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Full Length Research Paper

Physician prescription practice of antibiotics for upper respiratory tract infection at Kilimanjaro Christian Medical Centre Moshi, Tanzania

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Upper respiratory tract infection occurs commonly in both children and adults and is a major cause of morbidity worldwide. Inappropriate antibiotic prescription for upper respiratory tract infections is associated with increasing antibiotic resistance, healthcare costs, adverse events, and poor patient outcomes. The objective of this study was to determine physician prescription practices of antibiotics for upper respiratory tract infections at Kilimanjaro Christian Medical Center hospital in Moshi, Tanzania. This was a retrospective hospital-based cross sectional study which systematically sampled files of patients with diagnosis of upper respiratory tract infection. Information from a total of 300 patients’ prescriptions were collected, reviewed and analyzed. The most common infections diagnosis was non-specific upper respiratory tract infections accounting for 102 (34.0%) followed by rhinitis and tonsillitis both accounting for 52 (17.3%) with the least being common cold 22 (7.3%). Antibiotics were prescribed to 200 (66.7%) patients with upper respiratory tract infections. Amoxicillin alone was the most preferred drug for all upper respiratory tract infections accounting for 91 (31.5%). In the multivariable logistic regression analysis, patients with cough and running nose (AOR=16.41, 95% CI: 1.95-138.19) had higher odds of being prescribed with antibiotic as compared to those without such symptoms (AOR=1.98, 95% CI: 1.04-3.77), respectively. Antibiotics are being over-prescribed among patients with upper respiratory tract infection. Interventions to reduce the over-prescription and hence overuse of antibiotics for upper respiratory tract infections are urgently needed.

Key words: Antibiotics prescribing, upper respiratory tract infection, Tanzania.

INTRODUCTION

Upper respiratory tract infections (URTIs) is a term used to describe acute infections involving the nasal cavity,

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pharynx and larynx (Mungrue et al., 2009; Yoon et al., 2017). URTIs are classified according to the area of inflammation, which are tonsillitis, pharyngitis, laryngitis, sinusitis, common cold, influenza and otitis media (Centre for Clinical Practice, 2008). Moreover, Otitis media has not been classified as URTIs, although it has been linked with the upper respiratory tract, since it occurs as a complication of URTIs hence tends to be identified within URTIs (Chomnaitree et al., 2008).

Most of the URTIs are of viral etiology and these include viruses in the family rhinovirus, coronavirus, parainfluenza, respiratory syncytial virus, adenovirus and influenza (Cotton et al., 2008). Occasionally, bacteria may develop after a viral illness such as a cold or the flu. The significance of pathogenic bacteria in the upper respiratory tract is yet to be characterized (MacIntyre et al., 2017), since positive nasopharyngeal bacterial culture is a weak predictor of URTIs because healthy individuals often carry pathogenic bacteria (Ouédraogo et al., 2014). People are generally thought to be asymptomatic carriers of bacteria (Faden et al., 1997; Givon-lavi et al., 2002; Bogaert et al., 2004). Most episodes of URTIs are typically self-limiting, thus do not require physician visit or antibiotic(s) prescription (Peroš-Golubič and Tekavec-Trkanjec, 2015; Lior et al., 2017). Acute respiratory infections which are divided into URTI and lower respiratory tract infections, have been established as one of the leading causes of childhood morbidity and mortality in Africa (Symekher et al., 2009). It has been estimated that up to 1.9 million children die each year from acute respiratory with nearly 70% of deaths occurring in Africa and South East Asia (Simoes et al., 2006; Symekher et al., 2009). However, the burden of URTI in most African countries including Tanzania has not been documented.

Despite consistent and continued education among healthcare professionals on antibiotic resistance, antibiotic prescribing rates for URTIs remain high in general practice (Fletcher-Lartey et al., 2016). World Health Organization (WHO) estimated that up to 60% of people with URTIs receive antibiotics inappropriately (Kunda et al., 2015). Inappropriately use of antibiotics contributes to the emergency of antimicrobial resistance (AMR), which is a major public health problem worldwide. Documented factors associated with antibiotic prescription for URTI by physicians include fever (temperature >38°C), fear of complications, inflamed eardrums, cough, and throat irritation as well as primary caregivers’ pressure and patients financial gains (Al-Enezi et al., 2011).

