Evaluation of Antibacterial Activity of Amitriptyline Hydrochloride.

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Abstract: Anti bacterial activities of several ‘non-antibiotic drugs’ used in treatment of a variety of non-infectious human diseases have been observed. Such an effect has been noticed for barbiturates, beta-adrenergic receptor antagonists, diuretic drugs, H$_1$ antihistamines, mucolytic agents, non-steroidal anti-inflammatory drugs, proton pump inhibitors and psychotherapeutic drugs. A synergic or antagonistic effect with antibiotics of several of these drugs have also been noticed. Amitriptyline hydrochloride, an anti depressant drug, was screened for its possible antimicrobial property against seven strains of bacteria, belonging to Gram positive and Gram negative genera, by disc-diffusion method at concentrations ranging from 5-25mg/ml. Amitriptyline HCl was found to possess moderate anti-bacterial activity. since the minimum inhibitory concentration (MIC) of amitriptyline, ranged from 1-2 mg/ml for the tested strains.

Key words: antibacterial activity, nutrient agar medium, amitriptyline, disc diffusion method.

Introduction

Many microorganisms produce infections in humans and other organisms. These infections are at present treated by using antibiotics and other antimicrobial agents. However, many microorganisms develop “resistance” to the above compounds due to their indiscriminate use. So, it is essential to develop a new antibiotic/antimicrobial compounds to combat the menace of resistance developed by microorganisms. But developing a new antibiotic or a antimicrobial compound and bringing them to market involves a lot of time, labour, money and failures. So, researchers across the world are trying to discover the antimicrobial activities of some drugs which are used for treating other diseases or disorders. Different studies on search of newer antimicrobials have revealed that moderate to remarkable antimicrobial action is present in several compounds[1] belonging to various pharmacological categories, such as the antihistamines tranquilizers, anti hypertensives, antipsychotics and the anti inflammatory agents[2] their pre designated pharmacological actions, have been christened ‘Non antibiotics’. Since many of these compounds possess two to three benzene rings, the present work was aimed to determine antimicrobial action of Amitriptyline(10,11-Dihydro-N,N-dimethyl-5H-dibenzo[a,d] cycloheptene-Δ5, γ-propylamine hydrochloride.), an anti depressant drug which is used for treating mental/mood problems such as depression.[3]

Materials and Methods

Chemicals and glasswares:

Amitriptyline was obtained as pure dry powder form from M/S Par Formulations Pvt ltd Chennai, India, Nutrient agar was obtained from M/S Hi Media Pvt Ltd, Mumbai, India .All other reagents (AR grade) were obtained from M/S SD fine chemicals, Mumbai. All glasswares were procured from M/S Borosil Ltd., Mumbai.
Collection of microorganism:

*Staphylococcus aureus* ATCC No.29737, *Bacillus subtilis* ATCCNO.6633,  *Micrococcus luteus/flavus* ATCCNO. 10240, *Bacillus pumilus* ATCCNO.12228(all gram positive bacteria), *Escheria coli* ATCCNO 8739, *Pseudomonas aeruginosa* ATCC NO25619,  *Klebsiella pneumoniae* ATCC NO 10031(all gram negative bacteria) were obtained from National chemical laboratory, Pune, India.

The culture medium(nutrient agar) was prepared as per the directions of manufacturer. All glass wares were sterilized in hot air oven at160°C for 2 hours. Nutrient agar and Whatman filter paper disc were sterilized in an autoclave in suitably packed conditions at 121° C (15psi) for 20 minutes[4]

**a)** Procedure for In vitro screening of Amitriptyline for antibacterial activity.

**Table-1 Results of in-vitro antibacterial activity of Amitriptyline**

<table>
<thead>
<tr>
<th>Name of the organism.</th>
<th>Zone of inhibition(mm) Average±S.D*</th>
<th>5mg/ml</th>
<th>10mg/ml</th>
<th>15mg/ml</th>
<th>20mg/ml</th>
<th>25mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>10±0.752</td>
<td>13±0.814</td>
<td>16±0.732</td>
<td>17±0.752</td>
<td>22±0.752</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>8±0.752</td>
<td>10±0.752</td>
<td>12±0.732</td>
<td>13±0.752</td>
<td>17±0.752</td>
<td></td>
</tr>
<tr>
<td><em>Micrococcus luteus/flavus</em></td>
<td>10±0.752</td>
<td>12±0.752</td>
<td>14±0.836</td>
<td>16±0.547</td>
<td>20±0.632</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus pumilus</em></td>
<td>11±0.814</td>
<td>12±0.513</td>
<td>14±0.752</td>
<td>15±0.836</td>
<td>17±0.894</td>
<td></td>
</tr>
<tr>
<td><em>Escheria coli</em></td>
<td>9±0.752</td>
<td>12±0.894</td>
<td>13±0.752</td>
<td>15±0.836</td>
<td>17±0.752</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>8±0.516</td>
<td>10±0.894</td>
<td>12±0.516</td>
<td>13±0.516</td>
<td>14±0.547</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>8±0.816</td>
<td>12±0.516</td>
<td>14±0.632</td>
<td>16±0.516</td>
<td>21±0.408</td>
<td></td>
</tr>
</tbody>
</table>

