International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.72.80

Research Article
A Novel Approach to Defeat Obesity: An *in vitro* and *in vivo* Evaluation of the Active Diterpene in *Coleus forskohlii* (Forcslim™)

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Abstract

Background and Objective: The scientific community is now looking into the possibility of herbal traditional healers for the global epidemic of obesity due to their safety and efficacy. Hence, this study aimed to evaluate the antiobesogenic and metabolic benefits of the ethanolic extract of *C. forskohlii* containing 10% forskolin (Forcslim™) *in vitro* and *in vivo* models. **Materials and Methods:** The *in vitro* anti-adipogenic activity of Forcslim™ was assessed using 3T3-L1 murine adipocyte cell lines. Further, the anti-hyperlipidemic activity was evaluated using pancreatic lipase inhibition on the cell line and lipid accumulation studies using Oil red O staining. For *in vivo* analysis, the rats were randomly divided into five groups of six animals as follows: Group I: Fed with a normal diet, Group II: Cafeteria diet, Group III: Simvastatin (50 µg, orally), Group IV: 50 mg/kg Forcslim™ and Group V: 100 mg/kg Forcslim™. Indicators of obesity such as food intake, body weight and alteration in serum lipid profiles were studied. **Results:** A dose-dependent increase in lipase inhibition, with 11.68% at 25 µg/mL and 54.82% at 400 µg/mL and a significant reduction of lipid accumulation in 3T3-L1 cell lines was observed with Forcslim™. Moreover, the administration of Forcslim™ significantly halted the increase in food intake and weight gain associated with a cafeteria diet. The development of dyslipidemia was also significantly inhibited. **Conclusion:** *Coleus forskohlii* (Forcslim™) has significant anti-obesity activity while maintaining normal levels of physical and biochemical parameters. The generated results suggest *C. forskohlii* as a personalized supplement and/or a pharmacological intervention.

Key words: Anti-obesity, *Coleus forskohlii* (Forcslim™), Forskolin, 3T3-L1 cell lines, cafeteria diet-induced obesity, *in vitro* and *in vivo*

Citation: Mirza, F.H.H., C.T. Sadashiva, N. Benny, R. Yoganand and N. Singh, 2024. A novel approach to defeat obesity: An *in vitro* and *in vivo* evaluation of the active diterpene in *Coleus forskohlii* (Forcslim™). Int. J. Pharmacol., 20: 72-80.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity is a serious health issue in the world and has been associated with an increase in morbidity, mortality and reduced life expectancy[1]. An imbalance between energy expenditure and energy intake leads to increased lipid concentration in the blood and causes enlargement of the fat mass[2]. Presently, 300 million people are medically obese while more than one billion adults are overweight[3,4]. Current therapies for obesity treatment include the reduction of nutrient absorption and the administration of drugs that affect lipid mobilization and utilization (e.g., orlistat and sibutramine)[5]. However, owing to the adverse side effects associated with many anti-obesity drugs, more recent trials have focused on screening natural sources that have been reported to reduce body weight with minimal side effects[6,7]. This could pose a great substitute approach for producing effective and safe anti-obesity drugs in the future[8].

A variety of natural products, including crude extracts and isolated compounds from plants have been widely used traditionally to treat obesity[9]. Several reports suggest that numerous bioactive substances derived from natural sources are beneficial in treating obesity. A good bioactive component is polyphenolic compounds exhibiting strong anti-obesity activity, including apigenin, genistein and catechins[10].

Coleus forskohlii belongs to the family Lamiaceae and is extensively used to treat a wide range of illnesses in several nations. The leaf is used in Egypt and Africa as an emmenagogue, expectorant and diuretic. In Brazil, it is used to treat intestinal diseases and stomach issues[11]. It is used as a condiment in India and the tubers are prepared as pickles for consumption. In traditional systems of medicine, *C. forskohlii* has been used to treat cardiac conditions, abdominal colic, respiratory issues, sleeplessness, angina, asthma, bronchitis, intestinal issues, burning experience, constipation and seizures[12]. The labdane diterpenoid, Forskolin, is found to be in abundance in tuberous roots. Many other phytochemicals are also found in the plant, such as terpenoids, monoterpenes, sesquiterpenes, glycosides and phenolic glycosides.

