**Title**
Composition for physiological increase of male and female hormones with diterpene forskolin and its derivatives

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Abstract

A composition for physiological increase of male and female hormones, e.g. testosterone, estrogen and HGH in an individual is disclosed, comprising an effective amount of forskolin and or its derivatives. Compositions suitable for the invention are also disclosed, comprising about 1 to up to 100% forskolin and or its derivatives in combination with at least one physiologically acceptable carrier or excipient. A method of preparing a forskolin and its derivatives from Coleus forskohlii plant is further disclosed, as well as a forskolin and its derivatives compositions prepared by the method.
AUSTRALIA
PATENTS ACT, 1990

COMPLETE SPECIFICATION

FOR A STANDARD PATENT

ORIGINAL

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Actual Inventors: MAJEED, Mohammed and BADMAEV, Vldimir
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Invention Title: COMPOSITION FOR PHYSIOLOGICAL INCREASE OF MALE AND FEMALE HORMONES WITH DITERPENE FORSKOLIN AND ITS DERIVATIVES

The following statement is a full description of this invention, including the best method of performing it known to us.
SPECIFICATION

Composition for physiological increase of male and female hormones with diterpene forskolin and its derivatives

BACKGROUND OF THE INVENTION

Most weight loss pharmaceutical compositions and nutraceutical aids are designed to decrease the amount of body fat in an individual by decreasing the individual's appetite for food, decreasing the amount of food absorption in the individual, slowing down the rate of fatty acid synthesis within the body, or increasing the rate of catabolism of fatty acids. The following are some examples of weight loss products and their mechanisms.

Dexfenfluramine increases the brain levels of serotonin, a neurotransmitter and neurohormone that quells the appetite. Sibutramine also increases the levels of serotonin, as well as noradrenaline, and works to quell the appetite. Neuropeptide Y inhibitors curb the appetite, as well as stimulating the body to burn more sugars and less fat.

Bromeriptine mimics the neurotransmitter dopamine, and may reduce blood sugar and fat production by the liver. Leptin, a hormone generated by adipocytes, affects the hypothalamus. Cholecystokinin, a hormone and neurotransmitter, acts to reduce appetite. Butabindide blocks an enzyme that inactivates cholecystokinin. Orlistat interferes with pancreatic lipase, which results in poor absorption of dietary fat. Insulinotropin is a glucagon-like hormone which prevents obesity by slowing down the emptying of the stomach. Bta-243 stimulates beta-adrenergic receptors on adipocytes, with a resulting increase in the burning of fatty acids. Troglitazone is a synthetic hormone which signals muscle cells to utilize fat for energy, rather than sugars. Cytokine regulators change the activity of hormone-like cytokines and alter the communication among cells, resulting in weight loss. Hydroxycitric acid acts as an inhibitor of enzyme citrate lyase, which subsequently slows down the synthesis of fatty acids and increases the rate at which fatty acids are burned.

The average amount of body fat in the American male is 22 to 25%, and in the American female, the average amount of fat is 33 to 35%. These values are far above optimal values, which are 15 to 19% for 20-29 year old males and 19 to 23% for 20-29 year old females.
females. Corresponding values for 40-49 year olds are 17 to 21% and 21 to 25%, respectively; and for 60 year olds, the corresponding values are 19 to 23% and 23 to 27%, respectively. In highly overweight individuals, fat tissue can constitute up to 70% of body weight.

The remaining percentage of body composition corresponds to the lean body mass. Lean body mass is composed of muscle, vital organs, bone, connective and other non-fatty tissues in the body, and most of the body water. The body's metabolic rate is in direct proportion to the amount of lean body mass. Therefore, considering the lean body mass is important for any weight loss strategy.

The aforementioned weight control means do not take into account the importance of maintaining or increasing the lean body mass in the process of weight loss. In fact, regimens to decrease body fat often contribute to the catabolic wasting of lean body mass. Increased lean body mass regulates body metabolism and helps in losing weight, as well as maintaining the accomplished weight reduction. On the other hand, diminished lean body mass slows down body metabolism and results in difficulties in maintaining an appropriate, healthy body weight. Thus, an ideal weight management approach should be to reduce body weight to acceptable levels by restoring the optimal proportions of fat to lean body mass. By maintaining or increasing the lean body mass while simultaneously reducing body fat, the weight loss regimen would serve the general purpose of improving the overall health of the individual.

Maintaining or increasing the lean body mass (for example, skeletal muscles) is one of the important considerations for any weight loss strategy because lean body mass determines the rate of metabolism and the body's thermogenic response to food, and food induced thermogenesis and the metabolic rate, in turn, control body weight by an increase in the catabolisim of body fat. This is so because thermogenesis is preferentially fueled by fatty acids derived from stores of body fat and from food. In addition, a high rate of thermogenesis contributes to more food being absorbed and to the preferential build-up of lean body mass, rather than adipose tissue.
One of the critical components of lean body mass maintenance and prevention of body composition decline is having a sufficient supply of endogenous testosterone, estrogen and human growth hormone. Testosterone and estrogens are the end products of a number of hormonal reactions in somatotroph axis. For example, the somatotroph axis for testosterone starts with gonadotropin-releasing hormone (GnRH) which is secreted by the hypothalamus and controls the pulsatile secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary. Luteinizing hormone regulates the production and secretion of testosterone by the Leydig cells of the testes, and FSH stimulates spermatogenesis.

