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Research Article

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Evaluation of antioxidant activity of pioglitazone: Nitric oxide scavenging activity (*In-vitro* method)

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ABSTRACT

Diabetes mellitus (DM) is an endocrine disorder characterized by abnormal carbohydrate, lipid and protein metabolism along with specific long-term complications which are associated with oxidative stress (Pandey A, 2011). *Hence, it is important to discover a hypoglycemic drug that reduces oxidative stress in diabetic patients. This study, therefore, was performed to investigate the antioxidant potential of Pioglitazone (PIO) by nitric oxide scavenging activity, an in –vitro method. Pioglitazone antioxidant property was analyzed with varying concentration from 100 to 1000 µg/ml using spectrophotometer while keeping ascorbic acid as the standard. The results of this study show that Pioglitazone, on comparison with Ascorbic acid, has dose dependent antioxidant property.*

Keywords: Diabetes mellitus, pioglitazone, antioxidant, nitric oxide

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INTRODUCTION

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane which may lead to cellular death. To prevent free radical damage the body has a defense system of *antioxidants.* (A. A. Hamid1, , August 2010) (PERCIVAL, 1998)

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Therefore, antioxidant has potential to prevent aging, cancer, cardiovascular disease, Alzheimer's disease. (. BayaniUttara, March 2009) (TPA Devasagayam*, OCTOBER 2004)

Free radicals production is increased in diabetes mellitus. The increase in glucose level leads to increase in oxidative stress thereby changing in antioxidant capacity. This plays a vital role in complication of diabetes. (Brownlee, 2010 Oct 29)

Thiazolidinediones, a group of oral hypoglycemic agents effectively improves the glycemic control in diabetes mellitus type 2. They act as agonist for nuclear transcriptase factor peroximase proliferative activator gamma which improve insulin sensitivity. (Auwerx, 2000) (Rama R. Bhosale, 2013)

EXPERIMENTAL SECTION

Materials and Methods:

1. Test sample: crude drug of Pioglitazone (15mg)
2. Reference antioxidant: Ascorbic acid
3. Solvent: sodium nitroprusside in phosphate buffer
4. Reagent: Griess reagent
5. Spectrophotometer

Nitric Oxide Scavenging Activity:

Procedure

Nitric oxide scavenging activity can be estimated by the use of griessillosovy reaction(Garrat ,1964) (rozina paurl, 2012) (Jagetia GC1, 2004).Nitric oxide scavenging activity was measured using a spectrophotometer. Drug was prepared in ethanol, was added to different test tubes in varying concentrations (100, 200, 400, 600, 800, 1000 µg/ml). Sodium nitroprusside (5mM) in phosphate buffer was added to each test tube to make volume up to 1.5ml. Solutions were incubated at 25ºC for 30 minutes. Thereafter, 1.5ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard. (Jagetia GC1, 2004 jul)

Calculation

The percentage of scavenging activity by the drug was calculated using the formula:

$$\% \text{ inhibition} = \frac{(\text{control} - \text{test})}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

Table 1: Comparison of percentage of inhibition of Pioglitazone (Pios-15) and Ascorbic acid

Sl. No.	% of Inhibition		
	Concentration (µg/ml)	Pios-15	Ascorbic acid
1	100	0.2±3.86	12±6.6
2	200	1.3±2.16	25.6±4.2
3	400	2.6±3.21	45.31±2.12
4	600	3.9±2.16	68.2±4.26
5	800	4.6.32±1.64	84.4±2.61
6	1000	5.3±1.6	99.2±4.2

The nitrogen oxide scavenging activity was recorded in terms of percentage Inhibition. It was observed from that pioglitazone (1000 µg /ml) has shown dose dependent nitrogen oxide scavenging activity (5.3%) (Table 1). The Results obtained were comparative to Ascorbic acid standard. Higher Percentage Inhibition indicates better scavenging activity or antioxidant potential. The given sample showed the dose dependent activity in scavenging the free radicals but insignificant when compared to that of the Ascorbic acid standard.