Forskolin's herbal preparations can act on various pharmacologic mechanisms as the key chemical constituent of the tuber and its primary mode of action is to increase cyclic adenosine monophosphate (cAMP) and cAMP-mediated functions through the activation of the adenylate cyclase enzyme. These increase cAMP levels to improve circulation, decrease the release of histamine and other allergic compounds, relax arteries and improve blood flow and pressure, relax bronchial muscles and aids in breathing, increases insulin secretion, controls blood sugar levels and finally, aids in fat breakdown. The direct action of Forskolin on adenylate cyclase, an enzyme that initiates the creation of cyclic adenosine monophosphate, or cAMP, in cells, is the most well-understood mechanism underlying its anti-obesity efficacy. The cAMP promotes the breakdown of stored fats in animal and human fat cells[13]. This study aims to elucidate the potential anti-obesity properties that *C. forskohlii* extract (Forcslim™) may possess by evaluating the inhibitory effects on adipogenesis and measuring metabolic obesity indicators in cafeteria diet-fed mice. In the present study, the effect of *C. forskohlii* extract (Forcslim™) inhibits adipogenesis in 3T3-L1 cells and ameliorates anti-obesity and associated metabolic indicators in cafeteria diet-fed mice.

MATERIALS AND METHODS

Study area: The *in vitro* studies were performed in August, 2021 at Stellixir Biotech Pvt. Ltd., Bangalore and *in vivo* pharmacological studies were carried out in June 2022 at PES College of Pharmacy, Bangalore.

Preparation of *C. forskohlii* extracts (Forcslim™): *Coleus forskohlii* extracts (Forcslim™) are manufactured and registered by Star Hi Herbs Pvt. Ltd., Jigani, Bangalore, Karnataka, India.

Cell lines and culture media: The 3T3-L1 cell line was procured from the National Centre for Cell Science (NCCS) in Pune, Maharashtra, India. Stock cultures of the cell lines were cultured in Dulbecco's Modified Eagle (DMEM) medium supplemented with 10% inactivated fetal bovine serum (FBS), penicillin (100 IU mL^{-1}), streptomycin (100 mg mL^{-1}) and amphotericin B (5 mg mL^{-1}) (Himedia, India) in a 5% CO_2 atmospheric incubator at 37°C until confluent. The cells were dissociated with 0.2% trypsin and 0.02% EDTA in PBS solution (Himedia, India). All the experiments were carried out in 96-well microtiter plates (Corning, USA).

***In vitro* cell viability of *C. forskohlii* extract (Forcslim™) treated 3T3-L1 cell line:** For evaluating the *in vitro* cytotoxicity of *C. forskohlii* extract (Forcslim™), an MTT assay was performed to assess the cell viability with concentrations of *C. forskohlii* extract (Forcslim™) ranging from 25-400 µg/mL. About 100 µL of the different test concentrations of *C. forskohlii* (Forcslim™) was added to the partial monolayer in microtiter plates. The plate was incubated for 24 hrs at 37°C. The MTT reagent, to a final concentration of 0.5 mg/mL of total volume was added to the wells and the plate was further incubated for 3 hrs. The MTT reagent was replaced with 100 µL of Dimethyl Sulfoxide (DMSO).

Simvastatin was used as a positive control. The absorbance was observed in the ELISA reader (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) at 570 and 630 nm. The IC_{50} value was determined using a linear regression equation. The percentage growth inhibition was calculated using the following formula[14]:

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

***In vitro* anti-obesity studies of *C. forskohlii* (Forcslim™) in pancreatic lipase inhibition assay:** The pancreatic lipase inhibitory activity of *C. forskohlii* (Forcslim™) was determined using p-Nitrophenyl Phosphate (p-NPP) as a substrate[15]. Briefly, the enzyme, under the reaction conditions, hydrolyses p-NPP to release p-nitrophenol, which is a colored substance and can be monitored at 410 nm wavelength. The cells were exposed to various concentrations of the crude extracts (25, 50, 100, 200 and 400 µg/mL) and fractions were prepared in DMSO (25-400 µg/mL). Lipase (0.1 mg) was dissolved in tris-buffer (50 mM, pH 8) and added to the cell supernatant. The mixture was stirred for 15 min and centrifuged at 2000 rpm for 10 min. The clear supernatant was recovered. Different concentrations of *C. forskohlii* extract (Forcslim™) (or, orlistat) were mixed with 0.5 mL lipase solutions and was incubated for 30 min at 37°C. Post-incubation, 1 mL of substrate p-NPP (3 mM in 2-propanol) was added to all the tubes. After incubating the mixture for 2 hrs at 37°C, its absorbance was read at a wavelength of 410 nm against a blank control. The test sample contained all constituents except controls. Orlistat was used as a positive control. The following formula was used to determine the percentage inhibition[16]:

$$\text{Lipase activity } (\%) = 100 - \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}} \times 100$$

***In vitro* anti-adipogenic activity of *C. forskohlii* (Forcslim™) treated 3T3-L1 cell line:** The Oil red O staining method was performed to determine the testing concentrations of *C. forskohlii* (Forcslim™) for anti-adipogenic studies based on the results obtained in the cytotoxicity studies. The tests were carried out on 3T3-L1 cells and to evaluate the suppressive action of *C. forskohlii* (Forcslim™) on fat droplet formation was determined by the quantification of the Oil red O staining method.

