Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2014, 5(3): 32-36

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Study of the effect of ethanolic extract of *Solanum xanthocarpum schrad & wendl* on Glu-4 and PPARγ gene expression in L6 cell lines

Anitha Mary Mathews[1]* and Christina A. J. M.[2]

[1]*Department of Pharmacology, Pushpagiri College of Pharmacy, Thiruvalla, Kerala, India*
[2]*Department of Pharmacology, Taylor's University, No. 1 Jalan Taylor's, Subang Jaya, Selangor Darul Ehsan, Malaysia*

ABSTRACT

Solanum xanthocarpum is a plant used in traditional and Alternative medicine in India and other parts of the world. The present study is aimed at investigating the effect of the ethanolic extract (SXA) on GLUT-4 and PPARγ gene expression. Type 2DM and obesity are associated with impairment in the regulation of GLUT 4 gene expression and elevated levels of free fatty acids. An increase in the expression of GLUT-4 gene is an index of adaptation to enhanced metabolic demand and improved metabolism of glucose. The action of PPARγ on muscle insulin sensitivity may be secondary to the lowering of circulating lipids on PPAR-gamma activation. Both GLUT-4 and PPARγ are regulators of glucose and lipid metabolism, allowing adaptation to the physiological environment. The results showed that the ethanolic extract of SXA in the dose of 100 µg/ml produced an upregulation of both GLUT-4 and PPARγ gene expression in L6 cell lines.

Key words: gene expression, *Solanum xanthocarpum*, L6 cell lines, GLUT4, PPARγ

INTRODUCTION

Type 2 DM is the most common endocrine disorder in India and elsewhere. Patients suffer from hypoinsulinemia and resistance to the actions of available insulin. Insulin-sensitive glucose transporter GLUT 4 is the glucose transporter in skeletal muscle, adipose tissue and heart and insulin helps in the translocation of GLUT4 isoform from the intracellular storage site to the cell membrane.(1)GLUT4 protein level is regulated by increased biosynthesis, reduced degradation or both.(2) Another mechanism may be activation of intrinsic activity.(3)The level of GLUT4 protein available for translocation is an important factor that determines the volume of glucose uptake. Studies in human as well as animal models have shown that GLUT4 gene expression is controlled at the transcription level. A number of studies have been carried out to show that GLUT4 knock-out results in insulin resistance (4) and over-expression results in restoration of insulin sensitivity and glucose disposal(5, 6). Since skeletal muscle is the major site of glucose utilization and disposal,L6 cell lines have been selected for studying the effect of test extract SXA on GLUT4 gene expression. Regulation of GLUT 4 gene expression is a potential target for the treatment of type 2DM.(7) The ethanolic extract of aerial parts of *Solanum xanthocarpum* was found to increase glucose uptake by GLUT4 in a dose of 100 µg/ml in a study conducted by the communicating author.(8)

PPARγ or glitazone receptor is a nuclear receptor encoded by PPAR-gamma gene. PPARγ regulates storage of fatty acids and glucose metabolism. It is implicated in the pathology of a number of diseases like diabetes, obesity and atherosclerosis. It is a key transcriptional factor that plays an important role in adipocyte differentiation in adipose

tissue and muscle.(9) We have enough evidence for a link between glucose homeostasis, lipid metabolism and mass of fatty tissue.(10)The receptor dependent activation of PPARγ genes might be responsible for the insulin sensitizing activity of potential drugs.(11)

MATERIALS AND METHODS

The aerial parts of the plant were collected when the fruits were ripe, shadow dried and extracted with ethanol to get a dry extractive. It was then diluted to get test samples of 1000 µg, 500 µg and 250 µg.

Chemicals and reagents

Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS) and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Sigma Aldrich Co, St Louis, USA. Amphotericin and EDTA from Hi-Media Laboratories Ltd., Mumbai. TRI Reagent from G Biosciences, USA, and Reverse transcriptase kit from Thermoscientific were used..Primers were bought from Eurofins India.