Aging is associated with decline in the somatotroph axis, decline in production of estrogen and testosterone, increase of detrimental forms of hormones e.g. dihydrotestosterone (DHT) – events that have been considered to cause many of the catabolic sequelae of normal aging. For example, loss of bone mass (osteoporosis) with age is an example of loss of lean body mass and results in deterioration in body composition in geriatric individuals. Decreases in GH (growth hormone(s), exemplified but not limited to IGF-I, HGH) secretion may partially explain the age-related changes in metabolism, bones, muscles, cardiovascular system, central nervous system, the immune system and sense of well-being. Owing to clinical similarities between aging and growth hormone(s) deficiency, the relative GH insufficiency of elderly subjects has been postulated as one important factor contributing to their frailty.

Another example of detrimental changes in body composition is in low functions of gonads. Males with hypogonadism and low levels of serum testosterone have negative alterations in body composition, such as decreased muscle mass, increased percentage of body fat, and alterations in body fat distribution. Independently of hypogonadism decreased lean body mass, increased body fat and total body weight contribute to hormonal imbalance and lower levels of serum testosterone. In addition serum levels of testosterone and estrogen correlate negatively with the levels of serum cortisol, especially in overweight and obese individuals.
The invention comprises diterpene forskolin obtained from roots of *Coleus Forskohlii, Briq.* (Syn. *C. barbatus* Benth., *Plectranthus barbatus*, Andr. also *P. forskohlii* and Willd., *P. comosus* Willems.), a member of the mint family (Fam. Lamiaceae) and its natural derivatives isoforskolin and 7-deacetylforskolin. Forskolin has been reported to possess antihypertensive activity, positive inotropic effects, and to inhibit platelet aggregation (de Souza *et al.*, 1983; Ammon and Müller, 1985). The mechanism of action of forskolin is thought to be related to its stimulatory action on adenylate cyclase, increasing the intracellular level of cyclic adenosine monophosphate (cAMP), which mediates a number of biological responses (de Souza *et al.*, 1983; Ammon and Müller, 1985; Dohadwalla, 1985; Seamon, 1985). Numerous pharmacological studies on the various effects of forskolin have been conducted in laboratory animals and humans following topical, intravenous (i.v.), intraarterial, intraperitoneal (i.p.), intratracheal (i.t.), and inhalation treatment. Physiological effects of cAMP, which also have been demonstrated by forskolin, include inhibition of platelet aggregation, increased adipocyte lipolysis *in vitro*, positive inotropic effects on heart muscle, potentiation of insulin secretion by the pancreas, increased secretion of thyroid hormones, increased steroidogenesis by the adrenal glands, increased adrenocorticotropic hormone (ACTH) release by the pituitary gland, and decreased intraocular pressure in topical application (Dubey *et al.*, 1981; Caprioli and Sears, 1983; Malaisse and Malaisse-Lagae, 1984; Ammon and Müller, 1985; Dohadwalla, 1985; Potter *et al.*, 1985). The secretion ability of aged rat Leydig cells was found lower than that of young rat Leydig cells with or without forskolin stimulation (Deng *et al.*, 2005). Forskolin also has been reported to induce bronchodilation in guinea pigs (i.v. doses of 1 mL/kg body weight for 10 minutes and i.t. administration of 30 μg) and in asthmatic patients (inhalation of 1 to 10 mg forskolin, as dry powder), with no significant side effects (Lichey *et al.*, 1984; Kreutner *et al.*, 1985; Bauer *et al.*, 1993). Both isoforskolin and forskolin have suppressing effects on ocular hypertension in rabbits (Li *et al.*, 2000). The 1-acety-7-deacetylforskolin Forskolin activated adenylyl cyclase in cultured human endothelial cells, whereas 1-acetyl-7-deacetylforskolin did not (Sasaki *et al.*, 1995). The 7-deacetylforskolin was prepared to provide water-soluble derivatives of the potent cardioactive diterpenoid forskolin (Khandelwal *et al.*, 1988). Forskolin but not isoforskolin or 7-deacetylforskolin has been also shown to increase lean body mass in overweigh and obese human subjects (Majeed, Badmaev, Rajendran US patent 1998).
SUMMARY OF THE INVENTION

Present invention demonstrates for the first time that administration of forskolin orally to overweight and obese young males increases levels of serum testosterone.

The present invention relates to a composition for physiologically increase serum levels of testosterone, estrogen or HGH, which comprises forskolin, isoforskolin or 7-deacetylforskolin.

The present invention further relates to a safe, physiological, method of increasing serum testosterone, estrogen and HGH in prevention of anticipated body composition deterioration as in the process of aging, among those with hormonal deficiencies or among those with high risk of being overweight and obese. A method to improve mental and physical stamina is disclosed. Compositions suitable for the invention are also disclosed, comprising about 1 to about <95% forskolin and or its derivatives in combination with at least-one physiologically acceptable carrier or excipient.

A method of preparing a forskolin and its derivatives from Coleus forskohlii plant is further disclosed, as well as a forskolin and its derivatives compositions prepared by the method.

BEST MODE FOR CARRYING OUT THE PRESENT INVENTION

It is preferable that forskolin, isoforskolin or 7-deacetylforskolin be used those extracted from Coleus forskohlii or related species.