Lipid (Oil red O) staining method: To perform the Oil red O lipid staining method, 1000 µL cell suspension was seeded in a 6-well plate at the required cell density (10^6 cells per well), without the test agent[17]. The cells were allowed to grow for 72 hrs. The sample extract and 2% inactivated FBS concentrations added to DMEM were combined to make a stock solution at 1000 µg/mL.

The plate was incubated for 24 hrs at 37°C in a 5% CO_2 atmosphere, following which the spent medium was removed. Cell fixing was done on the removal of the cells from the medium by gentle washing with PBS. Each well received 10% formalin and incubated for 30 min to 1 hr. Cell staining was performed based on the standard procedure. The absorbance was read on the ELISA reader at a wavelength of 492 nm. The Oil red O intensity in treated samples was calculated relative to untreated control samples with the following equation[18]:

$$\text{Intensity of Oil red O staining } (\%) = \frac{\text{Absorbance of treatment}}{\text{Absorbance of control}} \times 100$$

Animals: The *in vivo* studies were performed on female Swiss albino mice (5 weeks old). The animals were housed for 1 week under a 12 hrs/12 hrs light/dark cycle in a temperature-and-humidity-controlled room. The animals were given free access to food and water. Following a week of acclimatization to the lights, the healthy animals were employed in the *in vivo* models. The experimental protocols were approved by the Bharathi College of Pharmacy, Mandya, Karnataka, India (1135/PO/Re/S/07/CPCSEA).

Diets: The cafeteria diet consisted of three diets: (a) 24 g of condensed milk, 24 g of bread, (b) 9 g of chocolate, 18 g of dried coconut, 18 g of biscuit and (c) 24 g of cheese, 30 g of potatoes[19].

Preparation of cafeteria diets for obesity induction: The method described by Harris and Kulkarni, was followed with some modifications[20]. Three diets comprise the cafeteria diet (an extremely appetizing, high-energy animal diet that contains a range of human snack foods): 48 g of condensed milk and bread; 18 g of chocolate; 36 g of biscuits and dry coconut and 48 g of cheese and 60 g of cooked potatoes. For 40 days, the cafeteria diet was given to five groups of six mice each in the form of pellets.

Experimental procedure for anti-obesity activity: Thirty female albino mice (22-26 g) were separated into five groups of six mice in each and treated as follows:

Group I: Normal diet
Group II: Cafeteria diet

Group III: Simvastatin (50 µg, orally) was administered daily
Group IV: *Coleus forskohlii* (Forcslim™) (50 mg/kg, orally) was administered daily
Group V: *Coleus forskohlii* (Forcslim™) (100 mg/kg, orally) was administered daily

Parameters evaluated

Body weight: The mice's body weights (g) were noted on days 1, 10, 20, 30 and 40 for every group.

Body weight: The mice's body weights (g) were noted on days 1, 10, 20, 30 and 40 for every group. The initial and final body weight and height measurements were recorded according to the Body Mass Index (BMI) and lee index of obesity formulae (LIO) on days 1 and 40.

Body mass index and lee index of obesity formula

Food consumption: Food consumption was measured at 1, 2 and 3 hrs intervals during the course of experiments conducted on days 1, 10, 20, 30 and 40. The amount of food remaining on the grid was subtracted from the original food weight to estimate the amount of food consumed[20].

Statistical analysis: The average of all data was compiled and SEM was calculated. All the data were compiled using One-way ANOVA followed by Dunnett's multiple comparison tests. Statistical significance was defined as $p<0.05$-0.001.

RESULTS

Cell viability of *C. forskohlii* (Forcslim™) treated 3T3-L1cell line: The cytotoxicity effect of *C. forskohlii* (Forcslim™) was tested in 3T3 L1 cell lines at concentrations ranging from 25-400 µg/mL. The difference in percentage survival and cytotoxic effect of the 3T3 L1 cell lines treated with different concentrations of *C. forskohlii* (Forcslim™) has been shown in (Fig. 1(a-g) and Fig. 2). It was observed that the percentage of cell survival in adipocytes treated with 400 µg/mL of *C. forskohlii* (Forcslim™) had a significant decrease compared to the control group indicative of some cytotoxic activity.