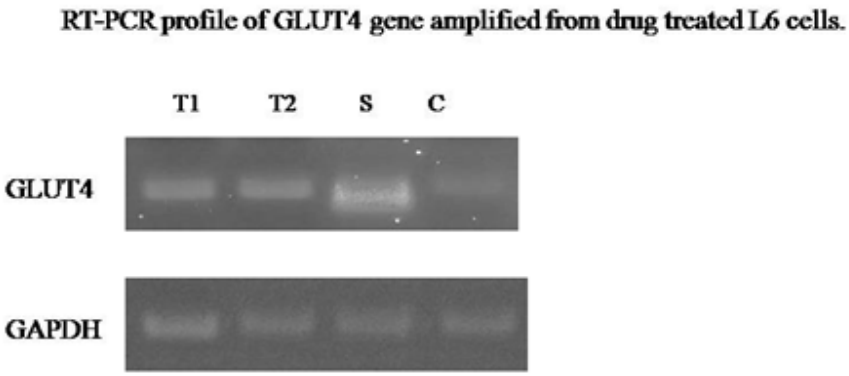
Cell lines and culture medium

L6 cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. Stock cultures of these cell lines were cultured in DMEM supplemented with 10% inactivated fetal bovine serum and Amphoterecin-B (5 mg/ml) in a humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in Phosphate Buffer Saline (PBS) solution. The stock cultures were grown in 60mm petridish.

Methodology for RT-PCR

The mRNA expression levels of Glut4 and PPARγ were carried out using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Briefly, the L6 cells were cultured in 60 mm petridish and maintained in DMEM medium for 48 hrs. The DMEM medium was supplemented with FBS and amphotericin. The required concentration of Test sample (1000 µg/ml) was added to the dish and incubated for 48 hours. 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 respective cDNAs using specifically designed primers procured from Eurofins India, As an internal control, the house keeping gene GAPDH was co-amplified with each reaction.

1.Effect of test extract SXA on GLUT4 gene expression



C- Cell control.
T1 - Cells treated with SXA 100 µg/ml
T2 - Cells treated with SXA 50 µg/ml
S - Cells treated with std. drug Rosiglitazone 50 µg/ml

Fig 1: RT-PCR profile of Glut4 gene amplified from SXA treated L6 cells

Amplification conditions for Glut4 gene:

95°C for 1 min followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 66°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: Random primer
For II strand synthesis:
5'CGGGACGTGGAGCTGGCCGAGGAG3'–Forward
5' CCCCCTCAGCAGCGAGTGA 3'- Reverse
Product size: 318 bp.

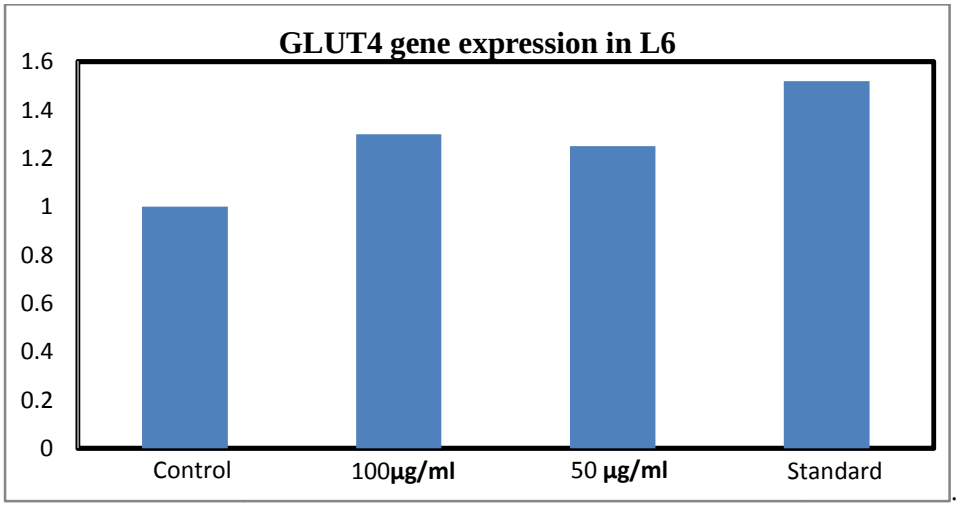
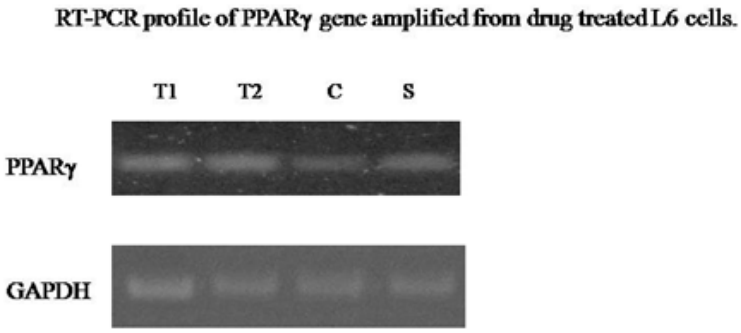


