

## Original Article

# Anti-adipogenic activity of I-SLIM on 3T3-L1 adipocytes

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### ABSTRACT

I-SLIM, a proprietary blend of three well-known natural ingredients, was evaluated for its anti-adipogenesis activity by oil red O staining and measuring the free triglyceride level in mouse fibroblast cells. I-SLIM was evaluated for cytotoxicity with different concentrations from 62.5 to 1000 µg/mL, the CTC50 value for which resulted to be >1000 µg/mL, and hence, the concentrations 150 µg/mL and 300 µg/mL were taken for anti-adipogenesis studies. In adipogenesis study, test doses 150 and 300 µg/mL exhibited 17.9% and 36% inhibition of lipid accumulation, respectively. Similarly, glycerol released was found to be 135.08 ± 4.24 and 115.77 ± 1.61% over control at 150 and 300 µg/mL, respectively.

**Keywords:** Adipogenesis, I-SLIM, lipid metabolism, obesity

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## INTRODUCTION

Obesity means a physiological state in which excessive body fat has been collected, and it may extend the hazard of various diseases such as hyperlipidemia, cardiovascular disease, type 2 diabetes, and coronary heart disease. For this reason, nowadays, obesity has become a serious worldwide problem in human health.<sup>[1,2]</sup> Numerous reasons, including genetic predisposition, excessive food intake, lack of exercise, and lifestyle, have been reported to induce obesity.<sup>[2-4]</sup> Most studies demonstrated that the storage of lipid in adipocytes by adipogenesis is one of the main causes of obesity.<sup>[5-8]</sup>

There are several pharmacologic substances available as antiobesity drugs; however, they have hazardous side effects including increased blood pressure, headache, dry mouth, insomnia, and constipation,<sup>[9,10]</sup> and thus obesity has been treated in Asian countries by various produce available naturally,<sup>[11]</sup> which is an alternative strategy for effective, safe antiobesity drug.<sup>[12]</sup>

I-SLIM, an Ayurvedic proprietary reconstituted health drink from Indusviva HealthSciences Pvt.

Ltd., Bangalore, is a polyherbal formulation in the management of obesity. With its unique combination of the antiobesity herbal active blend and with the goodness of proteins, multivitamins and enzymes, I-SLIM is considered safe and efficacious in obesity management.

I-SLIM is a proprietary blend of three well-known natural ingredients: *Coleus forskohlii*, *Salacia reticulata*, and *Sesamum indicum*. These ingredients are known to hinder the absorption of fat at distinct amount and kinetics independently. Individually, each of these components has been shown to inhibit fat absorption with differing degrees and dynamics. It helps in preventing the negative gastrointestinal and metabolic side effects often associated with fat blockers.<sup>[13]</sup>

*C. forskohlii* is well-studied plant for its supportive role in the management of weight, which has been used for centuries in traditional Indian medicine system. Torrents of chemical reactions are induced by *C. forskohlii* in fat reduction.<sup>[14,15]</sup>

An anti-diabetic plant, *S. reticulata* has a special place in the classical medicine of Ayurveda, with vested regulatory action on alpha-glucosidase, an enzyme that prevents absorption of excess dietary sugar,<sup>[16]</sup> and appears to have the ability to

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block fat and may reduce the rate of lipid (fat) absorption after a meal.

*S. indicum* also exerts this activity. This superfood is a rich source of protein and many essential minerals that are believed to support healthy cardiovascular and circulatory functions.<sup>[17]</sup>

In the present study, we investigated the anti-adipogenesis effect of I-SLIM by oil red O staining and measuring the free triglyceride level.

## MATERIALS AND METHODS

**Sample Preparation:** 10 mg of I-SLIM dissolved in dimethyl sulfoxide (DMSO) was taken for studies separately. DMEM high glucose along with 2% inactivated fetal bovine serum (FBS) is used to make up the volume to obtain a 1 mg/mL concentration stock solution and sterilized by 0.22  $\mu$  syringe filtration. For further studies, two-fold serial dilutions were prepared from the above mixture.

### Cell Culture

National Centre for Cell Science, Pune, India, provided the 3T3-L1 (Mouse embryo Fibroblast) cell line. 10% inactivated FBS with DMEM high glucose were used to culture the stock cells, amphotericin B (5  $\mu$ g/mL), penicillin (100 IU/mL), and streptomycin (100  $\mu$ g/mL) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% ethylenediaminetetraacetic acid, and 0.05% glucose in phosphate-buffered saline [PBS]). The stock cultures were grown in 25 cm<sup>2</sup> culture flasks, and all experiments were carried out in 96 mL plates (Tarsons India Pvt. Ltd., Kolkata, India).

### Cytotoxicity Studies

The monolayer cell culture was trypsinized, and the cell count was adjusted to 100,000 cells/ml using DMEM high glucose containing 10% FBS. To each well of the 96 well microtiter plate, 0.1 mL of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium, and 100  $\mu$ L of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 3 days in 5% CO<sub>2</sub> atmosphere, and microscopic examination was carried out, and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded, and 50  $\mu$ L of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO<sub>2</sub> atmosphere. The supernatant was removed, and 100  $\mu$ L of DMSO was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula

and concentration of test drug needed to inhibit cell growth by 50% (CTC50) values is generated from the dose-response curves for each cell line.