Pancreatic lipase inhibition activity of *C. forskohlii* (Forcslim™): The pancreatic lipase activity assay aimed to evaluate the ability of *C. forskohlii* (Forcslim™) to inhibit lipid absorption into the body by inhibiting the enzymatic lipase activity. Fatty acid and 2-monoacylglycerol are the two primary products created via the hydrolysis process of pancreatic lipase. Pancreatic lipase activity is known to act by promoting the absorption of monoglycerides and free fatty acids into the body, which is a known cause of obesity. The inhibition of pancreatic lipase activity of *C. forskohlii* (Forcslim™) prevents lipid accumulation in the body. Orlistat was chosen as the standard drug of comparison, which was approved by the FDA and available for obesity treatment, apart from centrally acting anti-obesity drugs. Untreated cell lines demonstrated 0% lipase inhibition activity; orlistat demonstrated 49.83% lipase inhibition activity and *C. forskohlii* (Forcslim™) demonstrated a dose-dependent increase in inhibition activity, with 11.68% lipase inhibition activity at 25 µg/mL and 54.82% lipase inhibition activity with 400 µg/mL (Fig. 3).

***Coleus forskohlii* (Forcslim™) elicits *in vitro* anti-adipogenic activity in 3T3-L1 cell line:** The anti-hyperlipidemic activity was performed using the Oil red O staining method on 3T3-L1 cell lines. The adipogenesis was substantially inhibited by *C. forskohlii* (Forcslim™) (400 µg/mL) with anti-adipocyte accumulation decrease of up to 49.98%. The control cell line did not show inhibition of fat accumulation; while simvastatin (50 µg/mL) showed that anti-adipocyte accumulation was decreased by 33.43%. These observations demonstrated the effect of *C. forskohlii* (Forcslim™) on anti-adipogenesis activity compared to the standard drug, which significantly reduced the lipid accumulation in 3T3-L1 cell lines, suggesting anti-obesity activity.

Effect of *C. forskohlii* (Forcslim™) on mice body weight: The effect of *C. forskohlii* (Forcslim™) on body weight in the normal and experimental mice groups was evaluated. When comparing the normal diet group to the cafeteria diet fed groups on days 1, 10, 20, 30 and 40, there were substantial ($p<0.001$) increases in body weight. Simvastatin-treated mice fed with cafeteria meals showed a significant ($p<0.05$-0.001) reduction in body weight in comparison to the high fat diet. Interestingly, body weight at days 20, 30 and 40 was significantly ($p<0.05$-0.001) reduced in the *C. forskohlii* (Forcslim™) treated groups also when given 100 mg/kg compared to the group eating the cafeteria meal (Table 1).

Effect of *C. forskohlii* (Forcslim™) Body Mass Index (BMI) and lee index of obesity (LIO): The impact of *C. forskohlii* (Forcslim™) on BMI and LIO in the groups of normal and experimental mice has been displayed in Table 2. When mice were fed with the cafeteria diet, their final BMI and LIO were found to be considerably ($p<0.001$) higher than when they were provided a normal diet. When compared to the cafeteria