Fig.2. Densitometric analysis of gene transcripts. The relative level of Glut4 gene expression is normalized to GAPDH
Values shown depict arbitrary units

Test sample	Control	SXA 100µg/ml conc.	SXA 50 µg/ml conc.	Rosiglitazone 50 µg/ml
Regulation of GLUT4 in terms of Folds	1	1.29	1.20	1.52

2.Effect of test extract SXA on PPARγ gene expression



C- Cell control.
T1 - Cells treated with SXA 100 µg/ml
T2 - Cells treated with SXA 50 µg/ml
S - Cells treated with std. drug Rosiglitazone 50 µg/ml
Fig 3: RT-PCR profile of PPARγ gene amplified from drug treated L6 cells

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: Random primer
For II strand synthesis:
5' GGATTCATGACCAGGGAGTTCCTC 3'–Forward
5' GCGGTCTCCACTGAGAATAATGAC 3'- Reverse
Product size: 155 bp.

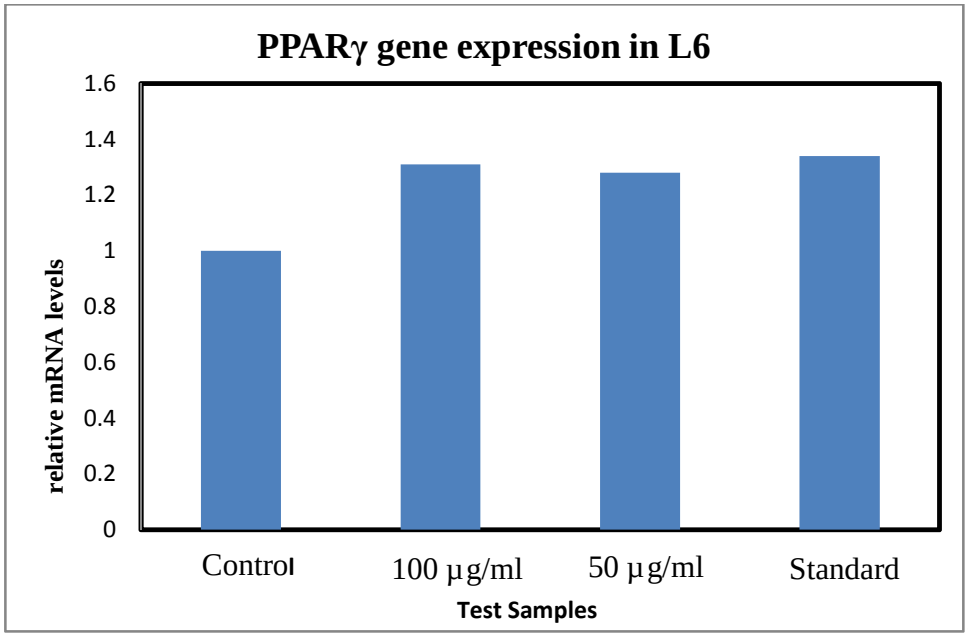


Fig.4. Densitometric analysis of gene transcripts. The relative level of PPARγ gene expression is normalized to GAPDH
Values shown depict arbitrary units

