

Inhibition of free radical activity by dual PPAR α and PPAR γ agonist using analytical assay methods

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ABSTRACT

Background: Overproduction of free radicals involved in the pathology of a wide variety of clinical disorders. Poor glycaemic control in diabetic people leads to free radical production responsible for diabetic related complications. Antioxidants produces resistance against the oxidative stress by scavenging free radicals may useful in treating diabetic related complications. Saroglitazar is a newer antidiabetic drug act on dual Peroxisome Proliferator Receptor Agonist α (PPAR α) and PPAR γ agonist with protection effect on Diabetes mellitus induced lipid dystrophy. Our study was done to evaluate the in vitro antioxidant effect Saroglitazar by 1, 1 Diphenyl 2 Picryl hydrazide (DPPH) and Nitric Oxide (NO) method.

Methods: In this study, we demonstrated invitro antioxidant activity by using 10 mg/dl stock solutions of Saroglitazar. DPPH and NO free radical scavenging test were done for different concentration of Saroglitazar.

Results: Saroglitazar showed concentration dependent free radical scavenging activity in DPPH assay. In DPPH assay at higher concentration 1000ug concentration showed 49.18% free radical scavenging activity. At lower concentration 10ug showed 17.18% free radical scavenging activity. NO scavenging activity at lower concentration 100ug showed 55.15% activity. But the higher concentration (1000ug) only slight increase in 60.15% activity.

Conclusions: Thus Saroglitazar invitro antioxidant analysis proved that it is a potent antioxidant.

Keywords: Diabetes mellitus, 1,1 Diphenyl 2 Picryl hydrazide, Free radicals, Nitric oxide, Peroxisome proliferator receptor agonist, Saroglitazar

INTRODUCTION

Type 2 diabetes mellitus is a chronic progressive disease is due to pancreas does not produce enough insulin or resistance to the insulin it produces or both. The prevalence diabetes among adults aged 18 years and older was 8.5% in 2014. Diabetes was the direct cause of 1.5 million deaths and high blood glucose was the cause of another 2.2 million deaths in 2012.¹ The number of diabetes patients in India is estimated to 68 million in 2025.² Free radicals can be defined as reactive chemical species having a single unpaired electron in an outer orbit. This unstable electron creates energy which is

released through reactions with adjacent molecules, such as proteins, lipids, carbohydrates, and nucleic acids. Free radicals involved in pathological conditions such as anaemia, asthma, inflammation, neurodegenerative disease and Parkinson's disease.³ Free radicals also play important role in Diabetes Mellitus. Hyperglycaemia produces glucose auto oxidation and glycation of proteins. This glycation of proteins leads to into free radical species.⁴ Antioxidants protect body from free radical induced damage. Endogenous antioxidant like Vit E and glutathione involved in free radical scavenging system.⁵ Peroxisome Proliferator Receptor Agonist (PPAR) are nuclear transcription factors. It regulates the expression of various genes involved in control of lipid,

lipoprotein metabolism and glucose haemostasis and inflammatory process. PPAR alpha activation reduces the synthesis and secretion of Triglycerides and increases the hepatic oxidation of Fatty acids. PPAR- α activation increases the fatty acid oxidation process in the liver and also reduces secretion of triglycerides and VLDL. It also promotes lipolysis by activating lipoprotein lipase and inhibiting the production of Apo lipoprotein C-III. Apo lipoprotein C-III is an inhibitor of lipoprotein lipase. Apo lipoprotein A-I, A-II and HDL-C (high density cholesterol) are also elevated by PPAR- α . It results in reduction of inflammation. PPAR- γ activation, activates the genes involved in glucose metabolism (adiponectin, CD 36 etc.) and increases the insulin sensitivity, glucose uptake and utilization. It also increases fatty acid uptake and decreases post-prandial surge in their levels. As a result, metabolic burden on liver and muscle is reduced.⁶

Saroglitazar is a novel glitazar compound developed in India and gained regulatory approval from the Indian regulatory authority DCGI in June 2013. It is also the first member of the glitazar class to gain approval to be discovered in India. It is beneficial anti-dyslipidemic and anti-glycaemic effects while minimizing the harmful side effects that plagued the other members of this class. Saroglitazar is a dual peroxisome proliferator activator receptor alpha and gamma agonist activity.⁷

Till Now no studies available on antioxidative effect of saroglitazar. Present study was aimed at invitro antioxidant effect by DPPH assay and NO method.

METHODS

DPPH Radical antioxidant activity

This assay is based on the principal of reduction of absorbance of ethanol solution of DPPH by free radical scavenger. This assay activity was done using the method Yahozowa et al 10mg/ml of stock solution of lipaglyin (Saroglitazar) were prepared with ethanol (60 μ L).⁸ Reagents required for this assay were DPPH and Ethanol. The Reagent mixture containing 1ml of DPPH solution (200 μ m in ethanol) and different concentration of the sample. Test drug lipaglyin (Saroglitazar) serial dilutions (100 μ g to 1000 μ g done) were taken and they are incubated separately in the dark for 20 min at room temperature. By using spectrophotometer serial dilution absorbance were recorded at 517nm. Control sample contained 1.9ml of DPPH and 0.1ml of solvent. Saroglitazar free radical scavenging activity by using DPPH assay were calculated using this formula.