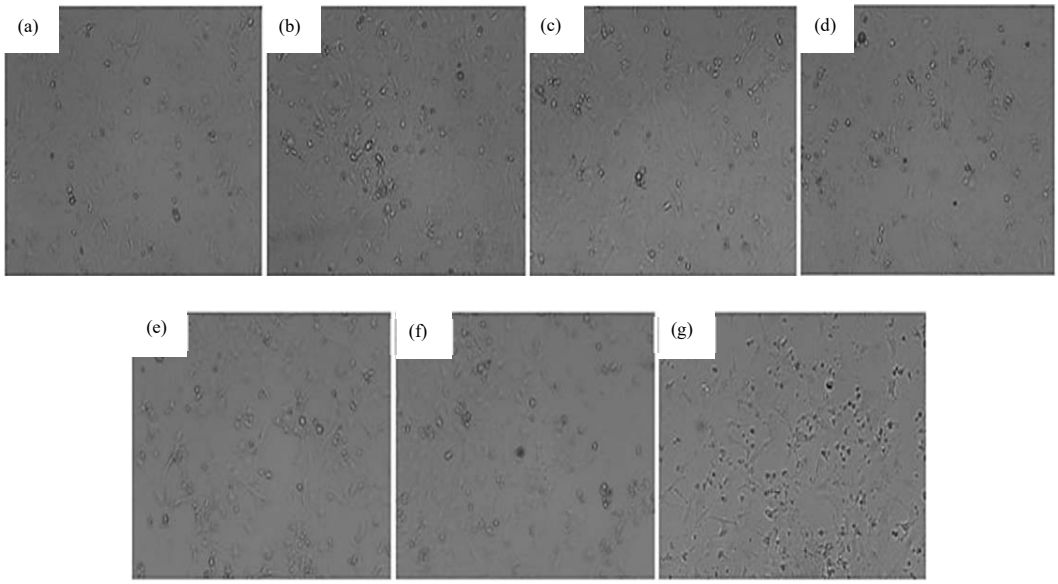

Fig. 1(a-g): Images of 3T3-L1 cells after exposure to *C. forskohlii* (a) Control, (b) 25 µg/ mL, (c) 50 µg/mL, (d) 100 µg/mL, (e) 200 µg/mL, (f) 400 µg/mL and (g) Simvastatin (standard) treated cells for 24 hrs

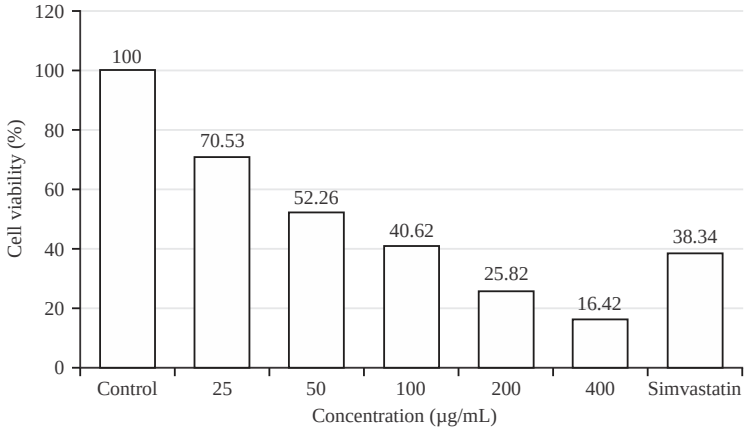

Fig. 2: MTT cell viability assay of 3T3-L1 cells treated with *C. forskohlii* (Forcslim™) (25-400 µg/mL)
Data are shown as Mean±SEM where n = 3

food-fed group, the mice treated with *C. forskohlii* (Forcslim™) and simvastatin (100 mg/kg) groups displayed a significant ($p<0.001$) drop in the final BMI and LIO.

Effect of *C. forskohlii* (Forcslim™) on food consumption: The impact of *C. forskohlii* (Forcslim™) on the amount of food consumed by the experimental and control groups was assessed. On days 1, 10, 20, 30 and 40, food consumption was found to be considerably ($p<0.001$) higher than that of a group fed a typical diet. When comparing the food consumption of the *C. forskohlii* (Forcslim™) (100 mg/kg b.wt.,) treated mice to the group given a cafeteria meal, there was a substantial ($p<0.05$, $p<0.01$) drop observed between days 30 and 40 as shown (Table 3). Similarly, mice given simvastatin for days 30 and 40 had a significant ($p<0.01$, $p<0.001$) reduction in food intake as compared to the group fed cafeteria diet.

***Coleus forskohlii* (Forcslim™) alters cafeteria diet fed mice biochemical profile:** The impact of *C. forskohlii* (Forcslim™) on the biochemical profile in both the experimental and normal mice groups have been displayed in Table 4. When compared to mice on a regular diet, feeding the cafeteria diet to mice resulted in a significant ($p<0.001$) increase in serum glucose,

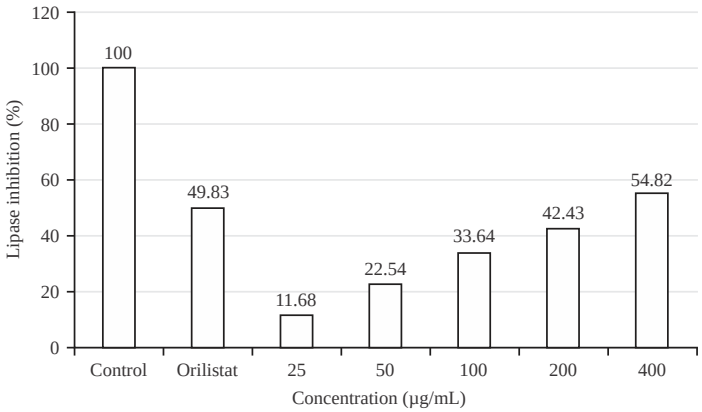

Fig. 3: Inhibitory effect of different concentrations of *C. forskohlii* (Forcslim™) on pancreatic lipase enzyme activity