Test sample	Control	SXA100µg/ml	SXA50µg/ml	Rosiglitazone 50 µg/ml
Regulation of PPARγ in terms of Folds	1	1.33	1.24	1.34

DISCUSSION

The activation of PPARγ alters the transcription of several genes involved in glucose and lipid metabolism and energy balance. *Solanum xanthocarpum* is a plant with great potential in the management of type2 DM since it promotes the up-regulation of PPARγ as well as GLUT4 glucose transporter genes. The study proved that the plant extract can be used to reduce insulin resistance associated with type2 DM since the plant possess good insulin sensitizing properties. The plant promotes glucose uptake too, thereby improving glucose utilization and disposal in skeletal muscles. The insulinogenic property of the plant extract in rats had been proved by the communicating author in her previous studies.

CONCLUSION

In this study the results show up- regulation of GLUT4 gene in L6 cell lines in the presence of SXA and Standard drug rosiglitazone. When compared with the control, the test samples of 100 µg/ml and 50 µg/ml are 0.29 folds and 0.2 folds up- regulated respectively. The Standard drug rosiglitazone up- regulated GLUT4 expression by 0.52 folds from control L6 cell lines.

Results show up - regulation of PPARγ gene in cell lines treated with SXA and standard, when compared with the control. The test sample of 100 µg/ml and 50 µg/ml is 0.33 folds and 0.24 folds up – regulated the gene expression, respectively compared to control. The Standard drug rosiglitazone up- regulated PPARγ expression by 0.34 folds from control. The ethanolic extract of *Solanum xanthocarpum* in a dose of 100 µg/ml produced up-regulation of

both GLUT4 and PPARγ genes and the effects were only slightly lesser than that produced by rosiglitazone. It has been inferred that one of the mechanisms of action of SXA may be similar to that of thiazolidinediones.

REFERENCES

[1] Kahn, B. B. (**1996**) Lilly lecture 1995.. *Diabetes* 45, 1644 – 1654
[2] Watson, R. T., Kanzaki, M., and Pessin, J. E. (**2004**). *Endocr. Rev*. 25, 177 – 204
[3] Somwar R, Niu W, Kim DY, Sweeney G, Randhawa VK, Huang C, Ramlal T, Klip A. *J Biol Chem*. **2001** Dec 7;276(49):46079-87. Epub 2001 Oct 11.
[4] .ZismanA.Peroni,O.D.,Abel,E.D.,Michael,M.D.,MauvaisJarvis,F.,Lowell,B.B.,Wojtaszewski,J.F.,Hirshman,M.F. , Virkamaki, A.,Goodyear,L.J.,etal.(**2000**).*Nat.Med*.6,924-928.
[5] Carvalho,E.,Kotani,K.,Peroni,O.D.,andKahn,B.B.(**2005**).*Am.J.Physiol.Endocrinol.Metab*.289,E551-561
[6] Michael LF, Wu Z, Cheatham RB, Puigserver P, Adelmant G, Lehman JJ, Kelly DP and Spiegelman BM . Proc Natl Acad Sci USA 98:3820–3825
[7] R. Anandharajan, K. Pathmanathan, N.P. Shankernarayanan, Ram A.*Journal of Ethnopharmacology* 97 (**2005**) 253–260
[8] Anitha Mary Mathews,K Sujith,Santosh Pillai,AJM Christina, *European Journal of Biological Sciences* 5(3):77-81,**2013**.
[9] Fajas, L., Auboeuf, D., Raspe, E., Schoonjans, K., Lefebvre, A. M., Saladin, R., Najib, J., Laville, M., Fruchart, J. C., Deeb, S., et al. (**1997**) *J. Biol. Chem*. 276, 46079 – 46087.
[10] Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. (**1996**) *Endocrinology* 137, 354 – 366.
[11] Gurnell M, Savage DB, Chatterjee VK, O'Rahilly S . *J Clin Endocrinol Metab* 88:2412–2421