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Modulation of adipogenesis and glucose uptake by *Curcuma longa* extract in 3T3L1 and L6 cell lines – An *in vitro* study

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ABSTRACT

Objective: To evaluate the effects of ethyl acetate extract of *Curcuma longa* against modulation of glucose uptake and adipogenesis in cell line models. **Methods:** We used 3T3L1 and L6 cells to investigate cytotoxicity, glucose uptake with 2-NBDG as probe and adipogenesis. All the analysis was done with flowcytometry. **Results:** The results showed that the extract did not possess any significant glucose uptake activity but it exhibited significant adipocyte differentiation potential. **Conclusions:** Ethyl acetate extract of *Curcuma longa* exhibits significant antiadipogenesis and can be used as an effective drug for the treatment of obesity and other associated complications.

1. Introduction

Metabolic syndrome describes the human condition characterized by the presence of coexisting traditional risk factors for cardiovascular disease, such as hypertension, dyslipidemia, glucose intolerance, and obesity, in addition to non traditional risk factors, such as inflammatory processes and abnormalities of the blood coagulation system[1]. Obesity currently affects over a billion of people. It is the growing health problem in many of the richest nations of the world and should now be considered as a chronic disease that is reaching epidemic proportions[2]. It acts as a major risk factor for type 2 diabetes and cardiovascular diseases. Most drugs used for treating type 2 diabetes causes obesity as a side effects by reducing blood glucose level and inducing adipogenesis. Traditional medicinal can serve as an ideal candidate in treating obesity and type 2 diabetes by acting on adipocytes and can act as a better alternative for the treatment of metabolic syndromes.

Curcuma longa (Family: Zingiberaceae) is reported to possess many medicinal properties including cardioprotective, hypolipidemic, antibacterial, anti-HIV, anti-tumour, anti-carcinogenic and antiarthritic activities[3].

So the present study aims to evaluate the modulatory effects of *Curcuma longa* extract against glucose uptake and adipocyte differentiation in 3T3L1 and L6 cells

2. Materials and methods

2.1. Chemicals

All the chemicals were purchased from Sigma-Aldrich (Saint Louis, MO, USA) unless or otherwise stated.

2.2. Preparaton of *Curcuma longa* extract

Fresh turmeric rhizomes were dried at 40–45 °C, ground into powder and extracted (250 g) successively with hexane, ethyl acetate, methanol and water at room temperature. Ethyl acetate fraction contains highest amount of curcuminoids was further taken to evaluate its biological

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properties.

2.3. Determination of the subtoxic concentration of *Curcuma longa* by MTT assay

Cytotoxicity of the extract was evaluated against L6 and 3T3L1 after 24 h incubation by MTT assay. The method was carried out as per previously described protocol[4].

2.4. Glucose uptake assay by 2-NBDG

Glucose uptake assay was performed according to the previous method[5] in L6 rat skeletal muscle cells with fluorescent analogue of glucose, 2-NBDG (10 μ M) using flow cytometry (BD FACS Aria II, USA). Insulin (100 U) and rosiglitazone (100 nM) served as control.

2.5. Adipocyte differentiation assay

Effect of *Curcuma longa* extract on inhibiting differentiation of 3T3-L1 preadipocyte to mature adipocyte was carried out based on reported protocol[6] using flow cytometry (BD FACS Aria II, USA).

2.5. Statistical analyses

The experimental results were expressed as mean \pm standard deviation (SD) of triplicate measurements. The data were subjected to one way analysis of variance and the significance of differences between means were calculated by Duncan's multiple range test using SPSS for Windows, standard version 7.5.1, and the significance accepted at $P < 0.05$.

3. Results

3.1. MTT assay

To determine the sub toxic concentrations against 3T3L1 and L6 cell lines, *Curcuma longa* extract was evaluated at 10, 50, 100 and 200 μ g/mL concentrations and cell viability was determined by MTT assay. Results showed *Curcuma longa* extract exhibited toxicity in both the cell lines after a concentration of 100 μ g/mL.

