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COMPARATIVE EFFICACY OF CARVEDILOL AS ANTIOXIDANT AGAINST VITAMIN C ON OXIDATIVE STRESS – DPPH ASSAY (IN-VITRO METHOD)

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ABSTRACT **Background:** Hypertension is one among the leading disease causing morbidity and mortality irrespective of other co morbid conditions. Hypothetically the culprit behind has been the accumulation of free radicals causing oxidative stress. Oxidative stress plays a vital role in pathophysiological changes in hypertension. Polypharmacy is one among the cause for major drug interaction and hence to prevent it, necessity of the pertained drug for the disease to act as a scavenger is the need of the hour in current scenario. **Objective:** In this study we are intended to find antihypertensive drug Carvedilol (3.125mg) antioxidant action by DPPH assay against the standard commercially available vitamin C. **Result:** It is found that the antioxidant activity of Carvedilol (3.125) is quite appreciable (83.02%) when compared to standard commercially available vitamin C(86.56%).

KEYWORDS : DPPH ASSAY, CARVEDILOL, ANTIOXIDANT IN HYPERTENSION

Introduction

Hypertension contributes to the pathophysiology of various diseases like renal failure, cardiovascular disease and cerebrovascular accident. Vascular remodeling, endothelial dysfunctions are few among those which contributes to the pathogenesis of hypertension and are basically due to oxidative stress caused by free radical accumulation. Among the free radicals producing oxidative stress, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the major culprit. These are responsible for the decreased nitric oxide bioavailability as well as decreased antioxidant capacity in vascular structures.

Oxidative stress is defines as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS)2. ROS include free radicals such as superoxide (O2-), hydroxyl (OH), peroxyl (RO2), hydroperoxyl (HRO2-) as well as nonradical species such as hydrogen peroxide (H2O2) and hydrochlorous acid (HOCl)2,3. RNS include free radicals like nitric oxide (NO) and nitrogen dioxide (NO2-), as well as nonradicals such as peroxynitrite (ONOO-), nitrous oxide (HNO2) and alkyl peroxynitrates (RONOO)2,3.

Oxidative stress causes healthy cells of the body to lose their function and structure by attacking them. Thus it causes damage to DNA, proteins, and other macromolecules which may lead to cellular death. To prevent oxidative stress, the body has a defense system of antioxidants.

Antioxidants are chemical or biological agents able to neutralize the potentially damaging action of free radicals. It exerts its effect by scavenging the species that initiate peroxidation, quenching singlet oxygen, chelating metals, breaking free radical chain reactions, and reducing the concentration of .O2-.

Carvedilol (3.125mg) and vitamin C

The test drug (Carvedilol (3.125mg)) is a potent α 1 adrenoreceptor antagonist , competitive β adrenoreceptor antagonist (a calcium channel antagonist at high concentration).The renal sparing effect of Carvedilol plays a vital role in choosing this very own drug as antihypertensive for the elderly. It reduces the mean arterial pressure without altering or compromising the renal blood flow and urinary sodium excretion. Many studies revealed the antioxidant property of Carvedilol on ROS (reactive oxygen species).This study contributes for the nitric oxide scavenging action of Carvedilol 3.125mg.

Vitamin C is a much known potent antioxidant.
Both the drugs are compared with the standard to reveal the percentage of DPPH activity to detect antioxidant property.

DPPH ASSAY: (Molyneux, 2004)

DPPH (1,1-diphenyl-2-picrylhydrazyl) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Blois,1958) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).

Representing the DPPH radical by Z and the donor molecule by AH, the primary reaction is

$Z + AH = ZH + A$

where ZH is the reduced form and A is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant.

CHEMICALS:

1. 1,1 – diphenyl -2- picrylhydrazyl (DPPH)
2. Dimethylsilphoxide (DMSO)
3. BHT (standard)-1.6mg/ml in methanol
4. Samples desired concentration from 1 mg /ml –max of 5mg / ml (in /DMSO)

PROCEDURE

Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank.

Add 100µl of BHT to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm.

Table 2: Procedure for DPPH Assay

S.NO	REAGENTS	BLANK	STANDARD	TEST
1	Methanol	3.8ml	3.7ml	3.7ml
2	BHT	-	100µl	-
3	Sample	-	-	100µl
4	DPPH	200µl	200µl	200µl

Incubation at dark for 30 minutes

O.D at 517 nm

CALCULATION:

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance at blank) - (Absorbance at test)}}{\text{(Absorbance at blank)}} \times 100$$

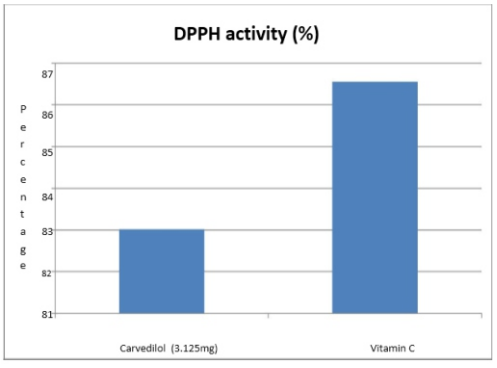
RESULTS:

FIGURE 1: The Antioxidant activity of given samples using DPPH Assay method.

S.NO	SAMPLE	Concentration (µg/ml)	O.D	DPPH activity (%)
1	Carvedilol	1000	0.139	83.02
	(3.125mg)			
2	Vitamin C	1000	0.110	86.56

Blank O.D: 0.819

FIGURE 2: Bar Chart Comparing antioxidant activity of Carvedilol 3.125mg and Vitamin C by DPPH Assay.



DPPH Assay shows Carvedilol have antioxidant property (Table.2). Carvedilol 3.125 mg has significant (83.02%) compared with vitamin C (86.36%) (Figure.2).DPPH assay provided an easy and rapid way to determine the antioxidant property of many substances and can compare their efficacy.

It is inferred that Carvedilol 3.125 mg at even low dose has appreciable antioxidant property which not only plays a significant role in reducing pathophysiological process of hypertension, it has other role in preventing aging, diabetic complications, myocardial damage etc which is primarily due to oxidative damage.

In hypertensive patients, those who are taking Carvedilol it reduce the blood pressure without affecting total peripheral resistance, maintaining the renal blood flow and perfusion. This additional property of antioxidant further prevents renal damage due to ROS (Reactive oxygen species).

Thus Carvedilol, a vasodilating non cardio selective β and an α blocker have significant role in controlling major mortality and morbidity diseases like hypertension,diabetes,angina,Myocardial damage which are basically due to oxidative damage. Hence the need for adding additional antioxidant drug is of no specific need as Carvedilol 3.125mg has significant antioxidant property when compared to the potent antioxidant Vitamin C.

CONCLUSION

In this study Carvedilol 3.125mg possess significant antioxidant activity compared to the ore potent antioxidant vitamin c. This additional antioxidant activity of Carvedilol makes it as novel therapy for the management of hypertension in elderly.

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