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The Dose-Dependent Antioxidant Capacity of Bezafibrate: An In-Vitro Analysis.

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ABSTRACT

The present study was conducted to evaluate the antioxidant activity of peroxisome proliferator-activated receptors alpha (PPARα) agonist, Bezafibrate. In-vitro analysis of the antioxidant capacity was assessed using two assays – Nitric oxide and Hydrogen peroxide scavenging activity. The ethanol extract of the drug Bezafibrate, taken in increasing concentration (µg/ml) shows increasing scavenging effect in both nitric oxide and hydrogen peroxide assay. This exceptional characteristics of dose- dependent antioxidant activity of Bezafibrate is beneficial in preventing oxidative cell damage and vascular complication in hyperlipidemic patients.

Keywords: Antioxidant capacity, In-vitro analysis, Bezafibrate, PPAR-α agonist, Nitric oxide scavenging activity, Hydrogen peroxide scavenging activity.

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INTRODUCTION

Reactive oxygen species (ROS) - like hydroxyl (OH-), superoxide anion(O2-), nitric oxide(NO) & peroxyl(RO2) are highly reactive molecules continuously produced during normal physiological events/ aerobic metabolism and are inactivated and scavenged by our antioxidant defence mechanism. The oxidation reaction produce free radicals, which in turn can start chain reaction and cause damage/ injury to cells. They play an important role in many diseases. Under pathological condition, ROS are over produced and disturb the homeostasis of the intracellular milieu by reacting unfavourably with cellular macromolecules including DNA, proteins and lipids. The imbalance between ROS and antioxidant defence mechanism leads to oxidative stress, associated with many of the age related degenerative diseases including atherosclerosis and other diseases like cancer, trauma, stroke, asthma, emphysema, arthritis, heart attack, dermatitis, retinal damage, hepatitis and liver cirrhosis [1-2].

Protective against free radicals can be enhanced by ample intake of dietary antioxidants. Dietary antioxidants, such as the vitamin A, C & E act as free radical scavengers by donating an electron to provide chemical balance. Sometimes these protective mechanisms are found not to be sufficient when compared to the damage occurred to the cells. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. Bezafibrate (Hypolipidemic drug), activator of Lipoprotein Lipase (LPL), a key regulatory enzyme responsible for the hydrolysis of Triglyceride (TG) rich lipoproteins. Many of the effect exerted, by Fibrates, on blood lipids are mediated by their interaction with Peroxisome Proliferator Activated Receptors (PPARs), which regulates gene transcription. Among the three PPARs isotopes (α β ϒ), fibrates bind to PPARα, expressed in liver and brown adipose tissue, to lesser extent in heart, kidney, skeletal muscle. Fibrates are the drug of choice for treating hypertriglyceridemia's, particularly those associated with low levels of HDL-C, familial hypertriglyceridemia (type IV) and dysbetalipoproteinaemia (type III). In this present study Bezafibrate is assessed for its antioxidant capacity with radical scavenging activity. [2-5]

MATERIALS AND METHODS

Materials

The test sample Bezafibrate tablet is used, to prepare its ethanolic extract for the assessment of the scavenging activity. Reference antioxidants used in this study are ascorbic acid, butylated hydroxytoluene (BHT) with phosphate buffer as solvent and the two reagents used in the assay are sodium nitroprusside and hydrogen peroxide respectively. The extract (100-1000µg/ml) is then spectrometrically measured for its scavenging effect [4].

Procedure

Nitric oxide scavenging activity

Nitric oxide is generated in biological tissues by specific nitric oxide synthases. The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing Nitric oxide. Under aerobic condition, nitric oxide reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using griess reagent.

Nitric oxide scavenging activity is measured spectrometrically. The drug Bezafibrate was prepared in ethanol, 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 up the volume to 1.5ml. Solutions were incubated at 25º c for 30minutes. 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 546nm and the percentage of scavenging activity was measured with reference to ascorbic acid as standard [4].