It is preferable that the composition is in a form to be administered in a daily dose of about 10 to about 100 mg. Furthermore, it is preferable that the composition contains about 1 to about 100% forskohlin. Preferred amounts are about 5 to about 20% forskohlin, more preferred about 8 to about 15%, most preferably about 10%.

The composition of the present invention can be used in prevention of body composition deterioration in subjects in need thereof, and in improvement of mental and physical stamina.
Yet another subject matter of the invention is a method of preparing a forskolin composition from a forskolin extract of Coleus forskohlii plant, comprising:

(a) providing forskolin, isoforskolin and 7-diacetoforskolin extract of Coleus forskohlii plant in supercritical extraction process;

(b) providing isoforskolin and 7-diacetoforskolin extracted from Coleus forskohlii plant with water and organic solvents;

(c) preparing a forskolin composition by combining the amount of forskolin obtained in steps (a) and/or (b) with at least one physiologically acceptable carrier or excipient to produce a forskolin composition having a predetermined forskolin content.

The present invention also includes compositions prepared from the above method, as well as methods using the compositions thus prepared.

The present invention further discloses a commercial process for making isoforskolin consisting of: pulverizing dried Coleus roots; extraction with a solvent selected from a mixture of water and alcohol, C1-C4 alcohols, methylene dichloride, toluene, or hexane; Concentration of the extract and precipitation with a non-polar solvent selected from heptane, pentane, hexane; filtration; back extraction with a mixture of water and alcohol; and crystallization in alcohol.

The present invention further discloses a commercial process for making isoforskolin consisting of: pulverizing dried Coleus roots; extraction of the root powder with supercritical carbon dioxide and cosolvent ethanol at a temperature of 45° to 55° C and pressure 300 bar; and crystallization in ethanol.

The present invention further discloses a commercial process for making 7-deacetylforskolin consisting of pulverizing dried Coleus roots; extraction of the root powder with supercritical carbon dioxide and cosolvent ethanol at a temperature of 45° to 55° C and pressure 300 bar; hydrolysis with lipase enzyme; and crystallization in ethanol.
The present invention further discloses a commercial process for super or subcritical extraction of dried Coleus roots to remove essential oil.

The present invention further discloses a commercial process with supercritical carbon dioxide with varying amounts of ethanol present in the supercritical gas. The pressure ranged from 110 bar to 400 bar below the critical temp. Ethanol was used as the solvent modifier. A wide range of composition was used wherein ethanol quantity was altered from 0.01 mol% to 10 mol%.


The randomized, double-blind clinical study of the invention examined oral ingestion of 250 mg of 10% forskolin bid (standardized extract of Coleus forskohlii roots) for 12 weeks in 30 overweight and obese young men (forskolin, n=15; placebo, n=15). The effect of forskolin on body composition, testosterone, metabolic rate, and blood pressure in overweight and obese (BMI > or = 26 kg/m(2)) men. Forskolin was shown to elicit favorable changes in body composition by significantly decreasing body fat percentage (BF%) and fat mass (FM) as determined by DXA compared with the placebo group (p < or = 0.05). Additionally, forskolin administration resulted in a significant increase in bone mass for the 12-week trial compared with the placebo group (p < or = 0.05). There was a trend toward a significant increase for lean body mass in the forskolin group compared with the placebo group (p = 0.097). Serum free testosterone levels were significantly increased in the forskolin group compared with the placebo group (p < or = 0.05). Table 1 and Table 2.
Body Composition and Forskolin Consumption, Godard, Johnson, and Richmond

Table 1. Body composition values including body weight, LJM, and fat mass at each time-point

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Mid</th>
<th>Post</th>
<th>Change (pre - post)</th>
<th>Percent change (pre - post)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forskolin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>100.98 ± 14.89</td>
<td>101.23 ± 15.04</td>
<td>101.91 ± 15.05</td>
<td>-0.07 ± 2.39</td>
<td>-0.08 ± 2.44</td>
</tr>
<tr>
<td>LJM (kg)</td>
<td>65.61 ± 5.94</td>
<td>67.32 ± 8.29</td>
<td>67.32 ± 8.29</td>
<td>0.71 ± 4.07</td>
<td>5.65 ± 6.33</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>37.43 ± 12.65</td>
<td>32.91 ± 11.02*</td>
<td>32.91 ± 11.02</td>
<td>-4.52 ± 5.74*</td>
<td>-11.23 ± 13.20*</td>
</tr>
<tr>
<td>Bone mass (g)</td>
<td>3.41 ± 0.43</td>
<td>3.68 ± 0.43</td>
<td>3.68 ± 0.43</td>
<td>0.28 ± 0.31</td>
<td>8.63 ± 10.46</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>100.95 ± 9.30</td>
<td>102.09 ± 9.75</td>
<td>102.15 ± 9.65</td>
<td>1.20 ± 2.33</td>
<td>1.20 ± 2.33</td>
</tr>
<tr>
<td>LJM (kg)</td>
<td>61.52 ± 6.44</td>
<td>63.39 ± 7.07</td>
<td>63.39 ± 7.07</td>
<td>1.87 ± 2.86</td>
<td>2.96 ± 4.39</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>35.65 ± 9.59</td>
<td>33.14 ± 10.56</td>
<td>33.14 ± 10.56</td>
<td>-0.51 ± 1.91</td>
<td>-1.73 ± 5.64</td>
</tr>
<tr>
<td>Bone mass (kg)</td>
<td>3.41 ± 0.35</td>
<td>3.60 ± 0.51</td>
<td>3.60 ± 0.51</td>
<td>0.20 ± 0.53</td>
<td>7.46 ± 10.78</td>
</tr>
</tbody>
</table>

The actual change from pre- to post-measurement and the percent change are also included.

