EVALUATION OF ANTI-OXIDANT POTENCY OF SMALLER CHAIN PEPTIDES USING DPPH FREE RADICAL SCAVENGING ASSAY AND PHOSPHOMOLYBDENUM METHOD

*KANDASAMY NAGARAJAN1, MAYURI MISHRA2, VINAY KUMAR3, PARUL GROVER 4, SADAF JAMAL GILANI5, SHADAB AHMAD SIDDIQUI6

ABSTRACT

1-Diphenyl-2-picryl-hydrazyl (DPPH) assay and Phosphomolybdenum Method assay are in-vitro assay, employed to determine the antioxidant potency of test compound 1, 2, 3 and 4 [Arg-lys. (RK), Lys-Hist. (KH), Arg-Arg-Hist. (RRH) and Arg-Arg-Hist. (RRK)] using ascorbic acid as the standard drug. The percentage scavenging and total antioxidant capacity of different concentrations of the test drugs were determined and IC50 values of the test compound were subsequently compared with that of standard, ascorbic acid. Among the compound tested in both the methods, test drug-1 (RK) has shown maximum potency with an IC50 value of 4.2 µg/ml and 4.1 µg/ml; whereas test drug-2 (KH) have 7.4 µg/ml and 6.3 µg/ml, test drug-3 (RRH) have 4.1 µg/ml and 8.4 µg/ml and test drug-4 (RRK) have 6.0 µg/ml and 8.2 µg/ml and all have shown almost comparatively better antioxidant capacity with that of the standard drug ascorbic acid (IC50 value of 5.2 µg/ml and 8.1 µg/ml). Based on the above results, Arg-lys. (RK) could be considered for various formulations of antioxidant effect suitable for prevention of human diseases.

KEY WORD

1, 1-Diphenyl-2-picryl-hydrazyl, Phosphomolybdenum reagent, Antioxidant, Peptide, Ascorbic acid.

AUTHORS AFFILIATION

*Main author for correspondence.

1. Professor & Head, Research Laboratory of Central Instrumentation Frontiers, Department of Pharmaceutical Chemistry, KIET School of Pharmacy, 13km. Stone, Ghaziabad Meerut Road, Ghaziabad-201206, India.

2. PG Research Scholar, M.Pharm Research lab-2, Department of Pharmacology, KIET School of Pharmacy, 13km. Stone, Ghaziabad Meerut Road, Ghaziabad-201206, India.

3. Assoc. Professor & Head, Department of Pharmacology, KIET School of Pharmacy, 13km. Stone, Ghaziabad Meerut Road, Ghaziabad-201206, India.

4. Assistant Professor, Department of Pharmaceutical Chemistry, KIET School of Pharmacy, 13km. Stone, Ghaziabad Meerut Road, Ghaziabad-201206, India.

5. Assistant Professor, Department of Pharmaceutical Chemistry, KIET School of Pharmacy, 13km. Stone, Ghaziabad Meerut Road, Ghaziabad-201206, India.

6. Assistant Professor, Department of Pharmaceutical Chemistry, KIET School of Pharmacy, 13km. Stone, Ghaziabad Meerut Road, Ghaziabad-201206, India.
INTRODUCTION

In living system, free radical of different forms are constantly generated for specific metabolic requirements. When the generation of these species exceed the levels of antioxidants mechanism, they cause extensive damage to cells leading to oxidative damage of tissue and biomolecules, eventually leading to disease conditions, especially leading to disease conditions like cancer [1], cardiovascular disease [2], neural disorder [3], Alzheimer’s disease [4], aging [5], etc.

Reactive oxygen species (ROS) are produced as a consequence of normal aerobic metabolism or host defence mechanism. Excessive free radical species attack cellular components that cause damage to lipids, proteins, and DNA, which may initiate a chain of events resulting in the onset of a variety of diseases [6].

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, and peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of varieties of bio-macromolecules, such as enzymes, proteins, DNA and lipids. Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging [7].

A study was designed to investigate the in-vitro antioxidant activity of four peptide test compounds and to establish the most potent antioxidant drug having therapeutic value.

MATERIALS AND METHODS

Chemical

All chemicals were analytical grade and all chemical required for biochemical assay were obtained from Sigma Chemical Co., USA

DPPH Radical Cation Scavenging Assay

The free radical scavenging activity of test compounds was measured in-vitro by 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) assay. [8,9]. According to Manzocco et al., 1998 [10], the mixture of 0.5 ml methanol containing individual test compound at different concentrations (1-32µg/ml) and 2.5 ml of methanol containing 75µM 2,2-Diphenyl-1-picrylhydrazyl (DPPH: a stable free radical that has a typical absorbance at 517 nm) was at room temperature in dark maintained for 90 min, and the free radical scavenging activity was tested via measuring the absorbance at 517 nm using UV-visible double spectrophotometer (Shimadzu-1800). The % scavenging activity at different concentrations of test drug were determined and IC50 value of test drugs was compared with that of ascorbic acid, which was used as the standard.

Phosphomolybdenum Method

Total antioxidant capacity is a quantitative determination of antioxidant capacity and can be calculated by method described by Prieto et al. (1999) [11]. 0.1 ml of sample (100 µg) solution is mixed with 1ml of reagent (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The sample is incubated in a boiling water bath at 95°C for 90 minutes. After cooling, sample at room temperature the absorbance is recorded at 695nm nm
using UV-visible double spectrophotometer (Shimadzu-1800). The % scavenging activity at different concentrations of test drug were determined and IC\textsubscript{50} value of test drugs was compared with that of ascorbic acid, which was used as the standard.