### Anti-adipogenesis Assay

#### 3T3-L1 cell culture and differentiation

To induce differentiation of cells, the 3T3-L1 cells were cultured in 4.5 g/L glucose-DMEM with 10% calf serum, antibiotic solution in 30 mm plastic Petri dishes until they reached 100% confluence. After 2 days, the 2-day post-confluent cells were incubated for 48 h in DMEM with 10% FBS, antibiotics, and a differentiation cocktail termed MDI, which contained 0.5 mM isobutylmethylxanthine, 1  $\mu$ M dexamethasone, and 100 nm insulin. After 48 h, cells were maintained in DMEM with 10% FBS, insulin antibiotics for 6 days. After 6 days, the cells were maintained in DMEM with 10% FBS along with test samples (I-SLIM) for 24 h to investigate its anti-adipogenic activity.

### Oil red O Staining

To determine the inhibitory effect of I-SLIM on lipid accumulation in 3T3-L1 cells, we used oil red O staining after adipocyte differentiation. The cells were rinsed with PBS and fixed with 10% formalin for 30 min. Then, the formaldehyde was removed, followed by washing with 60% isopropanol. After washing, the cells were stained for 1 h at room temperature in freshly diluted oil red O containing 0.5% oil red O in isopropanol. Finally, the dye retained in the 3T3-L1 cells was eluted with isopropanol and quantified by measuring the optical absorbance at 500 nm.

### Triglyceride Assay

The cells were incubated with I-SLIM during adipogenesis. After differentiation, the cells were washed with PBS and lysed with lysis buffer, and the supernatants were collected and assayed for the triglyceride concentrations at 580 nm.

## RESULTS

### Cytotoxicity Effect of I-SLIM in 3T3-L1 Cell Line

The cytotoxic effect of I-SLIM was determined in 3T3-L1 cell lines, with different concentrations (62.5, 125, 200, 500, and 1000  $\mu$ g/mL) of I-SLIM for every 24 h interval till 72 h. According to the results, I-SLIM did not show any cytotoxic effect up to a concentration of 1000  $\mu$ g/mL, even when the cells were treated for 72 h [Table 1].

### Anti-adipocyte Activity of I-SLIM in 3T3-L1 Cell Lines

Once the cells were differentiated with two different concentrations (150 and 300  $\mu$ g/mL) of I-SLIM, we used oil red O staining to know whether I-SLIM has an inhibitory effect on adipogenic differentiation. The result shows that I-SLIM inhibited lipid accumulation in 3T3-L1 dose-dependently

compared with fully differentiated adipocytes. As shown in the quantitative results [Table 2], retained oil red O dye and intracellular triglyceride values were decreased in a dose-dependent manner [Table 3] when cells were treated with I-SLIM. These results demonstrated that I-SLIM reduced the accumulation of lipid in 3T3-L1 during adipogenic differentiation without cell cytotoxicity.

## DISCUSSION

Obesity has dramatically increased in most developing nations and is a prevalent condition related to metabolic disorders worldwide. Because the antiobesity drugs available at present are known to have various hazardous effects.<sup>[18]</sup> Hence, there is an immense importance for the natural produce having therapeutics property, as it is much safer compared to synthetic compounds.<sup>[19]</sup> The antiobesity ability of the phytochemicals is evaluated based on its potential to hinder the adipogenesis.<sup>[20]</sup>

Overweight and obesity are of major concern all over the world in many countries. It is more prevalent in developed countries, as well as in developing countries. For the patients with a body mass index range of 28–34, with metabolic

complications, supplement therapy with weight management products is effective.<sup>[21]</sup>

I-SLIM regimen is a comprehensive lifestyle-based weight loss program which involves a synergy between herbal actives and nutrition, and thus, can initiate and maintain weight loss. I-SLIM supports overall holistic management of overweight and obesity.

Nowadays, investigation of the beneficial effects of several natural compounds in the adipocyte-related bioprocess is a new strategy of antiobesity research.<sup>[22]</sup> As shown in the oil red O staining and triglyceride assay results, I-SLIM decreased the lipid accumulation on the treated adipocytes compared with mature adipocytes.

## CONCLUSION

The present study has focused on the inhibition of the formation of the fatty substances characteristic of adipogenesis, through the use of an herbal blend (I-SLIM), in conclusion, this study demonstrated a potent anti-adipogenic effect of herbal blend (I-SLIM) on cellular levels, implicating a potential application of this cost-effective natural product in the prevention of obesity.

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**Table 1: Cytotoxic properties of I-SILM against 3T3-L1 cell line**

Test concentration (µg/mL)	% Cytotoxicity	CTC50 (µg/mL)
1000	41.44±1.3	>1000
500	26.82±0.9	
250	14.54±0.7	
125	7.85±2.4	
62.5	3.34±1.1	

**Table 2: In vitro anti-adipogenesis effect of I-SILM**

Test concentration (µg/mL)	Percentage inhibition over control (average±SD)		
	(N1)	(N2)	Average
300	35.7±0.92	36.2±2.52	36.0
150	17.8±1.53	18.0±1.93	17.9

SD: Standard deviation

**Table 3: Level of released triglyceride in cell supernatant**

Test concentration (µg/mL)	Triglyceride mg/dL (% control)
300	115.77±1.61
150	135.08±4.24

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