Antibacterial and Anti-Urease Activities of Chloropheniramine maleat, Paracetamol and Clarithromycin

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ABSTRACT

This paper presents anti-urease and antibacterial activities of chloropheniramine maleat, paracetamol and clarithromycin. Antibacterial activity of the compounds was investigated against Salmonella enterica, Enterobacter aerogenes, Bacillus subtilis, Proteus vulgaris, Gordonia rubripertincta, Klebsiella pneumoniae and Enterococcus faecalis. Moreover, anti-urease activity of the compounds was searched. All tested compounds demonstrated antibacterial action with varying degree except for chloropheniramine maleat. Chloropheniramine maleat, paracetamol and clarithromycin exhibited higher activity than thiourea. The data obtained from the study, chloropheniramine maleate, paracetamol and clarithromycin are thought to be useful as new urease inhibitors and the present study should be supported by further studies. Furthermore, it is thought that paracetamol has antibacterial properties and therefore can be used as an alternative to antibiotics.

Keywords: Anti-urease, Antibiotic, Antibacterial, Clarithromycin, Chloropheniramine maleat, Paracetamol

Klorofeniramin Maleat, Parasetamol ve Klaritromisin’in Antibakteriyal ve Anti-Ureaz Aktiviteleri

ÖZET


Anahtar kelimeler: Anti-üreaz, Antibiyotik, Antibakteriyal, Klaritromisin, Klorofeniramin maleat, Parasetamol

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I. INTRODUCTION

The number of multi-drug resistant bacteria has increased. This rise has been linked with indiscriminate utilizing of antibiotics and immunosuppressive medicines. Moreover, in synthetic medicines are not costly and insufficient to cure of illnesses in developing countries [1]. Eventhough resistance to antimicrobial agents amongst important bacterial pathogens throughout the world are increasing, the number of brand antimicrobials which present in market has undergone a stable decrease especially in the past decades [2]. Hence, there is demand to investigate new infection-fighting agents to combat microbial infections [1].

Gastrointestinal diseases like gastritis are commonly caused by Helicobacter pylori. This bacterium leads diseases such as urinary stone formation, peptic ulcer, pyelonephritis, and hepatic coma. H. pylori habitance in the acidic medium of the stomach is highly dependence on the urease enzyme activity. Ureas enzyme converts the stomach medium into a tolerable condition for the bacteria via neutralizing gastric acid through breakdown urea to form carbon dioxide and ammonia [3]. Hence, there is a demand to find brand urease inhibitors to avoid this illnesses. Recently, many medicines have been recommended as urease inhibitors [4].

Clarithromycin is a new semi-synthetic macrolide which derived from erythromycin [5]. Clarithromycin possess bactericidal action towards respiratory pathogens [6]. Chlorpheniramine maleate has been utilized to relieve symptoms of cold and cure the allergic illnesses [7]. Paracetamol generally utilized for acute musculoskeletal pains [8].

In the current survey, it was aimed to reveal antibacterial and urease enzyme inhibition activity of clarithromycin, chlorpheniramine maleat and paracetamol which are used as active ingredients of some medicines. This is the first study about antibacterial activity of chlorpheniramine maleat. Also, this is the first record related to anti-urease activity of clarithromycin, chlorpheniramine maleat and paracetamol.

II. MATERIAL AND METHOD

A. CHEMICALS

Chlorpheniramine maleat, Paracetamol and Clarithromycine obtained from Atabay pharmaceutical company, Turkey.

B. BACTERIA

Salmonella enterica ATCC 14028 was obtained from Giresun Province Control Laboratory; Enterobacter aerogenes CCM 2531, Bacillus subtilis IMG 22 and Proteus vulgaris FMC 1 were obtained from Frat University Department of Biology; Gordonia rubripertincta (lab isolate) and Klebsiella pneumoniae (lab isolate) were acquired from Yeditepe University Department of Genetic and Bioengineering; Enterococcus faecalis was obtained from Rize Tayyip Erdoğan University Department of Biology.
**C. ANTIBACTERIAL ACTIVITY**

Minimum Inhibition Concentration (MIC) assay was determined with microbroth dilution method [9].

**D. ANTI-UREASE ACTIVITY**

Urease inhibitor activity was determined using Van Slyke and Archibald's method [10]. Test compounds were prepared at 4 different concentrations (0.00001-0.01 μg/mL). 0.5 mL test compound was mixed with 0.5 mL of urease which prepared with solution (pH = 6.8) in 100 mM 16 mg/mL phosphate buffer. This mixture was incubated at room temperature for 15 min. Then, 0.4 ml phenol red prepared in urea-phosphate buffer was added to the mixture and absorbance was read at 570 nm. Inhibition was calculated from the equation 1 below:

\[
\text{% inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]  

[1]

\(A_0\): Absorbance of control  
\(A_1\): Absorbance of sample

IC\(_{50}\) value of the urease enzyme (the amount of substance required for 50% inhibition of the enzyme) was calculated from the regression equation.

**III. RESULTS AND DISCUSSION**

**A. ANTIBACTERIAL ACTIVITY**

MIC defines as the minimum concentration of the antibacterial compound which inhibits the bacterial growth after 24 h [11]. Table 1 shows antimicrobial action of the compounds and standard. Antimicrobial effect was expressed as MIC value. All tested compounds demonstrated antibacterial action with varying degree except for chloropheniramine maleat. The most compound was clarithromycine but all the compounds possess lower activity than ciprofloxacin which used as standard agent. According to obtained results, MIC values of the compounds varied within the range from 0.125 mg/mL to 0.25 mg/mL and from 0.0625 mg/mL to 0.125 mg/mL for paracetamol and clarithromycin, respectively.

