

Effect of Crocin on Adipogenic transcriptional factors in *in vitro* model of Obesity

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ABSTRACT

Objective:

To investigate the effects of Crocin treatment on key adipogenic transcriptional factors in 3T3-L1 pre-adipocytic cell line.

Material and methods:

To elucidate the mRNA expression levels of Peroxisome Proliferator Activated Receptor-gamma (PPAR- γ) and Sterol Regulatory Element Binding Protein 1c (SREBP-1c), RNA isolation followed by quantitative Real Time PCR was carried out.

Results:

Crocin treatment significantly down-regulates the expression levels of key adipogenic transcriptional factors.

Discussion:

Crocin has shown several beneficial effects like anti-cancer, anti-oxidant, anti-hyperlipidemic and anti-diabetic. Taken this into consideration, we wanted to explore its effect on adipogenesis in *in vitro* model of Obesity.

Keywords: Adipocyte differentiation, Crocin, 3T3-L1 pre-adipocytic cell line, Obesity.

I INTRODUCTION

Obesity is a condition characterised excessive storage of triglycerides in the adipose tissue that leads to severe consequences [1]. Obesity is associated with several medical disorders like Type 2 Diabetes, Hypertension, Cardiovascular diseases, liver abnormalities, respiratory problems and hormonal changes [2]. Obesity is increasing at huge numbers and the risk factors linked with the disease pose a great threat to the entire world [3]. There is increasing prevalence of obesity both in developed and developing countries as well. There is an urgent need to combat the disorder by screening the compounds from natural and synthetic sources. Currently, various natural anti-oxidants are screened to possible role in the treatment of metabolic disorders [4,5].

Among the different constituents of saffron, Crocin has been found to be the most important chemical constituent. Crocin (C₄₄H₄₀O₂₄) is a di-ester which is formed from the disaccharide gentiobiose and the dicarboxylic acid crocetin and is considered as one of the few naturally occurring carotenoids easily soluble in water [6-8]. Crocin has shown various pharmacological activities such as anti-oxidant [9], neuroprotective [10],

anti-hyperlipidemic [11, 12], anti-diabetic [13-15] and anti-carcinogenic [16] properties due to its pharmacological effects. The anti-hyperglycaemic effects of saffron and its constituents have been observed in alloxan induced diabetic rats [17]. However, effect on adipocyte differentiation by treatment with Crocin is little known. Thus, our present study will play an important role in understanding the effect of a natural compound and the mechanisms through which a particular nutrient affects the differentiation to adipocytes. This study would eventually help to look for the compounds that prevent the initiation and progression of obesity.

II MATERIALS AND METHODS

2.1. Cell Culture:

3T3-L1 mouse embryonic fibroblasts were obtained from National Centre for Cell Science (Pune, India), maintained at 37°C in a humidified 5% CO₂ atmosphere and cultured as per standard protocol. Briefly, cells were cultured in Dulbecco's modified Eagles medium (DMEM) containing 10% (v/v) Fetal Calf Serum (FCS), 100 U/ml of penicillin and 100 µg/ml of streptomycin, until confluent. Two days after confluence (Day 0), the cells were stimulated to differentiate with differentiation media (DI media) consisting of DMEM, 10% FCS, 167 nM insulin, and 0.5 µM Isobutylmethylxanthine and 1 µM Dexamethasone for two days (Day 2). The differentiation media was replaced by DMEM+10% FCS+ 167 nM insulin for next two days (Day 4), followed by culturing with DMEM+10% FCS for additional 4 days (Day 8), at which about 90% of cells were found to be mature adipocytes with fat droplets.

