overweight volunteers with 20 to less than 70 years of age were explained the objectives of the study in an informed consent document and agreed to participate in this study. The subjects had BMI of 25 and no higher than 30, not undergoing treatment for any medical condition, and meeting the inclusion criteria in prior screening, i.e. interview by the physician, physical examination and laboratory data. As a result of this selection, 14 subjects participated in the study.

2.2.2. *Test diet*

Four capsules per day were orally administered 30 minutes before meals, with 2 capsules taken in the morning and 2 at night (on the days of hospital visits, the capsules were administered after completion of examinations). Capsules were: two-piece hard gel capsules containing 250 mg *C. forskohlii* (10%) per capsule: 250 mg stored at room temperature; two-piece hard gel capsules containing maltodextrins matching appearance of test capsules and stored at room temperature.

The standardized botanical extracts used in the study were 1) *C. forskohlii* (Briq.) which was root extract standardized for 10, 40 and 98% forskolin; 2) *S. reticulata* (Wight.) which was root and leaves extract standardized for 10% polyphenols (total of gallocatechin gallate, catechin gallate and mangiferin) by high performance liquid chromatography (HPLC) method (an HPLC curve is shown in Supplementary Fig. S1); 3) white sesamin extract (70%) (*S. indicum* L.) which was seed extract standardized for 70% sesamin.

2.2.3. *HPLC analysis*

The *S. reticulata* extract was analyzed by HPLC using Shimadzu liquid chromatograph (Kyoto, Japan) with Shimadzu Shim-Pack C18 column (5 μm, 4.6 mm × 250 mm). The mobile phase consisted of Solution A (methanol, phosphoric acid 85% and water in 50:3.5:946.5) and Solution B (acetonitrile and methanol in 95:5) at a flow rate of 0.8 mL/min, with its absorbance detected at 278 nm. The linear gradient program in 90 minutes was used.

2.2.4. *Clinical protocol*

The subjects selected for the study were divided into two groups and given study diet and placebo capsules for 6 weeks continuously. The health status of study subjects and compliance with the regimen were monitored by the physician conducting the study at the time of the hospital visit, using the diet questionnaire and checking the intake records. The study group was evaluated at the baseline and after completion of the study. Physical examination included: height, body weight, body fat

percentage, BMI, body temperature, blood pressure and pulse, waist circumference, hip circumference, skinfold thickness (measurement at the bottom margin of the scapula on the back and the middle of the extension side of the upper arm, according to the caliper method). Measurement of abdominal fat was conducted with computerized tomography (CT): The area of fat (subcutaneous fat, visceral fat, and total fat) was measured at the umbilical cross-section for 3 subjects in the test diet group (2 males and 1 female) (Supplementary Fig. S2). Blood biochemistry included: Total protein, albumin, total bilirubin, AST (GOT), ALT (GPT), ALP, LDH, γ-GTP, T-CHO, HDL-C, TG, uric acid, urea nitrogen, creatinine, Na, Cl, K, and fasting blood glucose. Urine test included: Protein, sugar, urobilinogen, bilirubin, specific gravity, pH, ketone bodies, and occult blood.

2.2.5. *Diet and exercise*

Each subject recorded the intake of food and drugs that may affect the test diet and body lipid profile; the amount of exercise was tracked by pedometer.

2.2.6. *End points statistical analysis*

For all data obtained in the study, the mean and standard deviation were calculated for each group. The main endpoints were set as body weight and body fat percentage, and the mean values were compared from before and after administration using a paired t-test with a significance level of 5%. Furthermore, the comparison between the 2 study diets was tested for equality of variance using the F-test. If the variance was found to be equal, Student's t-test was used for comparison of mean values, and if the variance was not equal, Welch's t-test was used.

3. Results

3.1. *The biological properties of* C. forskohlii, S. reticulata *and* S. indicum *inhibiting pancreatic lipase* in vitro

The *in vitro* study evaluated three components of botanical formula i.e. *C. forskohlii*, *S. reticulata* and *S. indicum* extracts for their properties inhibiting pancreatic lipase activity (Table 1). All three extracts showed different degree and dynamics of pancreatic lipase activity inhibition. The stand-alone *C. forskohlii* extract (98%) in final concentrations of 0.1, 1, 10 and 100 μg/mL inhibited pancreatic lipase by 4.8, 4.9, 7.4, and 74.5%; 40% extract inhibited pancreatic lipase by 7.1, 8.4, 16.1, and 42.7%, respectively. *S. reticulata* extract (10%) in final concentrations 0.1, 1, 10 and 100 μg/mL inhibited pancreatic lipase by 2.9, 3.4, 16.7, and 53.6% respectively. *S. indicum* extract (70%) in final concentrations 0.1, 1, 10 and 100 μg/mL inhibited pancreatic lipase by 7.2, 15.1, 16.0, and 17.5% respectively.