Body Composition and Forskolin Consumption, Godard, Johnson, and Richmond

Table 2. Total testosterone and free testosterone values at each time-point

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Mid</th>
<th>Post</th>
<th>Change (pre - post)</th>
<th>Percent change (pre - post)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forskolin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total testosterone (ng/mL)</td>
<td>5.06 ± 1.21*</td>
<td>5.27 ± 1.03*</td>
<td>5.75 ± 1.50*</td>
<td>0.69 ± 1.26</td>
<td>10.77 ± 33.77</td>
</tr>
<tr>
<td>Free testosterone (pg/mL)</td>
<td>15.90 ± 13.39</td>
<td>15.67 ± 13.68</td>
<td>16.36 ± 13.32</td>
<td>0.46 ± 0.86*</td>
<td>2.91 ± 8.10</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total testosterone (ng/mL)</td>
<td>4.12 ± 0.82</td>
<td>3.97 ± 0.83</td>
<td>4.00 ± 0.89</td>
<td>-0.11 ± 0.95</td>
<td>-2.68 ± 18.35</td>
</tr>
<tr>
<td>Free testosterone (pg/mL)</td>
<td>13.28 ± 7.26</td>
<td>12.28 ± 7.44</td>
<td>12.77 ± 7.30</td>
<td>-0.51 ± 1.04</td>
<td>-3.81 ± 11.48</td>
</tr>
</tbody>
</table>

The actual change from pre- to post-measurement and the percent change are also included. All values are presented as means ± SD. * Significance between groups (p ≤ 0.05).

REFERENCES

EXAMPLE 1. Clinical effects of oral administration of forskolin in overweight women.

- 9 -
In a double blind and randomized manner, 23 females supplemented their diet with forskolin (250 mg of 10% Coleus forskohlii extract, n=7) or a placebo (n=12) two times per day for 12-wks. Body composition (DEXA), body weight, and psychometric instruments were obtained at 0, 4, 8 & 12 weeks of supplementation. No significant differences were observed in caloric or macronutrient intake. Forskolin tended to mitigate gains in body mass (-0.7±1.8, 1.0±2.5 kg, p=0.10) and scanned mass (-0.2±1.3, 1.7±2.9 kg, p=0.08). Subjects in the forskolin group tended to report less fatigue (p=0.07), hunger (p=0.02), and fullness (p=0.04). Results suggest that forskolin may help mitigate weight gain in overweight females with apparently no clinically significant side effects.

REFERENTIAL EXAMPLE 2. Comparative effect of Isoforskolin, 7-deacetylforskolin and Forskolin extract, and the composition on animal model of obesity. The study was done on Swiss Albino mice (Haffkine's Institute, Mumbai, India), aged between 25-30 weeks, on a total of 84 animals divided into 12 animals groups. The mice under treatment were fed with diet rich in carbohydrates and fats. The diet produced reliable weight gain over controls. Drug treatment was started only when the difference between the body weights of control and the treated mice exceeded 10g. The mice in respective groups, were given a daily fixed dose of Isoforskolin Extract, 10% 7-deacetylforskolin extract and Forskolin extract (1mg/ml) for a period of six weeks, by means of gastric intubation, twice a day. At the end of sixth week, six mice per group were put to sleep after taking body weight and flab measurements. The blood was drawn by cardiac puncture and immediately centrifuged to separate the plasma and analyzed for Cholesterol, tryglycerides, glucose, Thyroxine T3, T4 and TSH levels.

Abdominal fat and thyroid were fixed for histopathology. A separate sample of adipose tissue were collected and analyzed for total lipid content. The remaining mice from each group were continued on the same diet as given during the first six weeks of treatment. This group was maintained in this manner for additional six weeks at the end of which non-invasive parameters such as body weight and flab were once again assessed.
Results: Both control and treatment mice tolerated the procedure of gastric intubation throughout the six week treatment period. The extracts 10% isoforskolin, 10% 7-diacetoforskolin and 10% forskolin were well tolerated by the population of mice.

At the end of six weeks, six animals from each group, randomly selected, put to sleep and dissected to (a) excise adipose tissue and thyroid for histopathology (b) examine general anatomy and noting the changes, if any, in organs such as gut, heart, lungs, liver, pancreas, kidney, kidneys, reproductive organs etc. It was observed that none of the animals exhibited any abnormal anatomical features.