Guidelines by Centre of Disease Control (CDC) do not recommend antibiotic prescribing in non-specific URTIs because antibiotics neither enhance illness resolution nor prevent complications (CDC, 2017). One study showed that only 12% of physician would request laboratory tests such as culture and sensitivity before prescribing antibiotics, and 88% of the physician considered laboratory investigations as unnecessary (Mohan et al., 2004). Misdiagnosis and improper diagnosis leads to incorrect antibiotics prescription and so increases AMR in many parts of the world (Haque, 2017). For better treatment outcome, healthcare workers are encouraged to request for microbiological testing prior to start of antibiotics for a more rational antibiotic use in both children and adults (Levy-Hara et al., 2011; Chaw et al., 2018).

Several studies have shown the rate of antibiotic prescriptions in relation to the diagnosis of URTIs made which was high and inappropriate (Teng et al., 2004; Gwimile et al., 2012; Kunda et al., 2015). Inappropriate antibiotic prescription for URTI is a global public health problem, therefore URTIs are important for strategies aimed at reducing excess antibiotic use because antibiotics are frequently prescribed in these illnesses that are predominantly of viral etiology (Easton and Saxena, 2010; Kunda et al., 2015; Fletcher-Lartey et al., 2016; Zhang et al., 2017).

Although prescribing patterns generally differ between countries due to the national guidelines and drugs available, very little is known about antibiotics prescribing practices in Tanzania and specifically at Kilimanjaro Christian Medical Centre (KCMC). Despite the fact that awareness of the consequences of antibiotic misuse is increasing among population (Mbwambo et al., 2017), as well as among healthcare providers (Lyimo et al., 2018), in the northern part of Tanzania, overprescribing of antibiotics is still being practiced at high rate even before availability of laboratory results are made available (Chilongola et al., 2015; Kajeguka et al., 2017). Therefore, this study aimed at assessing physician prescription practice on antibiotics for URTIs.

METHODS

Study design and area

This was a retrospective hospital-based cross sectional study carried out from April 2017 to July 2017 at KCMC hospital in Moshi, Kilimanjaro region, Tanzania. Physicians’ antibiotic prescriptions from January 2015 to June 2017 were included. KCMC is a consultant referral hospital with 630 inpatient beds serving several regions in northern part of Tanzania. The study was conducted in three departments; which are Pediatric, Internal Medicine and Outpatient department (OPD).

Sample size determination and sampling

The following formula was used to obtain the required sample size:

\[ N = \frac{Z^2 \cdot P \cdot (1-P)}{d^2} \]

where N is the required sample, Z is the confidence level at 95% (1.96), P is the prevalence of 0.78, and d is the margin of error at 5% (0.05).
Table 1. General characteristics of study participants (N=300).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, Mean ± SD</td>
<td>2.02 ± 1.03</td>
</tr>
<tr>
<td>&lt;15</td>
<td>126 (42.0)</td>
</tr>
<tr>
<td>15-35</td>
<td>84 (28.0)</td>
</tr>
<tr>
<td>36-45</td>
<td>25 (8.3)</td>
</tr>
<tr>
<td>&gt;45</td>
<td>65 (21.7)</td>
</tr>
<tr>
<td>Age categories (in years)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>92 (30.7)</td>
</tr>
<tr>
<td>Female</td>
<td>208 (69.3)</td>
</tr>
<tr>
<td>Ward/Department</td>
<td></td>
</tr>
<tr>
<td>Pediatric</td>
<td>62 (20.7)</td>
</tr>
<tr>
<td>Internal medicine</td>
<td>42 (14.0)</td>
</tr>
<tr>
<td>Outpatient Department</td>
<td>196 (65.3)</td>
</tr>
</tbody>
</table>

The prevalence of antibiotics prescription on URTI was 78% (Kunda et al., 2015). A maximum of 300 patient files were systematically sampled. Records with missing data on URTIs were excluded.

Data collection methods and tools

All prescriptions of patient with the diagnosis of URTI from physician in selected departments/wards at KCMC hospital were included. Data was extracted from patient’s medical records (files). The following data were recorded: age, sex, diagnosis given, if laboratory test were requested, if antibiotic was prescribed, type of antibiotic prescribed, number of antibiotics prescribed and type of URTIs. Signs and symptoms that prompted antibiotic prescription were recorded, such as fever (Temperature >38°C), chest pain, cough, running nose, exudates in throats, inflamed ear and difficulty in breathing.