Percentage Inhibition= $(\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{Sample})}) / \text{Abs}_{(\text{control})} * 100$

NO radical scavenging activity

It was done by using the method of Alderston et al.⁹ It is based on the principal of inhibition of nitric oxide radical

(which is generated from sodium nitro pruside in phosphate buffed saline with addition of griess reagent).

The absorbance was measured at 546nm. Griess reagent contains 1% sulphilamide, 2% phosphoric acid and 0.1% naphthyl ethulene diamine dihydrochloride in 100ml of distilled water. 3ml of reaction mixture containing sodium nitropruside (10 milliMoles in phosphate buffered saline) and different concentration of test drug were incubated 37°C for 4 hours.

To the incubation solution 0.5ml of Griess reagent was added. By using spectrophotometer serial dilution absorbance were recorded at 546 nm. Free radical scavenging activity by Saraglitazar using NO assay were calculated using this formula.

Percentage Inhibition= $(\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{Sample})}) / \text{Abs}_{(\text{control})} * 100$

Statistical analysis

Experimental results were mean \pm S.E.M. of three parallel measurements. Statistical analysis was estimated by using Student's t-test followed by ANOVA method. The values for P <0.05 were considered as significant and values for P <0.001 as very significant

RESULTS

DPPH assay

DPPH free radical activity were tested different concentration of saroglitazar from 100 μ g to 1000 μ g concentrations. Saroglitazr (lipaglyin) showed dose dependent gradual increase in DPPH free radical activity. Percentage of inhibition varies from 17.83% to 49.214%. Maximum free radical scavenging activity exerted at higher concentrations (Table 1).

Table 1: DPPH assay.

Saroglitazar concentration	% Free radical scavenging activity
100 μ g	17.183 \pm 0.50
200 μ g	30.033 \pm 0.70
400 μ g	45.659 \pm 1.09
800 μ g	46.303 \pm 0.79
1000 μ g	49.214 \pm 1.10

Values are expressed as mean \pm S.D of three experiments

Control: 1.9ml of DPPH + 0.1ml of solvent=0.2737

Blank: 1.9ml of ethanol +0.1ml of solvent

% Scavenging Activity= Control- Test/Test X 100

Similarly, Sarogltazar (lipaglin) showed dose dependent very gradual increase in NO free radical assay from 10 μ g to 1000 μ g concentration. Percentage of inhibition varies from 55.491% to 61.1% (Table 2).

Table 2: NO assay.

Saroglitazar concentration	% Free radical scavenging activity
100µg	55.491±0.72
50µg	58.34±0.79
100µg	56.37±0.84
200µg	55.879±1.04
400µg	56.365±0.69
800µg	57.287±1.05
1000µg	61.1±1.15

Values are expressed as mean± S.D of three experiments

DISCUSSION

Free radicals produce oxidative stress. This is balanced by the body's endogenous antioxidant systems with an input from co-factors, and by the ingestion of exogenous antioxidants. If the generation of free radicals exceeds the protective effects of antioxidants, and some co-factors, this can cause oxidative damage which accumulates during the life cycle, and has been implicated in diseases such as cardiovascular disease, cancer, neurodegenerative disorders, and other chronic conditions.⁵

Chronic hyperglycaemic state in diabetes mellitus, glucose forms covalent adducts with the plasma proteins through known as glycation by non-enzymatic process. Formation of advanced glycation end products (AGEs) play an important role in the pathogenesis of diabetic complications like retinopathy, nephropathy, neuropathy, and cardiomyopathy. AGEs interfere with their normal functions by various methods like disrupting the molecular conformation, increasing or decreasing the enzymatic activity, and modifying the receptor functioning. AGE contribute in the development of diabetic complications by interfering with proteins, lipids and nucleic acids.¹⁰

Experimental data support AGEs generate free radicals and these free radicals involved in variety of diabetic complications. All glycation steps generate oxygen free radicals, mainly it is common with these of lipid peroxidation. AGEs induce an oxidative stress and a pro-inflammatory status by binding into membrane receptors such as RAGE. Glycated proteins modulate cellular oxidative functions.¹¹

Since DPPH is a relatively stable free radical, DPPH assay is considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants.^{12,13}

Excess concentration of NO is results in the cytotoxic effects observed in various disorders such as AIDS, cancer, Alzheimer's and arthritis. It is generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. Excess

NO generate nitrite and peroxynitrite anions by reacting with oxygen which act as free radicals.¹⁴

In this study, dual PPAR α and PPAR γ agonist showed significant free radical scavenging activity by DPPH and NO radical scavenging assays. Hence novel drug Saroglitazar is a potent free radical scavenging activity effective in diabetic induced chronic complications. Further studies are required for demonstration for antioxidant effect in vivo.

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