The abdominal fat shows interesting features in different groups: for example the control group mice under placebo or drug treatment showed good amount of abdominal fat distribution in bilateral lobes of adipose tissue. The animals from obese placebo group had significantly large quantity of abdominal fat with bilateral adipose tissue lobes extending into more than one third of the abdominal cavity. On the other hand, obese group animals treated with 10% isoforskolin, 10% 7-diacetylforskolin and 10% forskolin had practically exhausted the abdominal fat which was more pronounced in 10% forskolin and 10% isoforskolin group. In these groups of animals, there were significant loss of peritoneal fat as well as the fatty deposition in close association with uteri. Adipose tissue close to the kidney and ovary appeared to be adequate, though less in comparison with obese group under placebo treatment.
Table 3. Effect of treatment on the weekly record of body weights of experimental mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control-Placebo</th>
<th>Obese-Placebo</th>
<th>Obese-Extract 1</th>
<th>Obese-Extract 2</th>
<th>Obese-Extract 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25.23 ± 2.80</td>
<td>38.69 ± 3.71</td>
<td>37.18 ± 3.10</td>
<td>38.11 ± 3.51</td>
<td>37.33 ± 4.02</td>
</tr>
<tr>
<td>0 Week</td>
<td>26.26 ± 4.9</td>
<td>37.73 ± 2.28</td>
<td>34.93 ± 3.05</td>
<td>35.08 ± 3.56</td>
<td>36.73 ± 3.67</td>
</tr>
<tr>
<td>1 Week</td>
<td>26.41 ± 2.28</td>
<td>37.36 ± 2.29</td>
<td>34.39 ± 3.11</td>
<td>35.26 ± 3.88</td>
<td>34.82 ± 3.67</td>
</tr>
<tr>
<td>2 Week</td>
<td>26.18 ± 3.22</td>
<td>37.18 ± 2.27</td>
<td>33.00 ± 4.09</td>
<td>34.36 ± 4.11</td>
<td>34.58 ± 3.11</td>
</tr>
<tr>
<td>3 Week</td>
<td>26.90 ± 2.27</td>
<td>35.84 ± 2.69</td>
<td>31.88 ± 4.09</td>
<td>32.15 ± 3.91</td>
<td>32.67 ± 3.57</td>
</tr>
<tr>
<td>4 Week</td>
<td>27.4 ± 3.26</td>
<td>35.5 ± 3.68</td>
<td>31.69 ± 4.68</td>
<td>31.87 ± 4.81</td>
<td>32.21 ± 3.87</td>
</tr>
<tr>
<td>5 Week</td>
<td>28.33 ± 2.64</td>
<td>35.6 ± 3.10</td>
<td>28.61 ± 5.78**</td>
<td>30.78 ± 6.90**</td>
<td>31.54 ± 4.78**</td>
</tr>
<tr>
<td>6 Week</td>
<td>±12</td>
<td>±0.003</td>
<td>-20.37</td>
<td>-14.76</td>
<td>-15.51</td>
</tr>
</tbody>
</table>

** Significant reduction in weight by One Way Analysis of Variance (ANOVA)

Extract 1: Contains 10% Forskolin
Extract 2: Contains 10% 7-diacetoforskolin
Extract 3: Contains 10% Isoforskolin

As seen in the above table, there is significant reduction in the total body weight in all the treatment group, the percentage change being maximum in Forskolin group.

Table 4. Effect of treatment on the weekly record of Abdominal flab of experimental mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control-Placebo</th>
<th>Obese Placebo</th>
<th>Obese-Extract 1</th>
<th>Obese-Extract 2</th>
<th>Obese-Extract 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.81 ± 0.12</td>
<td>1.24 ± 0.12</td>
<td>1.42 ± 0.15</td>
<td>1.38 ± 0.14</td>
<td>1.35 ± 0.16</td>
</tr>
<tr>
<td>0 Week</td>
<td>0.91 ± 0.09</td>
<td>1.39 ± 0.08</td>
<td>1.27 ± 0.12</td>
<td>1.26 ± 0.17</td>
<td>1.30 ± 0.14</td>
</tr>
<tr>
<td>1 Week</td>
<td>0.92 ± 0.10</td>
<td>1.32 ± 0.09</td>
<td>1.20 ± 0.14</td>
<td>1.22 ± 0.15</td>
<td>1.23 ± 0.14</td>
</tr>
<tr>
<td>2 Week</td>
<td>0.93 ± 0.09</td>
<td>1.33 ± 0.07</td>
<td>1.11 ± 0.11</td>
<td>1.16 ± 0.12</td>
<td>1.28 ± 0.10</td>
</tr>
<tr>
<td>3 Week</td>
<td>0.94 ± 0.11</td>
<td>1.26 ± 0.11</td>
<td>0.99 ± 0.11</td>
<td>1.16 ± 0.12</td>
<td>1.17 ± 0.10</td>
</tr>
<tr>
<td>4 Week</td>
<td>0.97 ± 0.12</td>
<td>1.11 ± 0.09</td>
<td>0.91 ± 0.07</td>
<td>1.08 ± 0.13</td>
<td>1.10 ± 0.09</td>
</tr>
<tr>
<td>5 Week</td>
<td>0.97 ± 0.12</td>
<td>1.11 ± 0.12</td>
<td>0.91 ± 0.07</td>
<td>0.88 ± 0.11</td>
<td>0.94 ± 0.09</td>
</tr>
<tr>
<td>6 Week</td>
<td>0.97 ± 0.12</td>
<td>±0.003</td>
<td>0.82 ± 0.007</td>
<td>±0.08</td>
<td>±0.09**</td>
</tr>
<tr>
<td>% Change</td>
<td>+22.22</td>
<td>-13.00</td>
<td>-42.22</td>
<td>-34.81</td>
<td>-38.41</td>
</tr>
</tbody>
</table>

