Antiadipogenic activity of homoeopathic preparation of *Chelidonium majus* employing 3T3-L1 cell line as a model

Chinmay Gawade¹, Parth Aphale², Dharmendra Sharma³, Ramesh Bhonde⁴, Avinash Sanap⁵, Avinash Kharat⁶

1-III BHMS Student, Dr. D.Y. Patil Homoeopathic Medical College & Research Centre, Dr. D.Y. Patil Vidyapeeth (Deemed to be University), Pimpri, Pune, Maharashtra, India
2-Professor & HOD, Department of Homoeopathic Pharmacy, Dr. D.Y. Patil Homoeopathic Medical College & Research Centre, Dr. D.Y. Patil Vidyapeeth (Deemed to be University), Pimpri, Pune, Maharashtra, India
3-Principal, Professor & HOD, Department of Forensic Medicine & Toxicology, Dr. D.Y. Patil Homoeopathic Medical College & Research Centre, Dr. D.Y. Patil Vidyapeeth (Deemed to be University), Pimpri, Pune, Maharashtra, India
4-Scientist Emiritus, Regenerative Medicine Laboratory, Dr. D.Y. Patil Vidyapeeth, (Deemed to be University), Pimpri, Pune, Maharashtra, India
5,6-Scientist C, Regenerative Medicine Laboratory, Dr. D.Y. Patil Dental College and Hospital, Dr. D.Y. Patil Vidyapeeth, (Deemed to be University), Pimpri, Pune, Maharashtra, India

parth.aphale@dpu.edu.in – https://orcid.org/0000-0002-1004-2605

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ABSTRACT

Adipocytes are derived from mesenchymal stem cells through the process of adipogenesis. Adipocyte metabolism is abnormal in a range of disorders like obesity, nutritional insufficiency, and diabetes. The 3T3-L1 preadipocytes are most often used to create adipocyte models, and they can be differentiated into adipocyte cells under the right conditions. Homeopathic preparation of *Chelidonium majus* (HPCM) 30cH in the management of Obesity and Type 2 diabetes mellitus (T2DM) has much evidence in clinical practice. The 3T3-L1 preadipocyte cells were differentiated into adipocytes by using a differentiation cocktail. Cells were treated with HPCM 30cH attenuation at the concentration of 0.5%, 1%, and 2% for 15 days. Oil O Red Staining was used to assess lipid droplet (LD) accumulation. In order to determine the lipid content in 3T3-L1 adipocytes, cells were dissolved in isopropanol and the absorbance values were measured. Images were captured and analysed using ImageJ software. We investigated the action of HPCM in obesity using the 3T3-L1 adipogenesis model employing Human Mesenchymal Stem Cells (huMSC’s). After differentiation of adipocytes many LDs were formed in 3T3-L1 preadipocytes which can be compared with no lipid droplet in 3T3-L1 non-differentiated cells. Further, area of differentiated adipocytes was mapped and then compared for LD accumulation in control and HPCM to check its anti-adipogenic activity. A significant reduction in the accumulation of lipid droplets was seen in 0.5%, 1% and 2% concentration of HPCM as compared to control during the differentiation of 3T3-L1 preadipocytes into adipocytes. Excessive differentiation of cells and high fat accumulation in the adipose tissue are closely linked to obesity. Preadipocyte differentiation inhibitors may have preventive and therapeutic potential as anti-obesity drugs. HPCM 30cH has the potential to act as an antiadipogenic agent which can be used to combat various diseases like obesity, T2DM, and cardiovascular diseases.

Keywords: Adipogenesis, high-dilution, diabetes, obesity, homeopathy, *Chelidonium majus*
INTRODUCTION

