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# Antioxidant Role of Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Blockers in an *in Vitro* Biochemical Model

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## ABSTRACT

**Introduction:** Persistent activation of renin-angiotensin system causes oxidative stress resulting in cardiovascular derangements. Renin angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor antagonists (ARBs) are preferred over other antihypertensive drugs for hypertension and cardiac failure. Protective role of these drugs has been observed in cell lines (*ex vivo*) as well as in clinical settings. Limited work has been reported to evaluate antioxidant role of ARBs *in vitro*. **Aims & Objectives:** The aim of the study was to observe the antioxidant effect of ACEIs and ARBs *in vitro* which is claimed in clinical settings. **Place and duration of study:** This study was conducted in Pharmacology Department, University of Health Sciences during April-May 2017. **Material & Methods:** This work was designed to investigate antioxidant property of ACEIs as well as ARBs *in vitro*. Captopril, enalapril, lisinopril, quinapril, candesartan, losartan, olmesartan, telmisartan, irbesartan and valsartan of pharmaceutical grades were dissolved in dismethylsulfoxide (DMSO). Ascorbic acid was selected as control and 2, 2 diphenyl-1-picrylhydrazyl (DPPH) was used as oxidizing radical. Different dilutions of all the drugs included in the study were run in triplicate. The dilutions ranged from 0.75 µM to 200 µM. End point essay was used and absorbance was taken at 517 nm. **Results:** Significant antioxidant activity was observed by captopril and ascorbic acid. Ascorbic acid and captopril had $V_{max}$ 96.44 and 84.16 respectively. $IC_{50}$ of ascorbic acid was 3.84 µM and that of captopril was 17.34 µM. The results for other ACEIs and ARBs were not promising because $IC_{50}$ was more than 200 µM. **Conclusion:** DPPH radical scavenging activity of captopril and ascorbic acid were in conformity with other workers but nonthiol ACEIs and ARBs have non significant anti-oxidant activity *in vitro*.

**Key words:** DPPH activity, ACE inhibitors, ARBs, Oxidative stress, Coronary artery disease.

## INTRODUCTION

Angiotensin II (Ag II) activated by renin acts through angiotensin receptor 1 ($AT_1$) and angiotensin receptor 2 ($AT_2$) systems. Ag II activates NADPH oxidase and superoxide anions ($O_2^-$) which proceed as stimulant tools for cardiovascular functions. It also produces contraction of the vascular smooth muscles by activating protein kinase C as well as indirectly through autonomic nervous system.[1, 2]

Angiotensin II blockers and ARBs decrease peripheral vascular resistance resulting in fall in blood pressure. There is neither reflex tachycardia nor anginal ischemia.[3] ACEIs and ARBs are prescribed to patients having multiple pathologies such as diabetic nephropathies or chronic renal failures in addition to hypertension. Clinical improvements in such patients are claimed due to improved endothelial function, reduced intracellular oxidative stress and recovery of renal hemodynamics.[4, 5]

A number of researchers have reported radical scavenging activity of ACEIs *in vitro*. Benzie and Tomlinson[6] demonstrated reduction of $Fe_3(TPTZ)_3$ into $Fe_2(TPTZ)_3$ (FRAP) by ACEIs (captopril, enalapril, fosinopril, perindopril, quinalapril and ramipril). Chopra *et al.*[7,8] performed *in vitro* inhibition of riboflavin-mediated photo-oxidation of dianisidine, scavenging superoxide anion (STNB test), inhibition of microsomal lipid peroxidation and thiobarbituric acid reactive substances assay of captopril, fentiapril, enalapril, quinalaprilate, ramiprilate and zofenopril. ARBs acting through inhibition of angiotensin receptors are being promoted as an alternative to ACEIs and are equally prescribed by physicians for cardiovascular

diseases. A small number of *in vitro* studies have been noticed with ARBs, a newer group of drugs acting through AT receptors.

It was planned to evaluate the antioxidant activity of some ACEIs as well as ARBs being marketed for clinical use. We opted for 2, 2 diphenyl-1-picrylhydrazyl (DPPH); a cost effective, highly reactive compound for radical scavenging activity assay. It is single step protocol preferred for screening natural compounds.

## MATERIAL AND METHODS

**Study Design and Setting**

It was an experimental *in vitro* study carried out in the Department of Pharmacology, University of Health Sciences, Lahore during April-May 2017. The work was approved by Institutional Review Board.