** Indicates statistical significance in a One Way Analysis of Variance (AVOVA)

Extract 1: Contains 10% Forskolin
Extract 2: Contains 10% 7-diacetoforskolin
Extract 3: Contains 10% Isoforskolin

As seen in the above table, there is significant reduction in the abdominal flab in all the treatment group, the percentage change being maximum in Forskolin group.
Table 5. Effect of treatment in adipose tissue and fat content of experimental mice after six week treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average weight of Adipose Tissue</th>
<th>Average Volume (ml)</th>
<th>Average Weight of Fat (g)</th>
<th>Specific Density of Adipose Tissue</th>
<th>Specific Density of Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-Placebo</td>
<td>0.18±0.09</td>
<td>0.3±0.14</td>
<td>0.12±0.05</td>
<td>0.61</td>
<td>0.4</td>
</tr>
<tr>
<td>Obese Placebo</td>
<td>0.29±0.10</td>
<td>0.4±0.14</td>
<td>0.21±0.07</td>
<td>0.73</td>
<td>0.52</td>
</tr>
<tr>
<td>Obese-Extract 1</td>
<td>0.18±0.13</td>
<td>0.4±0.2</td>
<td>0.12±0.09</td>
<td>0.45</td>
<td>0.30</td>
</tr>
<tr>
<td>Obese-Extract 2</td>
<td>0.21±0.14</td>
<td>0.4±0.16</td>
<td>0.16±0.06</td>
<td>0.54</td>
<td>0.40</td>
</tr>
<tr>
<td>Obese-Extract 3</td>
<td>0.20±0.15</td>
<td>0.38±0.18</td>
<td>0.13±0.08</td>
<td>0.50</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Extract 1: Contains 10% Forskolin
Extract 2: Contains 10% 7-diacetoforskolin
Extract 3: Contains 10% Isoforskolin

REFERRENTIAL EXAMPLE 3. In Vitro, preclinical and clinical toxicology study of forskolin of the invention (standardized 10% extract of Coleus forskohlii roots).

Acute Toxicity Studies

In an acute toxicity study, male and female Wistar rats were given a single oral dose of 2,000 mg forskolin/kg body weight (Graver, 2000). No deaths occurred; however, diarrhea, soiling of the anogenital area, and wetness of the mouth and anogenital area were reported. No histopathological lesions were observed following necropsy. The LD₃₀ was reported to be >2,000 mg/kg body weight. Earlier studies by de Sousa et al (1983) showed the acute LD₃₀ of forskolin to be 3,100 and 2,550 mg/kg by oral administration and 105 and 92 mg/kg body weight when administered intraperitoneally, in mice and rats, respectively.
Subchronic Toxicity Study in Rats

Groups of 5 Sprague-Dawley rats/sex were administered doses of \( C. \) forskohlii 10% extract of 0, 100, 300, or 1,000 mg/kg body weight/day by gavage for a period of 28 days (Bhide, 2004). Two additional groups of rats (5/sex/group) were administered the extract at doses of 0 or 1,000 mg/kg body weight/day for 28 days, and were observed for a 14-day recovery period thereafter to assess the reversibility of any possible effects. Daily observations of clinical signs and mortality, and weekly measurements of body weight and food consumption were conducted. Ophthalmic examinations were conducted at the beginning and end of the dosing period and at the end of the recovery period. Hematological, biochemical, and urinary parameters were assessed at the end of the dosing and recovery periods. All animals were subjected to necropsy, and histopathological examinations of the organs were conducted. No toxicological effects were observed in any of the measured parameters at any dose. The no-observed-effect level (NOEL) of \( C. \) forskohlii 10% extract was considered to be 1,000 mg/kg body weight/day, the highest dose tested. This NOEL represents an appropriate high multiple (120-fold) of the intended human dose (approximately 8.3 mg/kg body weight/day).

Mutagenicity Study

The invention was reported not to be mutagenic in the bacterial reverse mutation assay with an independent repeat assay using \( S. \) typhimurium strains TA98, TA100, TA1535, and TA1537, and \( E. \) coli strain WP2 uvrA, both in the presence and absence of metabolic activation, at doses of up to 5,000 µg/plate (Wagner and Klug, 2001).