Data analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp, Armonk, NY, USA). Descriptive statistics was used to summarize data. Differences between categorical data were calculated using Pearson’s Chi-square test ($\chi^2$). Factors that were found to have a level of significance of $p<0.05$ were then entered into the final model of the multivariable logistic regression analysis, which was used to compute adjusted odds ratio (AOR) and 95% confidence intervals (95% CI) to assess the independent associations of these variables with outcome of interest (antibiotic prescription). A $p<0.05$ was significant.

RESULTS

Demographics characteristics and URTIs diagnosed

A total of 300 patient files were reviewed. The mean age in years (±Standard deviation) was 2.02 ± 1.03, with those aged 15 years and below constituting the highest proportion 126 (42.0%) followed by those aged 16 to 35 years 84 (28.0%). Two hundred and eight participants (69.3%) were female. Most of the participants were from OPD 196 (65.3%), followed by pediatric 62 (20.7%) and internal medicine 42 (14.0%) (Table 1). The most common URTIs diagnosed were non-specific URTIs 102 (34.0%) followed by rhinitis and tonsillitis, both 52 (17.3%). The least diagnosis assigned was common cold 22 (7.3%) (Figure 1).

Antibiotics prescribing pattern for URTIs

Two hundred patients with URTI (66.7%) received at least one antibiotic (Any antibiotic); with the highest in pharyngitis 47 (100%), followed by non-specific URTIs 82 (80.4%) and rhinitis 38 (73.1%) (Table 2). Amoxicillin alone was the most frequently prescribed antibiotic for common cold 16 (72.7%). Ampicillin was frequently prescribed for otitis media 7 (28.0%), followed by tonsillitis 14 (26.9), and pharyngitis 11 (23.4). Ampiclox was commonly prescribed for otitis media 10 (40.0%) (Table 2).

Signs and symptoms that influenced antibiotic prescription for URTIs

Multiple responses were allowed in this variable. Cough 236 (32.1%), running nose 114 (19.4%), fever (temperature >38°C) 115 (15.6%), exudates in throats 84 (11.4%), chest pain 70 (9.5%), difficulty in breathing 38 (5.2%), and inflamed ear 26 (3.5%) constituted the most common clinical presentations which affected the physician’s decision to prescribe antibiotics for URTIs.
(Figure 2).

The multivariable logistic regression analysis revealed that five independent variables had a significant influence on the antibiotic prescription. In the adjusted analysis (additional adjustment such as sex and age) patients who had fever, chest pain and difficulty in breathing had reduced odds of being prescribed with antibiotic as compared to those without such symptoms (AOR=0.02, 95% CI: 0.008-0.09), (AOR=0.29, 95% CI: 0.12-0.74) and (AOR=0.25, 95% CI: 0.07-0.85), respectively. Moreover, patients who had cough and running nose had higher odds of being prescribed with antibiotic as compared to those without such symptoms (AOR=16.41, 95% CI: 1.95-138.19) and (AOR=1.98, 95% CI: 1.04-3.77), respectively (Table 3).

**DISCUSSION**

The objective of the present study was to assess physician prescription practice on antibiotics for URTIs in order to have baseline information on antibiotic use and thereafter make recommendations to stake holders. It has been documented that most URTIs are of viral origin in 80% of cases (Kunda et al., 2015), however, physicians in many settings frequently prescribe antibiotics for these illnesses and contributing to increasing antibiotic over-prescribing is a problem in many settings (Easton and Saxena, 2010; Kunda et al., 2015; Fletcher-Lartey et al., 2016; Zhang et al., 2017) and the present results show that Tanzania is not an exception.

In this study, it was found that a significant number of patients with URTIs were prescribed with at least one antibiotic (66.7%). This prevalence was notably high and if not prevented it will continue to escalate the problem of antibiotic resistance. The present study, reports a relatively lower prevalence as compared to a studies by Gwimile et al. (2012) with a prevalence of 84.9%, a study in Namibia, with a prevalence of 78% among adults and children (Kunda et al., 2015) and in Malaysia, a prevalence of 68.4% (Teng et al., 2004). The reason for lower prevalence could be because of the availability of supportive environments such as laboratory facilities and effective hospital policies that influence antibiotic prescribing behaviors (Lyimo et al., 2018).