%inhibition = (control-test) / control X 100

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Hydrogen peroxide scavenging activity

Humans are exposed to H2O2 indirectly via the environment. Hydrogen peroxide may enter into the human body through inhalation of vapour or mist via skin or eye contact. It is then rapidly decomposed into oxygen and water which may produce hydroxyl radicals ($OH^{\bar{}}$) that initiates lipid peroxidation causing DNA damage in our body. The ability of any extract to scavenge hydrogen peroxide can be estimated according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (40mM) prepared in phosphate buffer (pH 7.4). Drug extract at the concentration of 10mg/10µl were added to 0.6ml of H2O2 solution. The total volume was made up to 3ml with phosphate buffer. The concentration of hydrogen peroxide is determined by absorption at 230nm using a spectrometer. Extract in distilled water is added to hydrogen peroxide and absorbance at 230nm is determined after 10min against a blank solution containing phosphate buffer without hydrogen peroxide [6].

The percentage of hydrogen peroxide scavenging is calculated as:

% scavenged (H2O2) = [(Ao – Ai) / Ao] X 100

Ao – Absorbance of control
Ai – Absorbance in the presence of drug

RESULTS

The antioxidant property of the drug Bezafibrate is dose-dependent. The increasing dose of Bezafibrate showed better scavenging effect. Of the two assay done in this study hydrogen peroxide had better scavenging activity compared to nitric oxide at the same concentration of the drug.

The table and the graph showing the scavenging effect of the Bezafibrate comparable to ascorbic acid and BHT respectively is given below:

Nitric oxide scavenging effect

Sl. No	% of Inhibition		
	Concentration (µg/ml)	Bezafibrate	Ascorbic acid
1	100	1.2±3.86	12±6.6
2	200	2.3±2.16	25.6±4.2
3	400	4.6±3.21	45.31±2.12
4	600	6.9±2.16	68.2±4.26
5	800	8.632±1.64	84.4±2.61
6	1000	15.3±1.6	99.2±4.2

Table 1: In-Vitro antioxidant effect by Nitric oxide scavenging activity

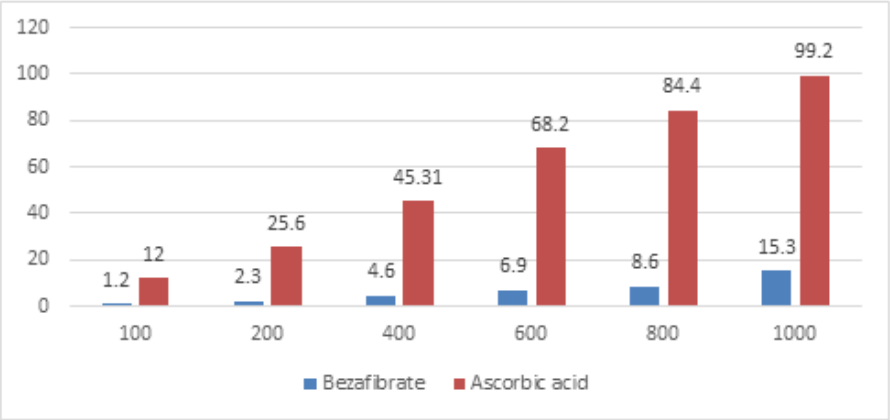

Figure 1: Bar chart comparing percentage inhibition of Bezafibrate with nitric oxide at various concentrations.

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The test drug Bezafibrate at the initial concentration 100 µg/ml had very mild percentage of inhibition compared to the standard ascorbic acid the scavenging effect. On increasing the dose of the test drug Bezafibrate there was mild and gradual increase in the percentage of inhibition. Dose concentration at 1000 µg/ml the percentage of inhibition was 15.

Hydrogen peroxide scavenging activity

This method to determine the antioxidant activity of Bezafibrate showed significantly better results. At the low dose 100 µg/ml of Bezafibrate the percentage of inhibition was mildly higher than the nitric oxide scavenging effect and on increasing the dose of Bezafibrate better scavenging activity was observed. At the dose of 1000 µg/ml the percentage of inhibition was 21.