**Chemicals and drugs**

2, 2 diphenyl-1-picrylhydrazyl and DMSO were purchased from Sigma Chemicals Co. (Germany). Ascorbic acid, captopril, candesartan, enalapril, irbesartan, lisinopril, losartan, olmesartan, quinapril, telmisartan and valsartan were procured from the local pharmaceutical firms.

**Preparation of Test Compounds**

Test compounds were dissolved in dimethylsulfoxide and different dilutions were made ranging from 0.75 µM to 200 µM as a final concentration in the reaction mixture. DPPH solution was prepared in 98% ethanol presenting 300 µM in the reaction mixture. Ascorbic acid was used as a standard.[9]

**Spectrophotometery**

An end point assay as described by Ahmad et al. (2008) was used. Test compounds and DPPH were thoroughly mixed and incubated for 30 minutes at 37 ˚C. The Spectrophotometer (BMS Shimadzu 1602 Germany) was set at 517 nm. Absorbance was noted manually and samples were run in triplicate.[10] DPPH 300 µM without any test compound was run as a control and the value was considered as 100%. Radical scavenging activity by DPPH was calculated by the following equation:

$$\text{Percent radical scavenging assay} = \frac{(A - B)}{A} \times 100$$

Where: A is absorbance of standard
B is absorbance of test compounds

**Statistical analysis:**

Mean ± SD of triplicate samples was taken. Vmax, Km and $IC_{50}$ of each sample were determined by Michaelis Menten equation. A software "EZ-Fit™ 5.03" (trial version) by Perella Scientific was used to work out these calculations.

## RESULTS

All the test compounds both ACEIs and ARBs had graded response relationship in DPPH radical scavenging assay as represented in Fig. 1 & 2. The thiol containing ACE inhibitor captopril showed $V_{max}$ 84.16, $K_m$ 12.47 and $IC_{50}$ was 17.34 µM. Captopril has less values as compared to ascorbic acid where $V_{max}$ was 96.44, $K_m$ 3.6 and $IC_{50}$ was 3.84 µM (Table-1). ACE inhibitors had better radical scavenging activity than ARBs (Fig-1,2). Although enalapril and olmesartan have lower $V_{max}$ and $K_m$ yet $IC_{50}$ was more than 100 µM. Rest of the ACEIs as well as all ARBs exhibited $IC_{50}$ more than 200 µM in this experimental model.

| Drugs | $V_{max}$ | $K_m$ | $IC_{50}$ (µM) |
|---|---|---|---|
| **ACEIs** | | | |
| Ascorbic Acid | 96.44 | 3.6 | 3.84 |
| Captopril | 84.16 | 12.47 | 17.34 |
| Enalapril | 45.11 | 14.3 | >100 |
| Lisinopril | 44.6 | 35.47 | >200 |
| Quinapril | 48.14 | 42.37 | >200 |
| **ARBs** | | | |
| Candesartan | 60.66 | 56.5 | >200 |
| Irbesartan | 35.52 | 61.82 | >200 |
| Losartan | 29.21 | 21.56 | >200 |
| Telmisartan | 34.41 | 16.5 | >200 |
| Olmesartan | 18.73 | 9.05 | >100 |
| Valsartan | 41.07 | 16.78 | >200 |

**Table-1:** Rate of DPPH radical scavenging activity by antioxidant role of angiotensin converting enzyme inhibitors and angiotensin receptor blockers

**Vmax.** Maximum Reaction Velocity
**Km.** Michaelis Menten constant
**$IC_{50}$.** Inhibitory concentration fifty percent

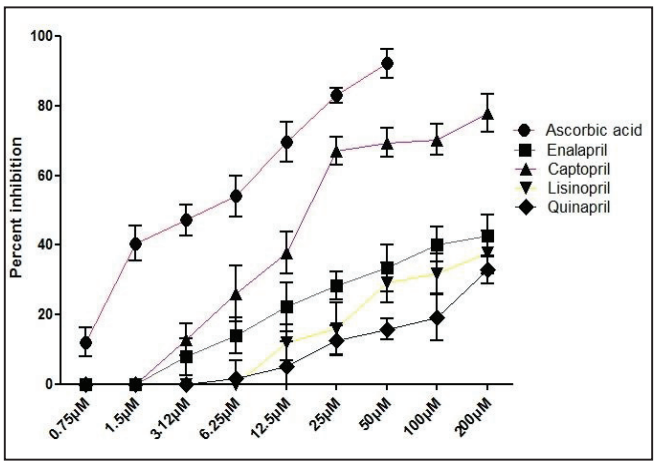


**Fig-1:** Concentration response curves showing DPPH radical scavenging activity of angiotensin converting enzyme inhibitors *in vitro*; Each point is the mean ± SD of three observations.