Table 1: Effect of *C. forskohlii* (Forcslim™) on the body weight of normal and experimental mice groups

	Days and body weight (g)				
Treatment groups	1 day	10 days	20 days	30 days	40 days
Normal diet	22.08±1.24	22.32±1.93	22.91±1.84	23.64±1.98	25.56±2.51
Cafeteria diet	23.87±1.64	26.32±2.93	27.61±2.45	29.43±2.83	31.43±71
Cafeteria diet+Simvastatin	23.47±1.56	22.98±2.42	21.03±2.24	21.52±1.67	20.02±1.72
Cafeteria diet+*C. forskohlii* (Forcslim™) 50 µg/mL	23.98±1.83	26.86±2.18	25.26±1.04	25.62±1.34	23.21±1.74
Cafeteria diet+*C. forskohlii* (Forcslim™) 100 µg/mL	23.28±1.83	25.47±2.10	24.42±1.77	23.89±1.64	22.56±1.33

All values are expressed as a Mean±SEM, n = 6, p<0.001 compared with normal diet group and p<0.005, p<0.001 compared with cafeteria diet group

Table 2: Effect of *C. forskohlii* (Forcslim™) on body mass index and lee index of obesity in normal and experimental mice groups

Treatment groups	Initial BMI (g cm^{-2})	Final BMI (g cm^{-2})	Initial LIO (g cm^{-2})	Final LIO (g cm^{-2})
Normal diet	0.40±0.02	0.44±0.03	60.1±3.1	65.6±5.2
Cafeteria diet	0.42±0.03	0.56±0.06	63.8±0.02	81.3±3.6
Cafeteria diet+Simvastatin	0.41±0.03	0.35±0.02	62.93±4.3	51.8±3.6
Cafeteria diet+*C. forskohlii* (Forcslim™) 50 mg/kg	0.42±0.03	0.42±0.03	61.8±4.8	64.2±4.6
Cafeteria diet+*C. forskohlii* (Forcslim™) 100 mg/kg	0.42±0.03	0.37±0.02	63.2±4	56.9±3.6

All values are expressed as a Mean±SEM, n = 6, BMI: Body Mass Index, LIO: Lee index of obesity, p<0.001 compared with normal diet group and p<0.005, p<0.001 compared with compared with cafeteria diet group

Table 3: Effect of *C. forskohlii* (Forcslim™) on food consumption in normal experimental mice groups

	Days and food consumption (g)				
Treatment groups	1 day	10 days	20 days	30 days	40 days
Normal diet	2.67±0.16	1.86±0.20	3.27±0.2	2.10±0.17	3.39±0.31
Cafeteria diet	15.9±1.74	14.5±1.42	13.1±1.23	11.92±1.03	10.2±.90
Cafeteria diet+Simvastatin	16.9±1.56	12.7±1.28	10.5±.98	8.90±.80	6.2±0.43
Cafeteria diet+*C. forskohlii* (Forcslim™) 50 mg/kg	17.2±1.73	13.9±1.84	13.8±1.29	10.6±1.04	8.93±0.85
Cafeteria diet+*C. forskohlii* (Forcslim™) 100 mg/kg	16.8±1.83	12.6±1.34	10.45±0.76	9.94±0.89	7.9±0.93

All values are expressed as a Mean±SEM, n = 6, p<0.001 compared to the normal diet group and p<0.005, p<0.001 compared to the cafeteria diet group

Table 4: The effect of *C. forskohlii* (Forcslim™) on biochemical profile in normal and experimental mice groups

Treatment groups	Glucose	TG	TC	HDL	LDL	AI
Normal diet	75.2±2.5	65.73±7.2	165.8±10.3	38.2±3.5	110.8±11.6	3.42±0.72
Cafeteria diet	95.3±7.9	98.87±8.9	195.6±14.9	29.3±22.8	156±0.6	7.07±0.8
Cafeteria diet+Simvastatin	78.4±9.6	71.2±4.7	173.8±13.8	36.2±2.1	124.2±10.2	3.98±0.5
Cafeteria diet+*C. forskohlii* (Forcslim™) 50 mg/kg	81.2±6.9	84.9±9.2	189.2±12.7	30.1±2.7	143.2±11.6 11.6	5.27±0.4
Cafeteria diet+*C. forskohlii* (Forcslim™) 100 mg/kg	75.9±8.5	76.9±6.3	178.2±12.8	35.2±2.4	129.4±11.3 11.3	4.29±0.7

