PPAR-γ down regulation in 3t3l1 adipocytes by *Solanum xanthocarpum* Schrad & Wendl

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ABSTRACT

Adipogenesis is a multistep process involving a number of transcription factors and cell cycle proteins that regulate gene expression leading to adipocyte development.PPARs are ligand activated and ligand binding nuclear receptors that respond to exogenous and endogenous ligand .They act by modulating genes related to lipid, insulin and glucose homeostasis. Solanum xanthocarpum is a plant with proven anti diabetic efficacy since it was found to possess and found to promote GLUT-4 uptake in L6 skeletal muscle cell lines and 3T3 L1 cell lines. The present study was aimed at examining its anti-dyslipedimic potential in T2DM.The results showed that an ethanolic extract of the plant Solanum xanthocarpum (SXA) in the dose of 100 µg/ml produced down-regulation of PPAR γ gene expression in 3T3L1 adipocytes reflecting its potential as an anti dyslipedimic agent.

Keywords: down regulation,dyslipidemia,Solanum xanthocarpum,gene expression.
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INTRODUCTION

PPAR-γ is a nuclear receptor expressed in adipose tissue and liver and regulates lipostorage in glucose metabolism and is a target of oral anti diabetes drugs thiazolidinediones (TZDs).First generation of TZDs like troglitazone, rosiglitazone and pioglitazone exhibit high levels of toxicity. There arised a search for newer drugs, that exhibit better efficacy and lesser toxicity. Oral anti diabetes agents in general are associated with a number of adverse reactions like cardio vascular disease, myocardial infarction, osteoporotic fractures and all cause mortality.Dyslipidemia is a complication of T2 DM and administration of oral anti diabetes agents may worsen the condition..A lot of research is going on, for the development of newer drugs like non-TZDs like PPAR γ agonists, PPAR α/ γ dual agonists, PPAR pan agonists, PPAR γ antagonists and selective PPAR-γ modulating drugs. These agents are supposed to have better insulin sensitizing activity like TZDs and lower toxicity. Activation of PPAR γ leads to adipocyte differentiation, It is a marker of adipogenesis.The differentiation prior to lipid filling involves PPARγ.Lipid accumulation is dependent on cellular uptake of fatty acids and glucose. PPAR signalling events are important prior to GLUT4 recycling and glucose transport as regulated by insulin. PPAR γ is an indirect regulator of itself through its action on C/EBPγ .Plants, their constituents and their derivatives may act as PPAR ligands,thereby, producing beneficial effects in T2DM.Solanum xanthocarpum is a plant with proven antidiabetic efficacy since it was found to promote GLUT-4 uptake in L6 skeletal muscle cell lines and 3T3 L1 cell lines.(1,2,3,4,5,6,7,)

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MATERIALS AND METHODS

Plant material
The plant Solanum xanthocarpum was collected in summer when the fruits were ripe,they were shade dried,coarsely powdered and extracted with ethanol and the extract was diluted according to the requirement.

Chemicals and reagents
Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), Dexamethazone, 3-iso butyl-1-methyl xanthine (IBMX), Insulin, Oil-O-red stain and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA and Antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India. PCR reaction- Aristogene,Primers-Eurofins,PCR- Mj mini, Bio Rad USA,Gel Doc- UVP, USA

Cell culture and treatment
Mouse 3T3-L1 preadipocytes cells were maintained in DMEM containing 10% calf serum.For the differentiation,postconfluent 3T3-L1 preadipocytes were treated with DMEM containing 10% FBS,1µg/mL insulin,0.5mM 3-isobutyl-1-methylxanthine ,and 1µM dexamethasone for 2 days and were then treated for 2 dats with DMEM containing 10µg/mL insulin and 10% FBS.Thereafter,cells were maintained and refed every 2 days with DMEM containing 10% FBS.With this protocol,>80% adipocyte differentiation was achieved.3T3-L1 cells were treated with a dose of 100µg/mL of SXA.Along with the differentiation medium containing insulin,dexamethazone and IBMX since day 0..Control cells were treated with the same volume of DMSO.On the sixth day,adipocytes were incubated in low glucose DMEM containing 2% w/v fatty acid free BSA and were serum starved for 24 hours.On the 10th day the RNA were extracted for further analysis.The reverse transcription quantitative PCR analysis were carried out using Mj mini ,Bio Rad USA. (8)

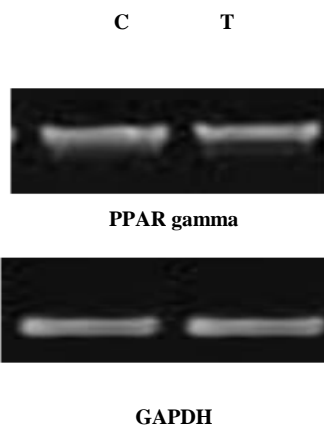| MEDIUM	FORMULATION
Pre-adipocyte Expansion	90% Dubelcco's Modified Eagle's Medium (DMEM), and 10% Bovine Calf Serum
Differentiation	90% DMEM, 10% Fetal Bovine Serum (FBS), 1.0 µM Dexamethasone, 0.5 mM Isobutylmethylxanthine (IBMX), and 1.0 µg/ml Insulin
Adipocyte Maintenance	90% DMEM, 10% FBS, and 1.0 µg/ml Insulin