In the *in vitro* experiments, when biologically inactive dose of 1 μg/mL of *S. reticulata* extract was combined with the suboptimal dose of 10 μg/mL of *C. forskohlii* (98%) extract, that resulted in synergistically enhanced 20.7% inhibition of pancreatic lipase activity (Table 2) compared to only 10.8% inhibition calculated from addition of % inhibition values for *C. forskohlii* (98%) extract and *S. reticulata* stand-alone (Table 1). Based on the described synergistic action of *C. forskohlii* and *S. reticulata* the two compounds of the botanical formula may work in pull and push mechanism preventing tachyphylaxis, or the repeated use, time-dependent, decrease of biological activity and age related resistance to the mechanism of *C. forskohlii*.

Table 1 – *In vitro* dose dependent inhibition of pancreatic lipase by botanical extracts stand alone and Tetrahydrolipstatin (Orlistat ®).

Group	Final conc. (μg/mL)	Inhibition ratio (%)
Control	–	–
Coleus forskohlii Extract 98%	0.1	4.8
	1	4.9
	10	7.4
	100	74.5
Coleus forskohlii Extract 40%	0.1	7.1
	1	8.4
	10	16.1
	100	42.7
Salacia reticulata Extract	0.1	2.9
	1	3.4
	10	16.7
	100	53.6
Sesamin Extract 70%	0.1	7.2
	1	15.1
	10	16.0
	100	17.5
Tetrahydrolipstatin	1	82.5
	10	91.8

On the other hand, *S. indicum* in the formula may play a dual role in its biological action on pancreatic lipase activity. The stand-alone *S. indicum* extract has shown different dynamics inhibiting pancreatic lipase depending on the low or high dose of the extract. In a low dose range, 0.1 and 1 μg/mL, *S. indicum* outperforms *C. forskohlii* and *S. reticulata* in inhibiting pancreatic lipase activity. However, with the increased dose i.e. 10 and 100 μg/mL, *S. indicum* gradually becomes less biologically effective inhibiting pancreatic lipase compared to the equivalent doses of stand-alone *C. forskohlii* or *S. reticulata*.

Interestingly, addition of 1 μg/mL *S. indicum* extract (70%) to 10 μg/mL *C. forskohlii* (98%) extract resulted in only 9.6% inhibition of pancreatic lipase (Table 2), contrary to the 22.5% inhibition calculated mathematically from sum of % inhibition afforded by either standalone compounds (Table 1). The above described biological response modifying action of *S. indicum* extract upon action of *C. forskohlii* indicates an important mechanism of the botanical composition which may harness the potential un-physiological inhibition of pancreatic lipase activity by the composition. Therefore *S. indicum* extract may exert dual action in the botanical composition enhancing pancreatic lipase inhibition but also preventing potential side effects of excessive pancreatic lipase inhibition of the formula. *S. indicum* works as a biological response modifier in a dose dependent manner controlling pancreatic lipase mechanism (Table 2). Tetrahydrolipstatin (Orlistat) was evaluated in the *in vitro* study to compare with three botanical extracts for the potential to inhibit pancreatic lipase. The inhibitory action of tetrahydrolipstatin on pancreatic lipase was approximately 5 to 10 fold stronger than the tested botanical extracts (Table 2).

Table 2 – *In vitro* inhibition of pancreatic lipase by combining botanical extracts.