At various sites in the body, adipogenesis takes place in which adipocytes store energy as fat (1). Adipocytes are the major energy storage sites in the body, and they also have critical endocrine functions (2). Adipocyte cells contain a large single lipid droplet surrounded by a unilocular layer of cytoplasm. A typical fat cell is 0.1 mm in diameter with some being half that size, and others twice that size. The nucleus is pushed to the periphery and flattened. However, these numerical estimates of fat cell size largely depend on the location of the adipose tissue and measurement method (3). The fat stored is in a semi-solid state and is composed primarily of ester and triglycerides (4). The proliferation and differentiation of adipose tissue are linked to the tissue’s function. The process by which adipocytes develop and accumulate as adipose tissue is called adipogenesis (5). Adipocytes are derived from mesenchymal stem cells. Adipocyte metabolism is abnormal in a range of disorders like obesity, diabetes, and nutritional insufficiency (6). Due to the rising prevalence of disorders of lipid metabolism in vitro studies of adipocytes are popular. The 3T3-L1 preadipocytes are used to create models of adipocytes, and they can be differentiated into adipocyte cells under the right conditions (7). The most important factor in the process of adipogenesis is the signalling pathway and this process could give effective methods for problems of lipid metabolism (8). Thus, preventive and therapeutic potential as anti-obesity drugs may be seen in inhibitors of preadipocyte differentiation. Adipocytes consist of adipose tissue which is a loose connective tissue. As an endocrine organ and reservoir of energy and through secretion of adipokines this tissue is known to play a role in the regulation of energy metabolism for the body. However, due to deregulated adipocyte differentiation, more expanse of the adipose tissue is linked to the development of obesity and related disorders (9).

Obesity is the increase in fat mass, through hypertrophy and, to a lesser extent, cell proliferation and hyperplasia (10). Adipocytes can synthesize estrogen from androgens potentially being the reason why being underweight or overweight are risk factors. Adipocytes are responsible for the production of the hormone leptin. Leptin is important in the regulation of appetite and acts as a satiety factor (11). Despite the evidence suggesting the role of homeopathic preparation of *Chelidonium majus* (HPCM) in the management of Obesity and type 2 diabetes mellitus (T2DM)- in clinical practice, its mechanism of action remains unclear (12). One such example is hypolipidemic effect of *Chelidonium* by analysing intracellular lipid content role of homeopathic medicine was established but mechanism of action remained unknown (13).

*Chelidonium majus* has the active compound berberine (BBR) which has been investigated earlier on various metabolic pathways. However, the active compound BBR in Homeopathic preparations of *Chelidonium majus* (HPCM) has not been investigated to manage T2DM and obesity (14). Therefore, we investigated the mechanism of action of HPCM 30cH using the 3T3-L1 adipogenesis model in obesity employing Human Mesenchymal Stem Cells (huMSC’s) (15).

The Oil red O staining is used in many different works to quantify the adipose conversion and triglycerides and accumulation of lipids in tissue. The integrated density of the lipid droplets (LDs) was used to quantify the lipid accumulation in adipocytes which was found significantly increased in obesity and reduced with weight loss (16).

The present study investigated HPCM in different concentrations and
studied its antiadipogenic effect on 3T3-L1 preadipocytes which was evidenced by mapping the area of lipid accumulation.

**AIM AND OBJECTIVES**

The aim of this work was to study the antiadipogenic activity of homeopathic preparation of Chelidonium majus employing a 3T3-L1 cell line as a model.

**METHODOLOGY**

**Cell line and Cell culture** (14)

To investigate the antiadipogenic effect of HPCM, we have employed 3T3-L1 cell line as a model.

3T3-L1 preadipocyte cells were differentiated into adipocytes by using a differentiation cocktail.

**Differentiation cocktail** (14)

It includes: (dexamethasone 1 mmol/L, IBMX 500 mmol/L, and insulin 100 nmol/L).

Cells were treated with HPCM attenuation at the concentration of 0.5%, 1% and 2% for 15 days. Oil O Red Staining was used to assess LD accumulation. Each experiment was performed in triplicate.

3T3-L1 preadipocytes were washed with Phosphate buffered saline (PBS).

**Oil O Red staining** (15)

Cells were washed with sterile double distilled water and subsequently with 60% isopropanol for 2 min and stained with a filtered 0.35% Oil Red O solution in 60% isopropanol for 10 min at room temperature.

**Staining** (15)

Stained with solution of oil O red for 10 minutes. The stained cells were washed four times with double distilled water.

To determine the lipid content in 3T3-L1 adipocytes, cells were dissolved in isopropanol and the absorbance values were measured.

**Images and statistical analysis**

Images were observed in an Olympus camera (JVC) and analysed and stored using the software image J. The data was reported as mean ± SD. Image J software was used to analyse the significant differences between the means of different groups through t-test. Each LD accumulation area was measured, and graphs were plotted as mapped area (pixel) versus test substance. Quantitative assessment for all concentrations in terms of LD accumulation was performed and Data was expressed as means ± SD. This was followed by one way ANOVA followed by the Tukeys Multiple Comparison test. For all results, p < 0.05 was considered statistically significant. Each experiment was performed in triplicates (n=3).