b) *n=6  p=0.05*

Gram positive bacteria and Gram negative bacteria were sub cultured in nutrient agar slants. The tubes were incubated at 37°C, examined for appearance of growth after 24 h and stored aseptically. The drug was dissolved in sterile distilled water to get concentrations of 5,10,15, 20,and 25mg/ml, under aseptic conditions. From the above sub-cultures, the organisms were directly suspended in 5mL of sterile distilled water. The turbidity of each suspension was adjusted to 0.5 McFarland Standard[4]. The suspensions were further diluted 1:100 with sterile distilled water. The inocula were mixed with molten nutrient agar medium(40°C) and poured into sterile Petri dishes(97mm in dia) aseptically and allowed to solidify. The drug dilutions(5…25mg/ml)[5] were impregnated into sterile filter paper disc and kept on the surface of the media and incubated at 37°C for 24 hour and then the diameters of zones of inhibition were measured.[6].

**c)** Determination of Minimum Inhibitory Concentration(MIC) of Amitriptyline.

Minimum Inhibitory Concentration (MIC) of Amitryptyline was determined by employing concentrations 1-5mg/ml of the drug. The procedure which is mentioned above was adopted to obtain zones of inhibition[7] .The results are presented as Table-2 and as figures 1-7

**Result and Discussion:**

**Discussion**

From the above results, it can be observed that dibenzocycloheptene derivative amitriptyline (tricyclic antidepressant) exhibited consistent antimicrobial activity against strains of *Staphylococcus aureus* ATCC No.29737,*Bacillus subtilis* ATCCNO.6633,  *Micrococcus luteus/flavus* ATCCNO. 10240, *Bacillus pumilus* ATCCNO.12228(all gram positive bacteria), *Escheria coli* ATCCNO 8739, *Pseudomonas aeruginosa* ATCC NO25619,  *Klebsiella pneumoniae* ATCC NO 10031(all gram negative bacteria). Result shows that amitriptyline inhibits the growth of bacteria (both gram positive and gram negative) by disc diffusion method[8,9] . The antibacterial activity is more pronounced in cases of gram positive bacteria than gram negative bacteria. The reason may be the better accessibility of cell walls of gram positive bacteria than that of
gram negative bacteria, by Amitriptyline. The MIC of the drug ranges from 1-2mg/ml, which indicates the suitability of the drug for treatment of infections

Table-2 Results of determination of MIC of Amitriptyline.

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Zone of inhibition(mm) Average±S.D*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1mg/ml</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>6±0.752</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>6±0.752</td>
</tr>
<tr>
<td><em>Micrococcus luteus/flavus</em></td>
<td>6±0.752</td>
</tr>
<tr>
<td><em>Bacillus pumilus</em></td>
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<td><em>Escheria coli</em></td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>6±0.516</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>6±0.816</td>
</tr>
</tbody>
</table>

* n=6  p=0.05.

Fig 1: Antibacterial activity of Amitriptyline against *Pseudomonas aeruginosa*;

Fig 2: Antibacterial activity of Amitriptyline against *Bacillus pumilus*

Fig-3: Antibacterial activity of Amitriptyline against *Klebsiella pneumoniae*

Fig 4 Antibacterial activity of Amitriptyline against *Staphylococcus aureus*

Fig-5: Antibacterial activity of Amitriptyline against *Micrococcus luteus/flavus*
Fig-6 Antibacterial activity of Amitriptyline against *Escheria coli*

Fig-7 Antibacterial activity of Amitriptyline against *Bacillus subtilis*

The drug Amitriptyline inhibits bacterial growth at 37°C had the most pronounced antibacterial effects. This drug is in routine therapeutic usage satisfying human toxicity tests, in course of time, be developed as the second or even the first line antimicrobial agent in many infections;(which is reflected from its MIC values) such properties would further enhance its applicability in humans[10]. Thus, the present study suggests that amitriptyline has a potential for being developed into a powerful antimicrobial agent, the efficacy of which may be enhanced further by various structural modifications and clinical or chemotherapeutic synergistic combinations of the drug with conventional antimicrobics and/or non-antibiotics.[11]

**Conclusion:**

The problem of bacterial resistance to almost all antibiotics calls for the necessity of finding out newer drugs. Although, there are continuous reports on newer antimicrobial agents, the search in completely different approaches have indicated antimicrobial property in a large number of compounds[12]. The results of the findings of these new classes of antimicrobial agents through in vitro screening viz, the antihistamines, tranquilisers, neuroleptics, antipsychotics, and antihypertensives have led to the recognition of phenothiazines as a class of compoundsexhibiting potentials for powerful antimicrobial activity[13].The tricyclic antidepressant amitriptyline possesses anti depressant properties. From our study, it can be concluded that Amitryptyline possesses antibacterial activity against selected strains of Gram-positive and Gram-negative bacteria, with MIC values ranging from 1- 2 mg/mL. The results have to be further confirmed by testing Amitryptyline against clinical isolates of the above bacteria for antibacterial activity[14].
References


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