RESULTS AND DISCUSSION

The results of DPPH radical scavenging activity of the subjected peptide leads are shown in table 1.

Table 1: DPPH Radical Scavenging for Peptides.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound Name</th>
<th>% Inhibition (µg/ml)</th>
<th>IC\textsubscript{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1.</td>
<td>Test compound (RK)</td>
<td>13.98</td>
<td>26.13</td>
</tr>
<tr>
<td>2.</td>
<td>Test compound (KH)</td>
<td>4.70</td>
<td>31.37</td>
</tr>
<tr>
<td>3.</td>
<td>Test compound (RRH)</td>
<td>28.72</td>
<td>45.14</td>
</tr>
<tr>
<td>4.</td>
<td>Test compound (RRK)</td>
<td>40.38</td>
<td>43.94</td>
</tr>
<tr>
<td>5.</td>
<td>Standard (Ascorbic acid)</td>
<td>19.6</td>
<td>31.24</td>
</tr>
</tbody>
</table>

*Value obtained from regression lines with 95% of confidence level. IC\textsubscript{50} is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%. All values given are mean of triplicate experiment at S.D. (5%) for the above table.

The graphs of uv-spectrophotometric determination of test drug and the standard with their linear regression and correlations coefficient are very well shown in figures 1-3.

Fig. 1: Graphical representation of linearity for the standard drug concentration with their corresponding values.
Fig. 2: Graphical representation of the test drug 1 (RK) concentration with their corresponding values.

Fig. 3: Graphical representation of linearity for the test drug 4 (RRH) concentration with their corresponding values.

All the test drug 1-4 demonstrated above the DPPH radical scavenging activity was detected in test drug-1 (RK) with an IC$_{50}$ value of 4.2 µg/ml (Table 1), which showed the antioxidant activity greater than that of standard ascorbic acid IC$_{50}$ value of 5.3 µg/ml.

Similarly test drug-2 (KH), test drug-3 (RRH) and test drug-4 (RRK) have shown almost comparatively better antioxidant capacity with that of the standard drug Ascorbic acid (IC$_{50}$ value of 5 µg/ml) by their corresponding IC$_{50}$ value are 7.4 µg/ml, 4.1 µg/ml and 6.0 µg/ml respectively.

The results of Phosphomolybdenum Method of the subjected peptide leads are shown in table 2.
Table 2: Phosphomolybdenum Method for Peptides.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound Name</th>
<th>% Inhibition (µg/ml)</th>
<th>IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1.</td>
<td>Test compound (RK)</td>
<td>30.87</td>
<td>38.55</td>
</tr>
<tr>
<td>2.</td>
<td>Test compound (KH)</td>
<td>19.29</td>
<td>43.91</td>
</tr>
<tr>
<td>3.</td>
<td>Test compound (RRH)</td>
<td>19.08</td>
<td>29.05</td>
</tr>
<tr>
<td>4.</td>
<td>Test compound (RRK)</td>
<td>19.42</td>
<td>29.72</td>
</tr>
<tr>
<td>5.</td>
<td>Standard (Ascorbic acid)</td>
<td>3.06</td>
<td>15.50</td>
</tr>
</tbody>
</table>

*Value obtained from regression lines with 95% of confidence level. IC₅₀ is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%. All values given are mean of triplicate experiment at S.D. (5%) for the above table.

The graphs of uv-spectrophotometric determination of test drug and the standard with their linear regression and correlations coefficient are very well shown in figures 4-6.

[Graph of Phosphomolybdenum Method for Ascorbic Acid]

Fig. 4: Graphical representation of linearity for the standard drug concentration with their corresponding values.
All the test drug 1-4 demonstrated above by the Phosphomolybdenum Method was detected in test drug-1 (RK) with an IC\textsubscript{50} value of 4.2 µg/ml (Table 1), which showed the antioxidant activity greater than that of standard ascorbic acid IC\textsubscript{50} value of 8.1 µg/ml.

Similarly test drug-2 (KH), test drug-3 (RRH) and test drug-4 (RRK) have shown almost comparatively better antioxidant capacity with that of the standard drug Ascorbic acid (IC\textsubscript{50} value of 8.1 µg/ml) by their corresponding IC\textsubscript{50} value are 6.3 µg/ml, 8.4 µg/ml and 8.2 µg/ml respectively.

Antioxidants are the compounds that protect cells against the damaging affects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radical, hydroxyl radicals and
peroxynitrite\textsuperscript{[12]}. An imbalance between antioxidant and reactive oxygen species results in oxidative stress, leading to cellular damage\textsuperscript{[13]}. Oxidative stress has been linked to cancer, ageing, artherosclerosis, neuro degenerative disease etc. The above research studies suggested that test drugs 2,3 and 4 (KH, RRH and RRK) possess a remarkable significant antioxidant property that may maintain good health by boosting the immune system and reducing inflammation and allergies.

**ACKNOWLEDGEMENT**

The authors are very much thankful to the Principal, KIET School of Pharmacy and The Director, Dr. S. Narendra Kumar, KIET Group of Institutions, Ghaziabad, India. Also we remain thankful to Mrs. M. Uma Maheswari, Professor, Department of Pharmacology, Sir Ramkrishna Institute of Paramedical Sciences, Coimbatore, India for her valuable technical suggestions.

**REFERENCES**