The most common URTIs diagnosed was non-specific URTIs (34.0%) followed by rhinitis and tonsilitis both 17.3%. This result was lower than studies conducted in Windhoek, Namibia and North Trinidad where it was 45and 54.5%, respectively (Mungrue et al., 2009; Kunda et al., 2015). This study shows that amoxicillin alone was the preferred drug for almost all URTIs. The same scenario was reported in Trinidad whereby amoxicillin alone or with clavulanate was the most frequently prescribed antibiotic for all URTIs (Mohan et al., 2004). This is of concern because prescribing antibiotics for these conditions in adults and children does not have any therapeutic benefits, but only increases the risk of developing antibiotic resistance. In addition, antibiotics do not warrant a better outcome in terms of cure or persistence of symptoms in patients who receive antibiotics compared to those who do not (Snellman et al., 2013).

Regarding signs and symptoms that influenced physician's decision to prescribe antibiotics for URTIs, fever (temperature >38°C), chest pain, cough, running nose and difficulty in breathing were the factors mostly affecting physician's decision to prescribe antibiotics. In this study, patients with fever, chest pain and difficulty in
breathing were less likely to be prescribed with antibiotics. In other settings, patients with fever and cough were reported to be prescribed with antibiotics assuming that they were more severely ill (Akkerman et al., 2005). In areas like Tanzania where malaria is common, symptoms of fever and cough may also be shared with those of malaria. In such scenario, it is likely that clients who presented with these symptoms may have been prescribed with antimalarial, and this has been evidenced in different studies conducted in Kilimanjaro region, Northern Tanzania (Hertz et al., 2012; Crump et al., 2013; Kajeguka et al., 2016, 2017).