All values are expressed as a Mean±SEM, n = 6, TG: Triglycerides, TC: Total cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein, AI: Atherogenic index, p<0.001 compared with normal diet group and p<0.005, p<0.001 compared with cafeteria diet group

Table 5: Effect of *C. forskohlii* (Forcslim™) on organ weight in normal and experimental group of mice

					Lungs (g)		Kidney	
Treatment groups	Brain (g)	Liver (g)	Stomach (g)	Heart (g)	Right	Left	Right	Left
Normal diet	0.29±0.03	0.98±0.04	0.28±0.03	0.08±0.004	0.16±0.002	0.16±0.005	0.12±0.004	0.12±0.004
Cafeteria diet	0.30±0.03	1.17±0.05	0.50±0.05	0.17±0.006	0.15±0.002	0.15±0.005	0.14±0.003	0.14±0.003
Cafeteria diet+Simvastatin	0.29±0.03	1.7±0.04	0.32±0.04	0.12±0.003	0.18±0.002	0.18±0.006	0.11±0.003	0.11±0.003
Cafeteria diet+*C. forskohlii* (Forcslim™) 50 mg/kg	0.28±0.03	1.18±0.04	0.37±0.03	0.14±0.004	0.17±0.002	0.17±0.004	0.11±0.003	0.11±0.003
Cafeteria diet+*C. forskohlii* (Forcslim™) 100 mg/kg	0.28±0.03	1.14±0.04	0.38±0.03	0.13±0.08	0.18±0.002	0.18±0.002	0.12±0.003	0.12±0.003

All values are expressed as a Mean±SEM, n = 6, p<0.001 compared with normal diet group and p<0.005, p<0.001 compared with cafeteria diet group

triglycerides, total cholesterol, LDL and atherogenic index levels and a significant (p<0.001) decrease in HDL values. Mice on a cafeteria diet were given *C. forskohlii* (Forcslim™) or simvastatin of 100 mg/kg b.wt., showed significant (p<0.001) reductions in serum glucose, triglycerides, total cholesterol, LDL and AI levels and significant (p<0.001) increases in HDL levels in contrast to the group fed a cafeteria diet.

Liver and small intestine weight decrease by *C. forskohlii* (Forcslim™): In both the group of normal and experimental mice, the impact of *C. forskohlii* (Forcslim™) on organ weight was quantified. Comparing the cafeteria diet fed group to the normal diet fed group, it was shown that the weight of organs such as the liver and small intestine had substantially (p<0.001) increased. In comparison to the group fed the cafeteria meal, the mice treated with *C. forskohlii* (Forcslim™) at a dose of 100 mg/kg exhibited a significant (p<0.05) loss of liver and small intestine weight. Considerable reduction in weight was observed also when the standard drug simvastatin was used. The weights of the brain, stomach, heart, lungs and kidneys in the cafeteria food fed group and the other experimental groups did not vary significantly (Table 5).

DISCUSSION

This research study elucidated the anti-obesity effects Forcslim™ may have on a mouse model study consisting of either having a normal or a cafeteria food diet for a period of 40 days. To evaluate these effects appetite suppression, nutrient absorption, thermogenesis and adipogenesis were analyzed. The results revealed that *C. forskohlii* administration to mice on the cafeteria food diet showed a significant decrease in the biochemical obesity marker profile, a reduction in liver and small intestine weight and lipase inhibition and anti-adipogenic activity. In general, anti-obesity effects are closely related to one of four different mechanisms. The first mechanism is to suppress the appetite of the central nervous system. By reducing the sensation of hunger and increasing the sensation of satiety, less food is consumed, resulting in anti-obesity effects. The second mechanism is to interfere with nutrient absorption. While the third mechanism is increasing energy expenditure (thermogenesis). The last mechanism is to decrease adipogenesis. These four mechanisms were evaluated through this experiment.