Clinical Studies

A number of clinical studies investigating the efficacy of the invention for weight loss have been conducted and are summarized in Table 1. It is important to note, although efficacy was the primary purpose of these trials, that parameters related to safety were also monitored. No clinically significant interactions were seen in metabolic markers, blood lipids, muscle and liver enzymes, electrolytes, red cells, white cells, hormones (insulin, TSH, T3, and T4), heart rate, blood pressure, or weekly reports of side effects.
<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>Dose of Coleus forskohlii Extract (mg/day)</th>
<th>Study Design</th>
<th>Study Length</th>
<th>Measured Outcome(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 overweight subjects (1 male, 13 female)</td>
<td>250 [25]</td>
<td>Open-field study</td>
<td>12 wk</td>
<td>No significant effects on systolic and diastolic blood pressure or pulse rate. No significant adverse effects.</td>
<td>Tsuguyoshi et al., 2001</td>
</tr>
<tr>
<td>6 overweight women</td>
<td>500 [50]</td>
<td>Open-field study</td>
<td>8 wk</td>
<td>No significant effects on systolic and diastolic blood pressure or pulse rate.</td>
<td>Badmaev et al., 2002</td>
</tr>
<tr>
<td>16 overweight men (8/group)</td>
<td>500 [50]</td>
<td>Randomized, double-blind, placebo-controlled study</td>
<td>8 wk</td>
<td>No significant effects on body weight, heart rate, mean arterial pressure, or systolic and diastolic blood pressure.</td>
<td>Agena, unpublished</td>
</tr>
<tr>
<td>19 women [n=12 (controls); n=7 (test)]</td>
<td>500 [50]</td>
<td>Randomized, double-blind, placebo-controlled study</td>
<td>12 wk</td>
<td>No significant differences between groups in metabolic markers, blood lipids, muscle and liver enzymes, electrolytes, red blood cells, white blood cells, hormones (insulin, TSH, T3, T4), heart rate, blood pressure, or reported side effects.</td>
<td>Kreider et al., 2004</td>
</tr>
<tr>
<td>60 obese men and women (30/group)</td>
<td>500 [50]</td>
<td>Randomized, double-blind, placebo-controlled study</td>
<td>12 wk</td>
<td>No significant effects on blood pressure, liver, kidney, and thyroid function or blood lipid profile, with the exception of increased HDL cholesterol and decreased ratio of total:HDLC cholesterol.</td>
<td>Bhandari et al., 2004</td>
</tr>
<tr>
<td>30 overweight or obese men (15/group)</td>
<td>500 [50]</td>
<td>Randomized, double-blind, placebo-controlled study</td>
<td>12 wk</td>
<td>Significant decreases in body fat percentage and fat mass and significant increases in serum free testosterone in the forskolin group compared to controls. No significant effects on body weight or systolic and diastolic blood pressure. The incidence of adverse effects was not reported.</td>
<td>Godard et al., 2005</td>
</tr>
</tbody>
</table>
### Table 6: Summary of Clinical Studies of Coleus forskohii Extracts

<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>Dose of C. forskohii Extract (mg/day) [dose of forskolin (mg/day)]</th>
<th>Study Design</th>
<th>Study Length</th>
<th>Measured Outcome(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 overweight or obese men and women (25/group)</td>
<td>500 [50]</td>
<td>Randomized, double-blind, placebo-controlled, multi-center study</td>
<td>12 wk</td>
<td>Significant increase in percentage of lean body mass, decrease in body weight, body mass index, and percentage body fat content in ForsLean group compared to placebo and control groups. No significant effects on renal function tests (urea and creatinine), liver function tests (bilirubin, SGOT, SGPT), or thyroid function tests (TSH, T3, T4). No significant changes in serum total cholesterol, HDL or LDL cholesterol, or triglyceride levels. No significant adverse effects.</td>
<td>Kamath et al., unpublished</td>
</tr>
</tbody>
</table>

**Notes:**

- Thyroid-stimulating hormone
- Triiodothyronine
- Thyroxine
- High-density lipoprotein
- Serum glutamic-oxaloacetic transaminase
- Serum glutamic-pyruvic transaminase
- Low-density lipoprotein
Preparation Example 1: Commercial process for making isoforskolin:

The present invention also includes a method of preparing a is forskolin composition from a forskolin extract of Coleus forskohlii plant. The method involves extracting the pulverized roots of the plant with a solvent selected from water, C1-C4 alcohols, chlorinated solvents like MDC, toluene or hexane or solvent is a mixture of water and alcohol of which the preferred extracting medium is toluene. The concentrated toluene extract is precipitated with more non-polar solvents of the type heptane, pentane, hexane; filtered, and the filtrate is back extracted with mixtures of aqueous alcohols in ratios of 10:90 to 90:10 to obtain the desired molecule isoforskolin which is further crystallized with alcohols to obtain the desired purity.

Preparation Example 2: Commercial process for making 7-deacetylforskolin:

Total extract from the above example is dissolved in a solvent medium which includes but is not limited to alcohols, toluene or hexane and treated with immobilized enzyme lipase in concentrations of 0.1-10%, preferably 1-5%, at 37°C under stirring for 12hrs. Once the reaction is complete, the material is back extracted with mixtures of aqueous alcohols in ratios of 10:90 to 90:10 to obtain the desired molecule 7-deacetylforskolin which is further crystallized with alcohols to obtain the desired purity.

Preparation Example 3: Commercial process for making Isoforskolin and 7-deacetylforskolin by Carbon dioxide extraction:
Supercritical fluid extraction using carbon dioxide with and without entrainer such as ethanol, acetone or ethyl acetate extract Isoforskolin and 7-deacetylforskolin from the roots of Coleus plant are described. The extracts were obtained at temperatures ranging from 25° to 120°C, preferably between 45°- 55°C, the extraction fluid pressure was maintained between 100 to 300 bar preferably at 300 bars with or without co-solvents, preferably 5% to 80% ethanol, preferably 30-100% ethanol, for 1-5 hrs, preferably for 3 hrs and at carbon dioxide flow rate of 1-4 kg/h, preferably at 2 kg/hr. The extract obtained is hydrolyzed with lipase enzyme in a liquid media and crystallized out from ethanol to obtain 7-deacetylforskolin of desired purity.