Table 2. Antibiotics prescribed for URTIs.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Pharyngitis</th>
<th>Rhinitis</th>
<th>Tonsillitis</th>
<th>Common cold</th>
<th>Otitis media</th>
<th>Non-specific URTIs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin No</td>
<td>24 (51.1)</td>
<td>32 (61.5)</td>
<td>36 (69.2)</td>
<td>6 (27.7)</td>
<td>23 (92.0)</td>
<td>84 (82.4)</td>
<td>206 (68.7)</td>
</tr>
<tr>
<td>Amoxicillin Yes</td>
<td>23 (48.9)</td>
<td>20 (38.5)</td>
<td>16 (30.8)</td>
<td>16 (72.7)</td>
<td>2 (8.0)</td>
<td>18 (17.6)</td>
<td>94 (31.3)</td>
</tr>
<tr>
<td>Ampicillin No</td>
<td>36 (76.6)</td>
<td>49 (94.2)</td>
<td>38 (73.1)</td>
<td>22 (100)</td>
<td>18 (72.0)</td>
<td>101 (99.0)</td>
<td>264 (88.0)</td>
</tr>
<tr>
<td>Ampicillin Yes</td>
<td>11 (23.4)</td>
<td>3 (5.8)</td>
<td>14 (26.9)</td>
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<td>7 (28.0)</td>
<td>1 (1.0)</td>
<td>36 (12.0)</td>
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<tr>
<td>Co-amoxiclav No</td>
<td>38 (80.9)</td>
<td>51 (98.1)</td>
<td>48 (92.3)</td>
<td>22 (100)</td>
<td>23 (92.0)</td>
<td>100 (98.0)</td>
<td>264 (88.0)</td>
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<td>Co-amoxiclav Yes</td>
<td>9 (19.1)</td>
<td>1 (1.9)</td>
<td>4 (7.7)</td>
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<td>2 (8.0)</td>
<td>2 (2.0)</td>
<td>36 (12.0)</td>
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<td>Cloxacillin No</td>
<td>46 (97.9)</td>
<td>52 (100)</td>
<td>52 (100)</td>
<td>22 (100)</td>
<td>21 (84.0)</td>
<td>100 (98.0)</td>
<td>282 (94.0)</td>
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<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>4 (16.0)</td>
<td>2 (2.0)</td>
<td>18 (6.0)</td>
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<tr>
<td>Ceftriaxone No</td>
<td>46 (97.9)</td>
<td>52 (100)</td>
<td>43 (82.7)</td>
<td>22 (100)</td>
<td>16 (64.0)</td>
<td>101 (99.0)</td>
<td>293 (97.7)</td>
</tr>
<tr>
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<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>9 (17.0)</td>
<td>0 (0.0)</td>
<td>9 (36.0)</td>
<td>1 (1.0)</td>
<td>7 (2.3)</td>
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<tr>
<td>Clarithromycin No</td>
<td>43 (91.5)</td>
<td>50 (96.2)</td>
<td>46 (88.5)</td>
<td>22 (100)</td>
<td>25 (100)</td>
<td>102 (100)</td>
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</tr>
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<td>2 (3.8)</td>
<td>6 (11.5)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>20 (6.7)</td>
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<tr>
<td>Ampiclox No</td>
<td>43 (91.5)</td>
<td>52 (100)</td>
<td>47 (90.4)</td>
<td>21 (95.5)</td>
<td>15 (60.0)</td>
<td>100 (98.0)</td>
<td>289 (96.3)</td>
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<td>11 (3.7)</td>
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<td>4 (9.57)</td>
<td>52 (100)</td>
<td>48 (92.3)</td>
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<td>24 (96.0)</td>
<td>102 (100)</td>
<td>278 (92.7)</td>
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<td>Chloramphenicol Yes</td>
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<td>Penicillin G No</td>
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<td>Gentamicin No</td>
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<td>52 (100)</td>
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<td>0 (0.0)</td>
<td>4 (1.3)</td>
</tr>
<tr>
<td>Doxycycline No</td>
<td>46 (97.9)</td>
<td>47 (90.4)</td>
<td>51 (98.1)</td>
<td>20 (90.9)</td>
<td>25 (100)</td>
<td>99 (97.1)</td>
<td>288 (96.0)</td>
</tr>
<tr>
<td>Doxycycline Yes</td>
<td>1 (2.1)</td>
<td>5 (9.6)</td>
<td>1 (1.9)</td>
<td>2 (9.1)</td>
<td>0 (0.0)</td>
<td>3 (2.9)</td>
<td>12 (4.0)</td>
</tr>
<tr>
<td>Azithromycin No</td>
<td>47 (100)</td>
<td>52 (100)</td>
<td>50 (96.2)</td>
<td>22 (100)</td>
<td>21 (84.0)</td>
<td>102 (100)</td>
<td>294 (98.0)</td>
</tr>
<tr>
<td>Azithromycin Yes</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (3.8)</td>
<td>0 (0.0)</td>
<td>4 (16.0)</td>
<td>0 (0.0)</td>
<td>6 (2.0)</td>
</tr>
<tr>
<td>Any Antibiotic No</td>
<td>0 (0.0)</td>
<td>14 (26.9)</td>
<td>23 (45.1)</td>
<td>9 (40.9)</td>
<td>9 (36.0)</td>
<td>20 (19.2)</td>
<td>100 (33.3)</td>
</tr>
<tr>
<td>Any Antibiotic Yes</td>
<td>47 (100)</td>
<td>38 (73.1)</td>
<td>28 (54.9)</td>
<td>13 (59.1)</td>
<td>16 (64.0)</td>
<td>82 (80.4)</td>
<td>200 (66.7)</td>
</tr>
</tbody>
</table>
Table 3. Signs and symptoms and antibiotic prescribed for URTIs.

<table>
<thead>
<tr>
<th>Categories</th>
<th>Frequency [n (%)]</th>
<th>COR (95% CI)</th>
<th>AOR (95% CI)</th>
<th>a AOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>115 (38.3)</td>
<td>0.02 (0.008-0.08)</td>
<td>0.03 (0.009-0.10)</td>
<td>0.02 (0.008-0.09)</td>
</tr>
<tr>
<td>No</td>
<td>185 (61.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>70 (23.3)</td>
<td>0.26 (0.13-0.55)</td>
<td>0.24 (0.10-0.58)</td>
<td>0.29 (0.12-0.74)</td>
</tr>
<tr>
<td>No</td>
<td>230 (76.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>236 (78.7)</td>
<td>43.96 (5.99-322.39)</td>
<td>12.89 (1.60-103.59)</td>
<td>16.41 (1.95-138.19)</td>
</tr>
<tr>
<td>No</td>
<td>64 (21.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running nose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>114 (48.0)</td>
<td>2.87 (1.73-4.74)</td>
<td>2.04 (1.08-3.86)</td>
<td>1.98 (1.04-3.77)</td>
</tr>
<tr>
<td>No</td>
<td>156 (52.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exudates in throat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>84 (28.0)</td>
<td>0.89 (0.53-1.49)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No</td>
<td>216 (72.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflamed ear</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (8.7)</td>
<td>2.76 (0.60-12.72)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No</td>
<td>274 (91.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficulty in breathing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38 (12.7)</td>
<td>0.31 (0.14-0.70)</td>
<td>0.28 (0.10-0.74)</td>
<td>0.25 (0.07-0.85)</td>
</tr>
<tr>
<td>No</td>
<td>262 (87.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