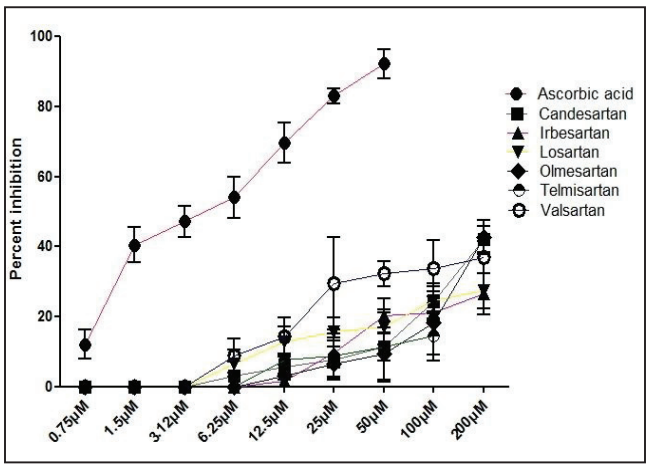


**Fig-2:** Concentration response curves showing DPPH radical scavenging activity of angiotensin receptor blockers *in vitro*; Each point is the mean ± SD of three observations

## DISCUSSION

The observations of this work are in agreement with the antioxidant activity assays reported by Benzie & Tomlinson[6] and Chopra et al[7,8]. Captopril, a thiol derivative ACE inhibitor exclusively showed positive results which were comparable with ascorbic acid. Whereas, remaining nonthiol ACEIs as well as ARBs studied showed low Vmax and $K_m$. $IC_{50}$ was more than 200 µM (Table-1).

A number of workers have performed *in-vivo* studies in which concentration of glutathiones and superoxide dismutases were increased by captopril and enalapril. Captopril and quinapril reduced reactive oxygen species in a number of disease models.[11,12,13] In spite of these findings enalapril and lisinopril, nonthiol ACEIs did not show antioxidant activity in parquat induced mitochondrial toxicity.[14] Captopril has presented higher DPPH radical scavenging assay in our experimental model but it is less than ascorbic acid. Reduction in angiotensin II, oxidant stress along with endothelin has been reported in hypertensive rat model.[15]

Lapenna *et al*[16] has paradoxical reports. Captopril has insignificant radical scavenging activity in human plasma *in vitro* as well as *in vivo*. The $AT_1$ receptor antagonists (candesartan, irbesartan, losartan, olmesartan, telmisartan and valsartan) had minimum DPPH scavenging effects; almost all the ARBs have $IC_{50}$> 200 µM (Table-1). ARBs selected for this work are biphenyl tetrazole imidazole derivatives but their structure activity relationship differs. Irbesartan decreased oxidative stress and endothelial dysfunction produced by metabolic syndrome in a clinical setting. Ferrari et.al (2016) observed 67% progress in endothelial functions and decreased IL6 and - PIA-1 inflammatory markers after 4 weeks treatment with irbesartan in atherosclerotic patients.[17,18] But in our study irbesartan has $V_{max}$ 36.52, $K_m$ 61.82 and $IC_{50}$ was > 200 µM (Table-1). Losartan has encouraging results during the management of chronic renal failure.[19] Glomerulonephritis and mesangial cell apoptosis produced by angiotensin II was successfully relieved by candesartan.[20] Neonatal endothelial cells apoptosis induced by oxidized low density lipoproteins was successfully ameliorated by olmesartan in an *in vitro* model.[21]

There are multiple confounding factors, which may affect the biological response of these drugs in a living system. Our results showed that ACEIs had more radical scavenging activity than ARBs. Antioxidant role of captopril in this biochemical setting is ascribed to the SH group which is lacking in other ACEIs and ARBs under study.

## CONCLUSION

DPPH radical scavenging activity of captopril was in conformity as reported earlier by other workers. The results of captopril were analogous to ascorbic acid but nonthiol ACEIs and ARBs in our study have shown non significant anti-oxidant activity.

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