3.2. Glucose uptake assay

Effect of *Curcuma longa* on glucose uptake was checked by 2-NBDG based assay. L6 cells were pre-incubated with extract at different concentrations from 1–100 μ g/mL. Results indicated that *Curcuma longa* was not able to enhance uptake of 2-NBDG at subtoxic concentrations (Figure 1). The effect was compared with standard drug rosiglitazone (100 nM), which showed 38.5% increase in probe uptake. Uptake potential of cells showed a reduction at 100 μ g/mL concentration compared to control. This might be due to slight toxic effect of extract against L6 cells.

3.3. Antiadipogenic effect of *Curcuma longa* extract

Around 10 000 events were recorded and analyzed by flow cytometer based on difference in size and granularity which gives a separation between undifferentiated cells and differentiated cells. Results showed that within a concentration 1 and 5 μ g/mL, the extract seemed to reduce adipocyte differentiation and lipid droplet content by 22% (Figure 2).

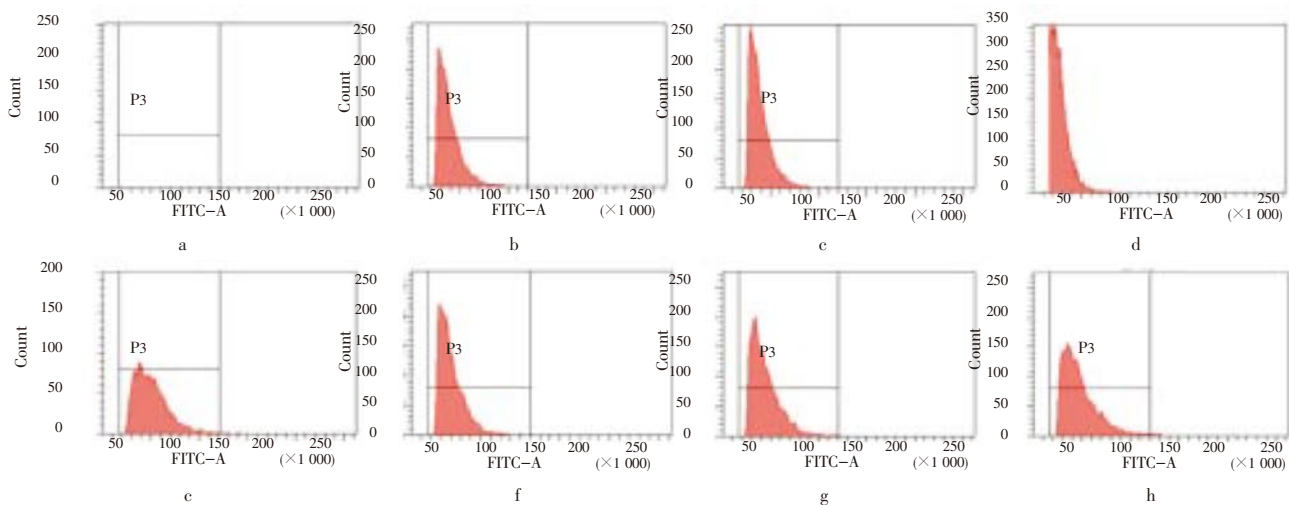


Figure 1. Glucose uptake effect of *Curcuma longa*.

a) Unstained control; b) NBDG control; c) Insulin treated; d) Rosiglitazone treated; e–h) Extract treated at 1, 10, 50 and 100 μ g/mL concentration.