**RESULTS**

After measuring the LD accumulation using mapped area of each group, it was evident that LD accumulation in control was the highest (2689059 ± 134453). LD accumulation was seen highest in HPCM 2% (1349029 ± 67451.45) among the HPCM attenuations and LD accumulation was lowest in HPCM 1% (352960 ± 17648). For HPCM 0.5% LD accumulation was seen more than in HPCM 1% (894959 ± 44747.95). The mapped area in the control was seen to be more in comparison to all HPCM attenuations. Mapped area of each group indicating LD accumulation was graphed in figure 1 and figure 2 shows images of 3T3-L1 adipocytes with accumulation of LDs.
**Figure 1.** Graph showing LD accumulation in 3T3-L1 via suppression of adipogenesis by HPCM. Data are expressed as mean ± SD (n = 3). (∗<0.05, ∗∗<0.01, and ∗∗∗<0.001 versus the control group). The control group was the cell lines treated only with differentiated medium.

**Figure 2.** Images of LD accumulation seen in Control, CM0.5%, CM 1%, CM 2%, and mapped area of each group for analysis. Red spots in images represent area stained by Oil Red O Dye.
DISCUSSION

Metabolism of Adipocytes is abnormal in disorders like obesity, nutritional insufficiency, and diabetes. Preadipocyte differentiation in excess will result in high fat accumulation in the adipose tissue which is closely linked to obesity development in the human body. Thus, preventive, and therapeutic potential as anti-obesity drugs may be seen in inhibitors of preadipocyte differentiation. Obesity is increase in size of the cells and increase in number of cells, to prevent, the drug should have the potential to inhibit differentiation of preadipocytes (15).

Obesity is forerunner of various metabolic disorders such as T2DM and cardiovascular diseases. *Chelidonium majus* 30cH (0.5%, 1% and 2%) was employed in this study and different concentrations showed strong antiadipogenic effect on 3T3-L1 preadipocytes which was evidenced by mapping area of lipid accumulation during the differentiation of 3T3-L1 preadipocytes into adipocytes (16).

On differentiation of huMSC’s into 3T3-L1 adipocytes, many LDs were accumulated in 3T3-L1 adipocytes which can be compared with no lipid droplet accumulation in undifferentiated cells. The mapped area in the control is more in comparison to Homeopathic preparation of *Chelidonium majus* 30cH (0.5%, 1% and 2%) which indicates that LD accumulation is more in control than that of HPCM which is evident of the antiadipogenic activity of HPCM.

CONCLUSION

In the present study, as detected by the Oil Red O staining, LD accumulation was markedly inhibited by treatment with HPCM. Quantitatively it was seen that LD accumulation is higher in control than in HPCM which shows an antiadipogenic activity of homeopathic preparation of *Chelidonium majus*. A significant reduction in the accumulation of lipid droplets was seen in 0.5%, 1%, and 2% concentrations of HPCM in comparison with the control, which shows that the given preparations are antiadipogenic.

In this experiment, homeopathic preparations of *Chelidonium majus* (HPCM) have been investigated which showed the potential to act as an antiadipogenic agent which can be used to combat various diseases like obesity, T2DM and cardiovascular diseases. In sum, we demonstrated that HPCM has antiadipogenic effects on 3T3-L1 preadipocytes. It will further help to study the action of these homeopathic medicines as a potential line of treatment in patients suffering from metabolic disorders such as T2DM. Further research is needed to study the potential of these and other homeopathic medicines as Antiadipogenic.

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Ethical Statement

The study was presented before the Institutional Ethics Committee which approved the study.

Abbreviations

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<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>huMSC’s</td>
<td>Human umbilical Mesenchymal stem cells</td>
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<tr>
<td>HPCM</td>
<td>Homeopathic preparation of Chelidonium majus</td>
</tr>
<tr>
<td>CM</td>
<td>Chelidonium majus</td>
</tr>
<tr>
<td>BBR</td>
<td>Berberine</td>
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<tr>
<td>LD</td>
<td>Lipid droplets</td>
</tr>
<tr>
<td>RT</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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References


10.1210/endrev/bnab018. PMID: 34100954; PMCID: PMC8755996.