2.2. Quantitative Real Time PCR:

To detect mRNA expression levels of key transcriptional factors, the cells were cultured and Crocin at different doses was also introduced into the media. Total RNA was extracted from differentiated cells on day 8. Trizol reagent was used for the extraction of RNA.. The quantity and integrity of total RNA was determined by 1.5% denaturing agarose gel. After DNase treatment, RNA was subjected to reverse transcription using Revert aid First strand cDNA synthesis kit (Thermo scientific) as per manufacturer's protocol. cDNA synthesis was performed as follows 25.0°C, 7 min; 40.0°C, 60 min; 65°C, 15 min. To determine the expression level of each gene qRT-PCR was done with specific primer pairs for each gene (PPAR- γ and SREBP1c). The gene-specific forward and reverse primers were designed using NCBI-Primer Blast software. β -actin was used as endogenous control. This was followed by PCR amplification using Maxima SYBR® Green mix (Thermo Scientific) in Real Time PCR 7500 as per manufacturer's protocol. All the reactions were run in triplicates. Specificity of PCR products was checked by met curve analysis and agarose gel visualisation of amplified products.

PPAR- γ	FORWARD PRIMER 5-AGGCCGAGAAGGAGAAGCTGTTG-3
	REVERSE PRIMER 5-TGGCCACCTCTTTGCTCTGCTC-3
	PRODUCT LENGTH 276bp
SREBP1c	FORWARD PRIMER 5-GGAGCCATGGATTGCACATT-3
	REVERSE PRIMER 5-GCTTCCAGAGAGGAGGCCAG-3
	PRODUCT LENGTH 182bp
ACTIN	Forward primer 5-TTCCTTCCTGGGCATGGAGT-3
	Reverse primer 5-CTGTGTTGGCGTACAGGTCT-3
	PRODUCT LENGTH 101bp

2.3 Statistical Analysis

Results represent means $\pm$ SD of three independent experiments. Statistical analysis was performed using One Way ANNOVA by Dunnett's multiple comparison tests utilizing Graphpad prism 5 software. Statistically significant differences are defined at the 95% confidence interval (* $P < 0.05$, ** $P < .01$).

III RESULTS

3.1 Crocin inhibits mRNA expression levels of Sterol Regulatory Element Binding Protein 1 (SREBP-1c)

SREBP-1c is a key protein required for glucose metabolism and fatty acid and lipid production. SREBP-1c is

responsible for regulating the genes required for *de novo* lipogenesis. It activates a cascade of genes required for endogenous lipogenesis and pre-adipocyte differentiation (fatty acid synthase, HMG-CoA synthase, LDL-receptor, adipocyte determination, and differentiation factor 1).

In the present study quantitative RT-PCR was used to examine the effects of different doses of Crocin on the expression levels of SREBP-1c in 3T3-L1 pre-adipocytic cell line. The mRNA was isolated and subjected to cDNA synthesis and finally real time PCR was performed as per section 2.2 SREBP-1c expression was significantly inhibited with increase in the concentration of Crocin. The values represent SREBP-1c expression levels normalized to β -actin relative to the normalized expression of SREBP-1c gene in control cells.

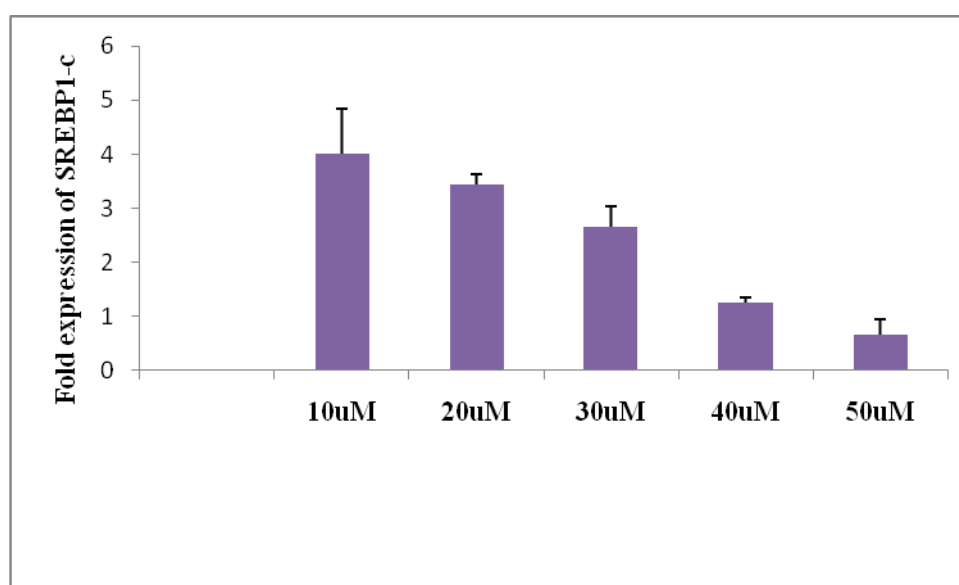


Fig 3.1: Quantitative Real-Time PCR to determine the relative expression of SREBP-1c in cells treated with increasing concentration of Crocin. Values are expressed as fold expression of SREBP-1c in different groups relative to normal group and expression in each group was normalized to β -actin.