Sl. No	% of Inhibition		
	Concentration (µg/ml)	Bezafibrate	BHT
1	100	3.2±4.36	40±2.6
2	200	5.4±3.12	65.6±1.4
3	400	8.36±2.46	77.21±3.42
4	600	11.46±1.06	84±1.62
5	800	16.42±4.37	92.2±6.13
6	1000	21.04±1.2	99.2±6.13

Table 2: In-Vitro antioxidant effect of Hydrogen peroxide scavenging activity

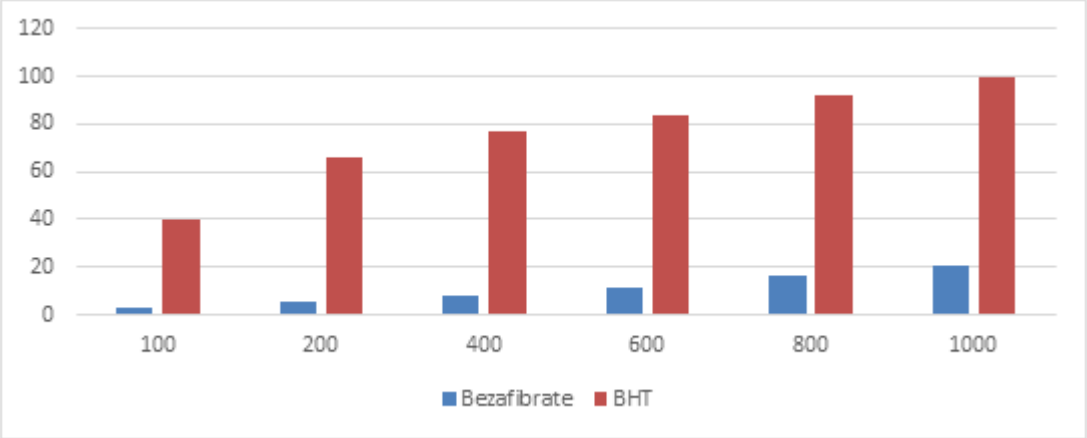

Figure 2: Bar chart comparing percentage inhibition of Bezafibrate with BHT at various concentrations.

DISCUSSION

The two most important risk factor associated with atherosclerosis are hemodynamic disturbances and effects of hypercholesterolemia. In hypercholesterolemia the final effect is accumulation of the lipoproteins in the vessel wall which is further modified through oxidation. In hemodynamic disturbances the ostia of the blood vessel is most affected where the blood flow is disturbed and turbulent. The normal laminar flow vessels has an inflammatory mechanism which induces the endothelial genes (products such as superoxide dismutase- antioxidant) protects against lesion formation [7].

Bezafibrate, activator of Lipoprotein Lipase (LPL), a key regulatory enzyme responsible for the hydrolysis of Triglyceride (TG) rich lipoproteins, and its treatment resulted in significant decrease in the serum concentrations of triglycerides, total cholesterol and LDL-Cholesterol and also VLDL, where HDL-Cholesterol serum levels increased. It is a very well-tolerated drug. [8-9]

Antioxidants have good protective effect over the damage done by free radicals in many cases. Under conditions, which promote oxidative stress, endogenous antioxidants may not be sufficient and dietary or

exogenous supplementation should may be required to maintain cellular function and also to prevent furthermore damage. Even the mild antioxidant effect exerted by Bezafibrate, as observed in this study is also beneficial to prevent oxidative stress and many other cardiovascular complications. [10]

CONCLUSION

Bezafibrate is an expensive oral hypolipidemic drug and used less commonly. The dose-dependent antioxidant effect, though mild, is much beneficial to combat the adverse effect produced by free radicals. Few researches have reported that Bezafibrate reduces blood glucose levels, also effects on insulin sensitivity. Several studies have also linked Bezafibrate in lowering biliary enzymes in primary cirrhosis. Encouraging more research works on Bezafibrate and promote its use widely in India is thus very necessary. [11-12]

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