Group	Inhibition ratio (%)
Control	–
Coleus forskohlii Extract 40% (10 μg/mL) + White Sesamin Extract 70% (1 μg/mL)	14.0
Coleus forskohlii Extract 40% (10 μg/mL) + Salacia reticulata Extract (1 μg/mL)	23.2
Coleus forskohlii Extract 98% (10 μg/mL) + White Sesamin Extract 70% (1 μg/mL)	9.6
Coleus forskohlii Extract 98% (10 μg/mL) + Salacia reticulata Extract (1 μg/mL)	20.7
White Sesamin Extract 70% (10 μg/mL) + Salacia reticulata Extract (1 μg/mL)	15.8

OD_0: These values were measured before the enzyme reaction proceeded (t = 0).
OD_{15}: These values were measured at the enzyme reaction was finished (t = 15).
Inhibition ratio (%) was calculated from the following formula:

$$\text{Inhibition ratio (\%)} = \left(1 - \frac{\text{Mean of } \Delta\text{OD after the sample was applied}}{\text{Mean of } \Delta\text{OD in Control}}\right) \times 100$$

3.2. *Clinical evaluation of* C. forskohlii *in body weight management*

C. forskohlii stand alone was clinically evaluated primarily for its effects on body fat and blood lipids. *C. forskohlii* extract standardized for 10% diterpene forskolin, has been evaluated in a 6 week clinical trial with 7 male volunteers, average age 41 ± 4 years old, body weight 77.5 ± 11.4, BMI 27.2 ± 0.9, and 7 female volunteers, average age 36 ± 9 years old, body weight 75.6 ± 7.3 kg and BMI 27.5 ± 1.4. The study subjects were randomized and received either 250 mg × 2 capsules of 10% *C. forskohlii* or 250 mg × 2 capsules of matching placebo 30 minutes before breakfast, and 30 minutes before a dinner. Total daily intake of *C. forskohlii*/placebo was 1000 mg (4 capsules) for six weeks. There was no dietary or life style modification in the course of the study. The six week administration of *C. forskohlii* diet or control (placebo) diet showed no objective or subjective side effects in the course of the study (Table 3).

3.2.1. *Percent body fat*

The percent of body fat for the *C. forskohlii* group was statistically significantly lower at the completion of administration compared to the baseline values ($p < 0.05$) (Table 3). The percent of body fat for the placebo group was statistically significantly higher ($p < 0.05$) (Table 3) at the end of administration compared to the baseline values. The percent body fat in *C. forskohlii* group at completion of the study (6 weeks) as compared to the percent body fat in placebo group at completion of the study (6 weeks) was statistically significantly lower ($p < 0.05$). The percent body fat in *C. forskohlii* group pre-administration was 34.1 ± 4.2 and post-administration 33.5 ± 4.4 vs. placebo pre-administration 35.0 ± 6.6 and post-administration 35.9 ± 6.5. The fat content change measured with computerized tomography in various anatomic areas was evaluated selectively in 4 subjects from *C. forskohlii* test diet group before and after 6 weeks of the regimen. The whole body fat and central abdominal fat were diminished with no change in the subcutaneous fat at the completion of the study compared to the baseline values (Table 4).

3.2.2. *Body weight*

C. forskohlii administration for 6 weeks did not affect body weight significantly, however numerically *C. forskohlii* group showed lower weight at the end of 6 weeks and in placebo group the numerical weight was increased at the end of 6 weeks as compared to the respective baseline values. The *C. forskohlii* group pre-trial weight was 77.5 ± 12.0 kg and post-trial weight 76.6 ± 12.1 kg; placebo pre-trial weight was 75.5 ± 7.2 kg and post-trial 76.0 ± 7.4 kg (Table 3).

3.2.3. *Waist circumference*

Waist circumference in *C. forskohlii* group was numerically lower compared to the baseline values, i.e. pre-trial was 93.9 ± 7.8 cm

Table 3 – Body measurement and physical parameters before and after 6 weeks of test diet vs. placebo regimen.

Item	Study diet	At commencement	At completion
Body weight (kg)	Test diet	77.5 ± 12.0	76.0 ± 12.1
	Control diet	75.5 ± 7.2	76.0 ± 7.4
Body fat (%)	Test diet	34.1 ± 4.2	33.5 ± 4.4*
	Control diet	35.0 ± 6.6	35.9 ± 6.5*
BMI	Test diet	27.2 ± 1.0	26.9 ± 1.0
	Control diet	27.5 ± 1.4	27.7 ± 1.6
Waist (cm)	Test diet	93.9 ± 7.8	93.2 ± 8.0
	Control diet	92.1 ± 7.1	92.4 ± 7.1
Hip (cm)	Test diet	101.0 ± 2.9	100.1 ± 3.0
	Control diet	100.2 ± 3.4	100.4 ± 3.3

N = 7 of subjects in test diet group and 7 subjects in control diet group. Data are presented as mean ± SD.
* $p < 0.05$, significant difference compared to the baseline and between the group values by Paired-t test.