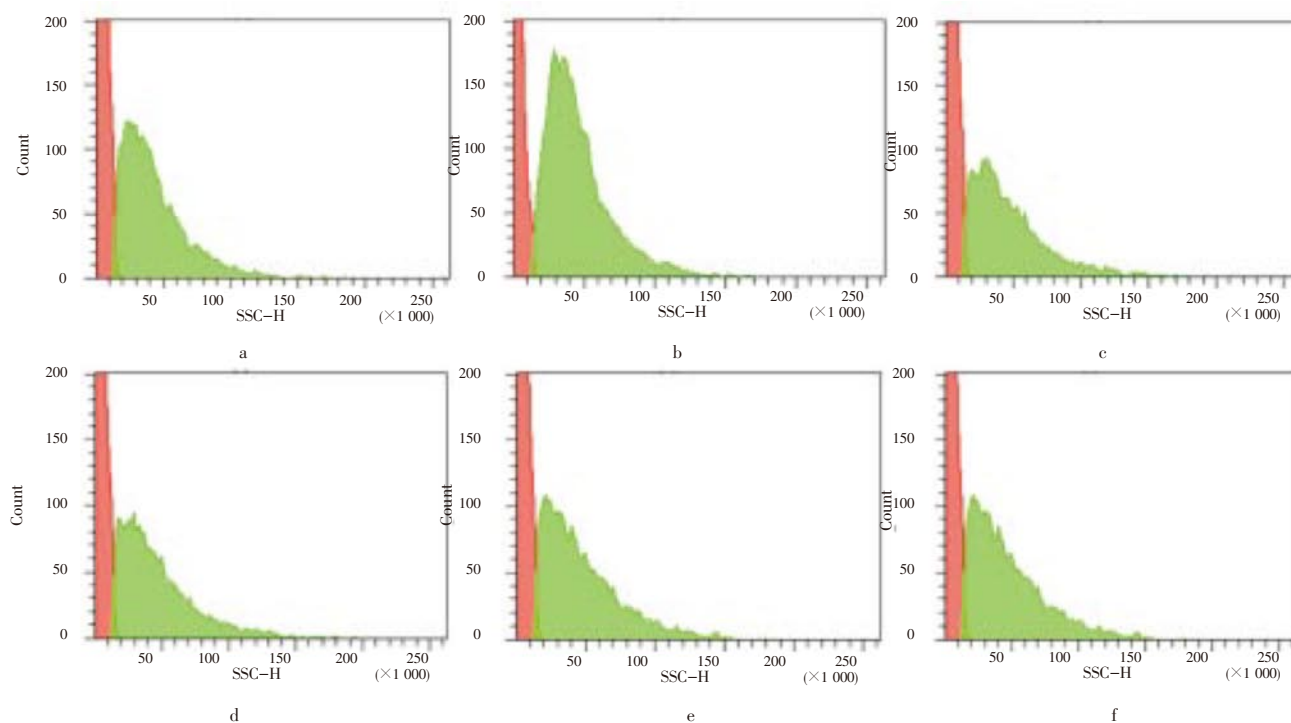


Figure 2. Change in percentage of differentiated cells upon extract treatment. a) Untreated control, b) MDI treated cells, c–f) cells treated with different concentration of extract (1–50 μ g/mL).

4. Discussion

Adipocyte differentiation inhibitors may be effective in preventing obesity, atherosclerosis, diabetes and other associated complications. 3T3–L1 cells are known to differentiate into adipocytes under the appropriate conditions[2] and have been useful as a model for adipose cells, which are one of the major sites of lipid and glucose metabolism. Overweight and obesity are the result of excessive adipogenesis and therefore inhibition of the differentiation of 3T3–L1 cells to adipocytes is beneficial for the prevention of obesity complicated by diabetes and atherosclerosis[2]. The inhibition potential of *Curcuma longa* was found to be dose dependant suggesting that *Curcuma longa* may have altered the expression of any genes involved in the adipocyte expression.

In conclusion, the present study reveals that *Curcuma longa* extract didn't exhibit any significant glucose uptake activity but it can inhibit adipocyte diffrenciation and can serve as an ideal candidate for the management of obesity and associated metabolic syndromes.

Conflict of interest statement

We declare that we have no conflict of interest.

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