3.2. Crocin inhibits adipogenesis by downregulating PPAR- γ in 3T3L1 cells

PPAR- γ is a key transcriptional factor involved in regulating adipocyte differentiation. It regulates fatty acid storage and glucose metabolism. The genes activated by PPAR- γ stimulate lipid uptake and adipogenesis by fat cells. As shown in Fig 3.2. There is a decrease in the expression levels of PPAR-gamma with Crocin at a dose dependent manner. The expression of PPAR gamma is highest in the cells which are treated with low doses of Crocin. However, there is a significant fold reduction in the mRNA expression at higher doses of Crocin.

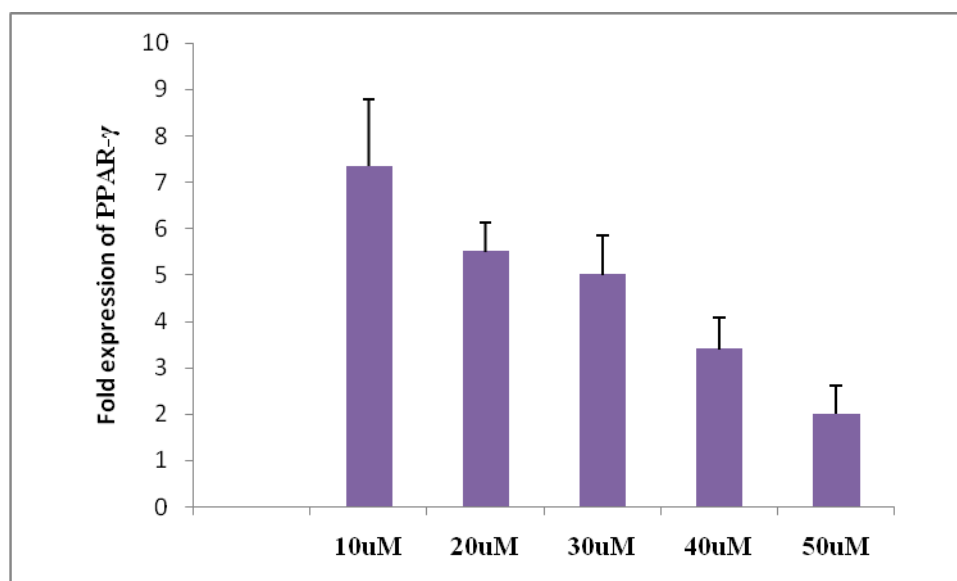


Fig 3.2. Quantitative Real-Time PCR to determine the relative expression of PPAR- γ in cells treated with increasing concentration of Crocin. Values are expressed as fold expression of PPAR- γ in different groups relative to normal group and expression in each group was normalized to β -actin.

IV DISCUSSION

The growth and proliferation of preadipocytes have a profound implication in the development of obesity therapeutics. The increase in adipose tissue mass is determined by the number and size of adipocytes which depends upon the proliferation and differentiation of preadipocytes into adipocytes [18]. To know the effect of test compounds on adipocyte differentiation, 3T3-L1 pre-adipocytic cell line was used as an *in vitro* model for the study. The pre-adipocytes were stimulated to differentiate into mature adipocytes by using a standard adipogenic medium that contains insulin, isobutylmethylxanthine and dexamethasone. Adipocytes precursor cells when exposed to differentiation media were able to undergo full maturation into adipocytes. Consequently, this study shows that Crocin has an anti-adipogenic effect and speculate that Crocin be an active medication to treat obesity and related diseases. However the study needs further investigation.

CONFLICT OF INTEREST: None

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