Table 4 – Evaluation of body fat area (cm^2) by computerized tomography in the *C. forskohlii* study group diet.

Item	At commencement	At completion	p-value[a]
Total fat	345.2 ± 59.7	330.1 ± 37.6	0.370
Subcutaneous fat	212.9 ± 55.5	215.2 ± 45.3	0.854
Visceral fat	132.3 ± 95.6	114.9 ± 73.5	0.355
Changes in total fat	–	–15.1 ± 22.8	
Changes in subcutaneous fat	–	2.3 ± 19.4	
Changes in visceral fat	–	–17.4 ± 25.3	

n = 3 (3 subjects from the test diet group). Data are presented as mean ± SD.
[a] *p*-value in paired-t test.

and post-trial 92.2 ± 8.0 cm. In the placebo group there was a numerical increase in the circumference at the end of trial compared to baseline values, i.e. pre-trial waist circumference was 92.1 ± 7.1 cm and post-trial was 92.4 ± 7.1 cm. Similar pattern of change to that of waist circumference was recorded in hip circumference, i.e. the *C. forskohlii* group pre-trial was 101.0 ± 2.9 cm and post-trial 100.1 ± 3.0 cm; in placebo group pre-trial hip circumference was 100.2 ± 3.4 cm and post-trial 100.4 ± 3.3 cm (Table 3).

3.2.4. *Blood lipids*

The blood lipid levels showed trends in *C. forskohlii* and placebo receiving groups, i.e. in the *C. forskohlii* group total cholesterol decreased from baseline value of 213 ± 28 mg/dL to post-trial value of 200 ± 15 mg/dL. In the placebo receiving group total cholesterol slightly decreased from the baseline value of 221 ± 35 mg/dL to post-trial value of 218 ± 38 mg/dL. In the *C. forskohlii* receiving group there was no change in high density lipoprotein levels compared to the baseline. In the placebo receiving group there was a statistically significant decrease in high density lipoprotein levels from the baseline 61 ± 7 mg/dL to post-trial 58 ± 7 mg/dL ($p < 0.05$). The *C. forskohlii* triacylglycerol levels decreased from pre-trial value of 137 ± 69 mg/dL to post-trial value of 120 ± 74 mg/dL. In the placebo receiving group triacylglycerol levels increased from the baseline value of 98 ± 33 mg/dL to post-trial value of 109 ± 54 mg/dL (Table 5).

3.2.5. *Blood chemistry and urine analysis*

The blood biochemistry and urine analysis values in *C. forskohlii* and placebo receiving groups were not clinically and statistically different from the baseline values and between the groups.

3.2.6. *Caloric intake*

The effects of 6 week administration of *C. forskohlii* on caloric intake of food was compared to the placebo group. In the placebo group caloric intake was increased by approximately 700 kcal by the end of 6 weeks, whereas in the *C. forskohlii* group caloric intake was decreased by approximately 300 kcal by the end of 6 weeks (Table 6). The difference between the baseline and end of the study (6 weeks) in caloric intake for *C. forskohlii* groups was statistically significant ($p < 0.05$). The statistically significant decrease in caloric intake with *C. forskohlii* as compared to the baseline may indicate a satiety effect with the herb. By comparison current generation of pancreatic lipase inhibitors, i.e. tetrahydrolipstatin shows tendency to increase levels of appetite (Ellrichmann et al., 2008).

The *in vitro* results show for the first time *C. forskohlii* inhibition of pancreatic lipase activity and the clinical effects of decreased total body fat with the herb and its satiety effects may support previous literature reporting application of *C. forskohlii* in body weight management (Badmaev et al., 2002; Godard et al., 2005).

4. Discussion

Excess dietary fatty acids from an unbalanced diet and overeating gradually overwhelm and incapacitate hepatocytes in obese patients. Their mitochondrial fatty acid beta-oxidation for energy is impaired. This biochemical burden to hepatocytes is further aggravated by the oxidative stress due to unbalanced nutrition and low antioxidant capacity. Over time the biochemical and oxidative stress to hepatocytes and the liver leads to the development of insulin resistance. Insulin

Table 5 – Results of blood lipid tests in *C. forskohlii* test diet vs. control (placebo) diet.