Preparation Example 4 Commercial process for making forskolin, isoforskolin, 7-deacetylforskolin, 1-deoxyforskolin, 9-deoxyforskolin and 1,9-dideoxyforskolin by carbon dioxide extraction:

The roots and stem of Coleus Forskollii are rich sources of Forskolin, Isoforskolin and 7-Deacetylforskolin. In addition minor constituents such as 1-deoxyforskolin, 9-deoxyforskolin and 1,9-dideoxyforskolin are also present along with oil.

The supercritical method is based on differential extraction of these constituents dependant on polarity. The polarity of carbon dioxide can be altered by changing several important parameters in the extraction process. Carbon dioxide has a critical pressure of 73.8 bar and a critical temperature of 31.06°C. This allows wide variations possible in the selection of experimental parameters. First parameter is pressure. Variation in the pressure of the extracting gas changes the properties of the extracting solvent. The second one is temperature range. The third important variable in altering the polarity of the supercritical carbon dioxide is addition of cosolvents. Several cosolvents are possible and were employed in the present work. Solvent modifiers constituted about 0.01% to 10.0 mol% and included solvents such as MeOH, EtOH, PrOH, iso-PrOH, BuOH, benzyl alcohol, acetone, acetophenone, N-methyl-2-pyrrolidone, Methylene ketone, DMSO, DMF, Ethylene glycol, and Acetonitrile. The use of ethanol is particularly adopted and preferred in the present work. Also flow rate of the gas as well
as the particle size of the plant material affect the extraction efficiency. Cosolvent has the maximum effect on the extraction ability of supercritical carbon dioxide.

The roots or stem parts of the plant were cleaned well with water and were then dried well devoid of moisture either by drying in the sun or in an enclosed oven.

In the actual method the dry parts of the plant are initially extracted with super or subcritical carbondioxide to remove the oil present in it. The resultant plant part appeared very powdery. This is again extracted with supercritical carbon dioxide with varying amounts of ethanol present in the supercritical gas. The pressure ranged from 110 bar to 400 bar below the critical temp. Ethanol was used as the solvent modifier. A wide range of composition was used wherein ethanol quantity was altered from 0.01 mol% to 10 mol%.

The forskolin, isoforskolin, 7-deacetylforskolin, 1-deoxyforskolin, 9-deoxyforskolin and 1,9-dideoxyforskolin compositions prepared by the above method are stable. The stability of the compositions has been determined by subjecting the compositions to normal ambient storage conditions, as well as to accelerated storage conditions. During this study, the quality has been tested for stability indicating parameters. As per the study, the extract is stable for a period of not less than 5 years, when it is stored under normal ambient storage conditions.

The present invention includes products (i.e., compositions) produced by this method. The products can usually contain about 1 to about 40% forskohlin, although up to 100% pure forskohlin is possible. Preferred amounts are about 5 to about 20% forskohlin, more preferred about 8 to about 15%, most preferably about 10%.

Reasonable modifications of the inventions disclosed herein are well within the scope of those skilled in the art, and are also intended to be within the scope of the present invention. The scope of the present invention is not intended to be limited by the specific examples set out herein, but rather is to be interpreted according to the following claims.
Throughout this specification, the word 'comprise', or variations such as 'comprises' or 'comprising', means the inclusion of a stated element, integer or step, or group of elements, integers or steps, but does not necessarily mean the inclusion of any other element, integer or step, or group of elements, integers or steps.
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A composition for physiologically increase serum levels of testosterone, estrogen or HGH, which comprises forskolin, isoforskolin or 7-deacetylforskolin.

2. The composition of claim 1, which is used to physiologically increase target tissue levels of testosterone, estrogen or HGH.

3. The composition of claim 1 or 2, which comprises 1-deoxyforskolin, 9-deoxyforskolin and 1,9-dideoxyforskolin.

4. A commercial process for making isoforskolin consisting of: pulverizing dried Coleus roots; extraction with a solvent selected from a mixture of water and alcohol, C1-C4 alcohols, methylene dichloride, toluene, or hexane; Concentration of the extract and precipitation with a non-polar solvent selected from heptane, pentane, hexane; filtration; back extraction with a mixture of water and alcohol; and crystallization in alcohol.

5. A commercial process for making isoforskolin consisting of: pulverizing dried Coleus roots; extraction of the root powder with supercritical carbon dioxide and cosolvent ethanol at a temperature of 45° to 55° C and pressure 300 bar; and crystallization in ethanol.

6. A commercial process for making 7-deacetylforskolin consisting of pulverizing dried Coleus roots; extraction of the root powder with supercritical carbon dioxide and cosolvent ethanol at a temperature of 45° to 55° C and pressure 300 bar; hydrolysis with lipase enzyme; and Crystallization in ethanol.

7. A commercial process for super or subcritical extraction of dried Coleus roots to remove essential oil.

8. A commercial process with supercritical carbon dioxide with varying amounts of ethanol present in the supercritical gas under the pressure ranged from 110 bar to 400 bar below the critical temp.
9. The composition of any one of claims 1 to 3 wherein the composition is in the form to be administered in a daily dose of about 10 to about 100 mg.

10. The composition of any one of claims 1 to 3 which contains about 1 to about 100% forskohlin.

11. A composition according to claim 1 substantially as herein described with reference to any example thereof.

12. A process according to any one of claim 4-8 substantially as herein described with reference to any example thereof.