In the current work, it was observed that the percentage of cell survival in adipocytes treated with 400 µg/mL of *C. forskohlii* (Forcslim™) was significantly better than the control group indicating some cytotoxic activity. Adipocytes are specialized fat-storing cells. The lipolysis in differentiated adipocytes may control adipocyte size. The inhibition of the differentiation and proliferation of adipocyte precursors reduces hyperplasia and hypertrophy of adipocytes, leading to a decrease in fat accumulation[21]. It has been reported that Forskolin activates the hormone-sensitive lipase enzyme activity in mature adipocytes to increase lipolysis. Also, the extract demonstrated a dose-dependent increase in lipase inhibition, with 11.68% at 25 µg/mL and 54.82% at 400 µg/mL. Interestingly, a significant reduction of lipid accumulation in 3T3-L1 cell lines was also observed. The inhibition of digestive enzymes such as lipase and amylase hinders the digestion of foods, which suppresses the intestinal uptake of fats and sugars from the diet.

The cafeteria diet has been linked to increased energy consumption and obesity in both humans and animals[22]. There have been reports that the cafeteria diet causes rats to experience hyperphagia, which leads to increased fat storage and an increase in body and organ weight[23]. A cafeteria diet-induced obesity model is the most basic model of obesity-induced and is considered the one that most closely represents human obesity in reality[24]. Current study results demonstrated that feeding *C. forskohlii* (Forcslim™) with a cafeteria diet to mice for 6 weeks significantly reduced food intake (an appetite suppressant effect) in this model. These effects were reflected in the reduced body weight and biochemical parameters of the mice. The most common complication of obesity includes significant alterations in lipid profiles. The activation of the Uncoupling Protein (UCP1–3) in the mitochondria elicits anti-obesity effects by enhancing

thermogenesis, which converts the energy obtained from foods to heat, rather than fat[25]. Apart from weight reduction, when compared to mice fed the cafeteria diet alone, *C. forskohlii* (Forcslim™) substantially stabilized the lipid profiles, resulting in a noticeable decrease in TC, LDL and TG contents and a rise in HDL. The increased proportion of HDL in *C. forskohlii* (Forcslim™) treated mice may be the cause of the observed reduction in the ratios of TC/HDL and LDL/HDL. Normal fat diet-fed mice are unaffected by *C. forskohlii* extract supplements for serum cholesterol, phospholipid and fatty acids levels. However, blood triglyceride levels are affected and induction of hepatic steatosis was noted[26,27].

The prevalence of overweight and obesity has continued to increase over the last several decades worldwide[28]. Obesity is a metabolic disorder that can lead to adverse metabolic effects on blood pressure, cholesterol, triglycerides and insulin resistance and also increases the risk of coronary heart disease, ischemic stroke and type 2 diabetes mellitus. Overweight and obesity are the fifth leading risk for global deaths. At least 2.8 million adults die each year as a result of being overweight or obese.

In the current study, *C. forskohlii* (Forcslim™) may have beneficial applications because of its hypophagic and anti-hyperlipidemic qualities, which prevent obesity and obesity-associated metabolic changes in mice, fed a cafeteria diet. The development of obesity brought on by the cafeteria diet and related metabolic abnormalities like dyslipidemia can be prevented by supplementing with *C. forskohlii* (Forcslim™).

CONCLUSION

Altogether the obtained data clearly shows that the test drug, *C. forskohlii* (Forcslim™) possesses marked anti-obesity potential against a cafeteria diet-induced obese mouse. It has been discovered that the test drug worked more effectively at the highest dose (100 mg/kg b.wt.). The anti-obesity potential of *C. forskohlii* may be mediated via delayed intestinal intake of dietary fat due to a reduced function of pancreatic lipase. The obtained results clearly suggested that *C. forskohlii* (Forcslim™) is a source of natural products that has the potential to be developed as medicinal ingredients in the prevention and treatment of obesity and other metabolic diseases in humans.

SIGNIFICANCE STATEMENT

This study explores the anti-obesity properties of ethanolic extract of *Coleus forskohlii* (Forcslim™). Forcslim™ is an innovative, patented product that contains 10% forskolin. Forcslim™ produces anti-adipogenic and anti-hyperlipidemic effects in 3T3-L1 cells. Results revealed considerable reduction in body weight, peri epididymal fat changes, liver weight and biochemical characteristics. This study shows the anti-obesity potential of Forcslim™ may be mediated via delayed intestinal intake of dietary fat due to a reduced function of pancreatic lipase. Forcslim™ data suggests marked anti-obesity potential working effectively at the highest dose and is effective and safe for long-term usage. The study highlights that future investigation on Forcslim™, as sustainable natural source used as a medicinal ingredient for preventing and treating obesity and other metabolic human diseases.

ACKNOWLEDGMENT

We are very thankful to Prof. M.N. Palaksha, Department of Pharmacology, PES College of Pharmacy, helped us with *in vivo* pharmacological studies.

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