Item	Reference values	Study diet	At commencement	At completion
T-Cho (mg/dL)	150–219	Test diet	213 ± 28	200 ± 15
		Control diet	221 ± 35	218 ± 38
HDL-C (mg/dL)	Male: 40–80; Female: 40–90	Test diet	57 ± 15	53 ± 14
		Control diet	61 ± 7	58 ± 7*
TG (mg/dL)	50–149	Test diet	137 ± 69	120 ± 74
		Control diet	98 ± 33	109 ± 54

N = 7 of subjects in each group. Data are presented as mean ± SD.
* $p < 0.05$, significant difference compared with baseline by Paired-t test.

Table 6 – Appetite effect measured by calorie intake in *Coleus forskohlii* test diet and placebo receiving subjects for 6 weeks.

Study groups	PRE (kcal)	POST (kcal)
Coleus forskohlii	5519 ± 1341	5222 ± 1301*
Placebo	5827 ± 1535	6527 ± 1210

N = 7 of subjects in each group. Data are presented as mean ± SD.
* $p < 0.05$, significant difference compared with baseline by Paired-t test.

resistance is related to elevated blood sugar and the hormone insulin, increased free fatty acids and their metabolites, metabolic and oxidative stress to hepatocytes and the liver, inefficient fat metabolism and the development of obesity and other comorbid conditions including pre-diabetes, diabetes and metabolic syndrome.

Addressing excessive dietary fat absorption and accumulating dietary liver fat as a cause of overweight and obesity has been increasingly discussed in the literature. The search for natural safe and effective food supplements preventing excessive dietary fat absorption including *C. forskohlii*, *S. reticulata* and *S. indicum* extracts combined with a healthy diet and active life style can lead to a sensible weight-loss strategy.

The *C. forskohlii* stand-alone test diet statistically significantly lowered body fat and resulted in decreased appetite as assessed by statistically significantly decreased caloric intake in the 6 week clinical study as compared to placebo receiving group. It is possible that *C. forskohlii* decreases levels of appetite regulating postprandial hormonal substance ghrelin associated with feeding and increased food intake and produced by endocrine cells in the gastric mucosa and pancreas.

As previously discussed the efficacy of available pancreatic lipase inhibitors, i.e. tetrahydrolipstatin tend to decrease with prolonged use and in addition may also increase levels of appetite, thus diminishing their weight loss potential (Ellrichmann et al., 2008).

In the current *in vitro* study *C. forskohlii* extract has been shown for the first time to inhibit pancreatic lipase activity. In addition in the clinical study, the daily intake of 1000 mg *C. forskohlii* standardized for 10% forskolin for a relatively short period of 6 weeks showed statistically significant lowering of total body fat as compared to the baseline and placebo group. The computerized tomography performed only on 4 subjects in the *C. forskohlii* test diet group showed decreased total body fat and abdominal fat in comparison to the baseline. These effects of stand-alone *C. forskohlii* have been emphasized by the *in vitro* synergy between *C. forskohlii* and *S. reticulata* inhibiting pancreatic lipase. The synergy between the two compounds is potentially important due to reported decrease of *C. forskohlii* weight loss potential with aging and depending on gender of the population, with male population being less responsive to the mechanism of *C. forskohlii* (Badmaev, 2011 personal communication). The addition of white *S. indicum* extract to the formula has been found to synergistically assist inhibition of the pancreatic lipase in a lower dose range, while moderating the pancreatic lipase inhibition in a higher dose range. This dual mechanism of *S. indicum* has been postulated as a safety mechanism preventing any potential side effects resulting from excessive inhibition of pancreatic lipase activity. Interestingly *S. reticulata* of the discussed three herb formula has well known anti-diabetic properties by inhibiting alpha-glucosidase, an enzyme which facilitates gastrointestinal absorption of dietary carbohydrates (Shivaprasad et al., 2013).

The results of current clinical study support the role of *C. forskohlii* in diminishing excessive body fat, with no objective and/or subjective side effects reported in the course of daily administration of 1000 mg *C. forskohlii* for 6 weeks. The *in vitro* study of the *C. forskohlii*, *S. reticulata* and *S. indicum* extract supports the rationale for three herb formula and its synergistic and complementary activity. The formula may provide safe and effective regimen preventing gastrointestinal absorption and accumulation of excess dietary fat, lowering appetite levels and decreasing total and abdominal fat potentially preventing obese and overweight conditions.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2015.05.027.

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