C   T

PPAR gamma

GAPDH
Fig: RT-PCR profile of PPARγ gene amplified from SXA treated adipocytes

C- Cell control.
T - Cells treated with test product 200 µg/ml

RT-PCR PROCEDURE
The mRNA expression levels of PPARγ carried out using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Briefly, the adipocytes were cultured in 60 mm petridish ,to the dish was added the required concentration of SXA (100 µg/ml) and incubated for 24 hr. Total cellular RNA was isolated from the untreated (control) and treated cells using Tri Reagent according to manufacturer's protocol. cDNA was synthesized from total isolated RNA by reverse trancriptase kit according to manufactures instructions (Thermo scientific). Then 20µl of the reaction mixture was subjected to PCR for amplification of PPARγ cDNA using specifically designed primers

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procured from Eurofins India, As an internal control, the house keeping gene GAPDH was co-amplified with each reaction.

Amplification conditions for PPARγ gene:
95°C for 1 min followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 64°C for 30 seconds and extension at 72°C for 45 sec. This was followed by final extension at 72°C for 10 min.

Primer used:
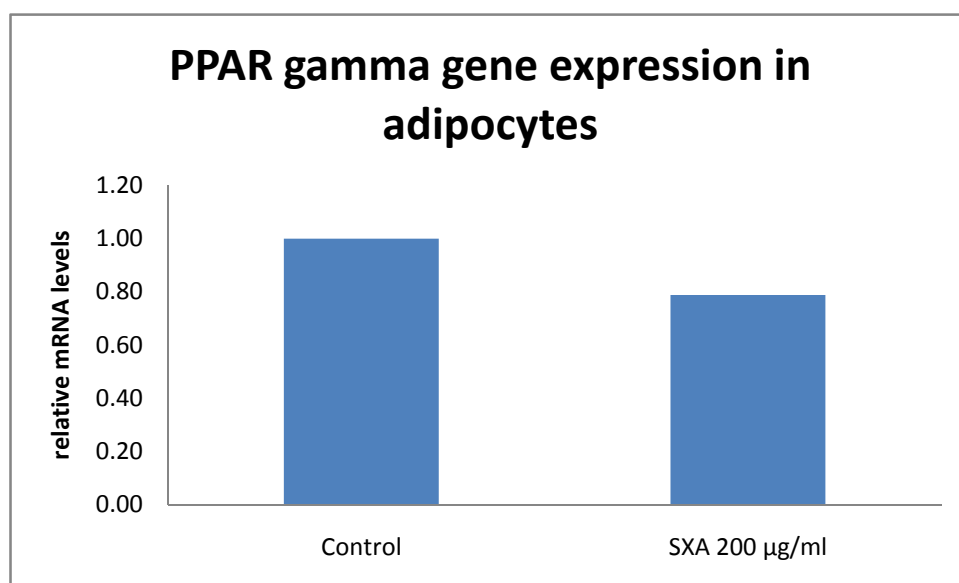*For I strand synthesis:*   oligo dT
For II strand synthesis:

    5' GGATTCATGACCAGGGAGTTCCTC 3'–Forward
    5' GCGGTCTCCACTGAGAATAATGAC 3'- Reverse

Product size: 155 bp.

Fig.2. Densitometric analysis of gene transcripts. The relative level of PPARγ gene expression is normalized to GAPDH
Values shown depict arbitrary units.

Test sample	Control	Treated ((100 µg/ml)
Regulation in terms of Folds	1	0.79

INFERENCE
In this study, the results showed down regulation of PPARγ gene in test samples (Treated with SXA) when compared with the Control. The test sample treaed 100 µg/ml of SXA WAS 0.27 folds down regulated compared to control.

CONCLUSION

Thazolidinediones like troglitazone produce downregulation of PPARγ proteins in 3T1L1 celllines due to a decrease in the transcription of PPARγ mRNA.The antidyslipidemic and antiadipogenic potential of the ethanolic extract of Solanum xanthocarpum is elucidated through its modulation of PPARγ gene expression in 3T3L1 adipocytes.A decrease in PPARγ number would increase insulin sensitivity through an increase in corepressor action.These data infer that the expression of PPARγ is auto-regulated in 3T3L1 cell lines.The downregulation of PPARγ gene is an indication of gene repression by the drug.Solanum xanthocarpum may be considered a potential drug because of the beneficial effects elicited ,in T2DM.The aerial parts of the plant is devoid of the atherogenic and cardiac adverse effects of oral antidiabetes drugs.(9,10)

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