**Table 1. MIC values of the tested compounds and standards (mg/mL)**

<table>
<thead>
<tr>
<th>G (+) / G (-)</th>
<th>Bacteria</th>
<th>Chlorpheniramine maleat</th>
<th>Paracetamol</th>
<th>Clarithromycin</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>G (-)</td>
<td><em>S. enterica</em></td>
<td>NA</td>
<td>0.25</td>
<td>0.125</td>
<td>0.00781</td>
</tr>
<tr>
<td>G (-)</td>
<td><em>E. aerogenes</em></td>
<td>NA</td>
<td>0.25</td>
<td>0.0625</td>
<td>0.25</td>
</tr>
<tr>
<td>G (+)</td>
<td><em>B. subtilis</em></td>
<td>NA</td>
<td>0.125</td>
<td>0.0625</td>
<td>0.015625</td>
</tr>
<tr>
<td>G (-)</td>
<td><em>K. pneumoniae</em></td>
<td>NA</td>
<td>0.125</td>
<td>0.125</td>
<td>0.03125</td>
</tr>
</tbody>
</table>
Table 1. (continue). MIC values of the tested compounds and standards (mg/mL)

<table>
<thead>
<tr>
<th>G (+) / G (-)</th>
<th>Bacteria</th>
<th>Chlorpheniramine maleat</th>
<th>Paracetamol</th>
<th>Clarithromycine</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>G (+)</td>
<td>G. rubripertincta</td>
<td>NA</td>
<td>0.25</td>
<td>NA</td>
<td>0.015625</td>
</tr>
<tr>
<td>G (-)</td>
<td>P. vulgaris</td>
<td>NA</td>
<td>0.25</td>
<td>0.0625</td>
<td>0.00781</td>
</tr>
<tr>
<td>G (+)</td>
<td>E. faecalis</td>
<td>NA</td>
<td>0.25</td>
<td>0.125</td>
<td>0.0039</td>
</tr>
</tbody>
</table>

NA: No Activity

This is the first record related to antibacterial efficiency of chlorpheniramine maleat.

Pathogenic bacteria have been thought as a major reason for mortality in humans. Although pharmaceutical industry have manufactured many brand antibacterial agents in last years but bacterial resistance to these antibacterial agents is public health concern [12].

There are some studies related to antimicrobial activity of paracetamol and clarithromycin. For example, Zimmermann and Curtis (2017) reported that MIC values of paracetamol as 2.5 mg/mL, 5 mg/mL, 2.5 mg/mL, 2.5 mg/mL and 1.25 mg/mL against Bacillus spp., Enterobacter cloacae, Escherichia coli, Salmonella enterica serovar typhi and Staphylococcus aureus, respectively [13]. In this current study, MIC values were found as 0.25 mg/mL against S. enterica and 0.125 mg/mL against B. subtilis.

Hussain and Al-Janabi (2010) declared that MIC values of paracetamol as 0.312 mg/mL against E. coli, Salmonella typhi, E. cloaceae, S. aureus and B. subtilis [14]. On contrast to this study, in our study, MIC values was detected for E. aerogenes as 0.25 mg/mL and for B. subtilis as 0.125 mg/mL.

Ferrero et al. (1996) reported that clarithromycin possess activity against S. aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Moraxella catarrhalis and Haemophilus influenza [15]. According to a study which was carried out by Bergman et al (1999) clarithromycin had great activity on S. pneumoniae [16].

Different antibacterial results could be explained with using different bacterial strains and different concentration of the test compounds.

B. ANTI-UREASE ACTIVITY

The data for the determination of anti-urease activity of the compounds are given in Table 2. It was found that anti-urease activity rised linearly with the rise in the concentration of the test compounds. Chlorpheniramine maleat, paracetamol and clarithromycin exhibited higher activity than thiourea.
Table 2. Anti-urease activity of the test compounds (µg/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>% inhibition</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpheniramine maleat</td>
<td>0.00001</td>
<td>23.24±3.15</td>
<td>0.008575±0.0002</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>35.45±2.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>46.51±1.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>53.92±1.70</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>0.00001</td>
<td>31.87±2.40</td>
<td>0.006345±0.0002</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>45.28±1.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>57.45±2.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>73.40±2.48</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.00001</td>
<td>18.72±2.17</td>
<td>0.00986±0.004</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>30.22±1.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>39.29±2.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>47.03±2.17</td>
<td></td>
</tr>
<tr>
<td>Thiourea</td>
<td>0.00001</td>
<td>30.78±0.47</td>
<td>0.01052±0.0003</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>34.90±0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>38.80±0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>43.76±1.54</td>
<td></td>
</tr>
</tbody>
</table>

Acetohydroxamic acid and phosphoramidates which are urease inhibitors have exhibited curative impact, restrictions are linked heavy adverse impacts like psycho-neurological symptoms and teratogenicity so they have restricted their utilizing in the cure of urinary and gastrointestinal tracts disorders. Hence, the exploration for different type of urease inhibitors which possess the lowest side effects has acquire much interest [17].

As far as we know, this is also the first record about anti-urease activity of chlorpheniramine maleat, paracetamol and clarithromycin.

IV. CONCLUSION

This is the first record of anti-urease inhibition of chlorpheniramine maleat, paracetamol and clarithromycin. Our results clearly demostrated urease inhibitory effect of the test compounds. According the data obtained from the study demonstrates that chlorpheniramine maleat, paracetamol and clarithromycin might be used new urease inhibitor. Also, it was found that paracetamol possess antibacterial property so it may be used as an alternative to antibiotics.

V. REFERENCES