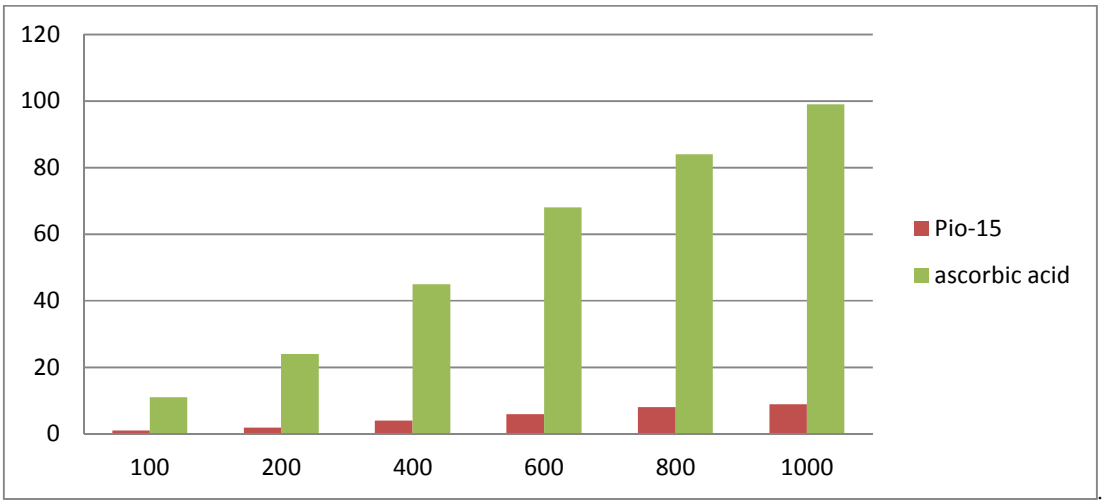
The antioxidant property of pioglitazone was assessed by percentage of inhibition (Table1). As the concentration of the drug increases, the percentage of inhibition increases, but insignificant when compared with ascorbic acid (Figure 1).Therefore, this study shows that Pioglitazone has minimal dose dependent Antioxidant property.

The 2, 4-thiazolidinedione structure is common in a variety of agents and difference in side chain modifications influences their pharmacological actions. Thiazolidinediones are believed to mediate their effects via a variety of targets: peroxisome proliferator activated receptor (PPAR), protein tyrosine phosphate 1B (PTP 1B), mitochondria. Their therapeutic attestation as antidiabetic, antioxidant, anti-inflammatory, antibacterial, anti-obesity agents point toward biodynamic nature of 2,4-thiazolidinedione. (Shom Prakash Kushwaha, 2011)

A series of 5-arylidene-2, 4-thiazolidinediones and its geranyloxy or prenyloxy derivative were synthesized and studied for their radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Their comparable scavenging activities were expressed as IC50 value. Compounds 2c, 2d, 4d, and 6a showed appreciable radical

scavenging activities. The vanillin based thiazolidinedione compound 2c displayed highest activity comparable to that of alpha-tocopherol. But in vivo, compound 6a showed better results in inducing phase II detoxifying/antioxidative enzyme. (Hossain SU, 2007)

Figure 1: Bar Chart Comparing Percentage of Inhibition Pioglitazone with Ascorbic Acid At Various Concentrations



CONCLUSION

Thiazolidinediones have been cited as the most costly oral anti-diabetic medications (L, 2009). Limiting glucose lowering efficacy (20% maximum decrease in fasting plasma glucose at the maximum recommended dose) and side effect profile (chiefly weight and fluid retention) confines the use of currently available thiazolidinediones. (Martijn van Doorn, 2005)

Therefore, novel thiazolidinediones compounds which have superior glucose lowering efficacy coupled with antioxidant activity are needed. This will help in the development of thiazolidinediones derivatives possessing a broad spectrum of activities as to counter major components of metabolic syndrome.

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