COR: Crude odds ratio, AOR: adjusted odds ratio. Only variables that were predictors of antibiotic prescription (set in the bivariate analysis to p<0.05) were included in multivariate analysis. *Includes additional adjustment such as sex and age.

The CDC and WHO recommend a performance of a group A β-hemolytic streptococci test prior to treatment of sore throat with antibiotics (Biezen et al., 2015). Laboratory test (such as culture and sensitivity) was obtained for investigation for about 136 (47.1%) patients diagnosed with URTIs before antibiotic therapy was prescribed. This indicates that most prescribers are still not well aware of the concept and implications of
antibiotic resistance. Therefore, they do not take laboratory tests into consideration when they decide whether or not to prescribe antibiotics for URTIs.

In order to reduce the irrational use of antibiotic prescribing in URTIs, it is of greatest importance that at the health facility level, the health care providers are trained on appropriate antibiotic prescription. This will assist in alleviating antibiotic overprescribing and the consequence of antibiotic resistance. Health workers’ continuing education should be strengthened through conferences and seminars. Equally important is the periodic antibiotic use review, which can provide feedback to prescribers in health facilities on antibiotic use expenditure and resistance patterns. Also, there is obvious need for an antibiotic stewardship committee that will follow-up closely and enable care providers to rationally prescribe antibiotics.

Additionally, Tanzanian Ministry of Health, Community Development, Gender, Seniors and Children should consider adopting a strategy of delayed prescription or delayed antibiotic use, which has shown to be effective in reducing antibiotic usage for URTIs (Spurling et al., 2013; Ryves et al., 2016). In a Cochrane Review it has been highlighted that delayed prescribing may be a suitable compromise in place of immediate prescribing to significantly reduce unnecessary antibiotic use and thereby reduce antibiotic resistance while maintaining patient safety and satisfaction levels (Spurling et al., 2017). Moreover, regular training in antibiotic management for healthcare professionals is paramount, also there should be an awareness regarding antibiotics among patients and the community (Mbwanbo et al., 2017). Further research is recommended to identify factors contributing to antibiotic over-prescribing in URTIs in Tanzania, which will also identify barriers of compliance to standard treatment guideline.

STRENGTHS AND LIMITATIONS
The study utilized systematic random sampling of prescriptions in KCMC and captured a broad view of antibiotic prescribing for URTIs in this setting. The study was conducted at one health facility. This prevented generalization of the results to the larger population of Tanzania. This study relied on the diagnosis written on the prescription and clinical presentation of the patient not the microbiological culture, which lead to a failure to comment on the appropriateness of the antibiotic prescription in relation to laboratory results.

CONCLUSION
In URTI treatment, use of antibiotic is always an area of concern. An irrational prescription practice of antibiotics is an important public health issue that affects the community. Use of antibiotic for the treatment of URTIs is evidently inappropriate unless the infection was proven to be bacteria.

CONFLICT OF INTERESTS
The authors have not declared any conflict of interests

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Simple bioanalytical method development and validation of micronised Domperidone 20 mg tablets using LCMS-MS and its pharmacokinetic application in Healthy Indian Volunteers

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The current investigation deals with an validated Liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) analytical method for the quantification of micronised domperidone in plasma of human volunteers. The validation of LC-MS/MS method was accomplished by evaluating the inter-day and intra-day precision and accuracy in a linear concentration range of 3.33-100 ng/ml. The entire study was an attempt to evaluate the comparison index of the bioavailability study of micronised domperidone tablet formulation with that of conventional domperidone tablet containing 20 mg of domperidone. Both the formulations were given orally as a single dose cross over design. The washout period was taken as 1 week. A single-dose, two-sequence, two-treatment, two-period crossover Bioequivalence study of two formulation were performed on 12 Indian healthy male volunteer. The estimation of domperidone concentration in human plasma was determined by the validated LC-MS/MS method. The various pharmacokinetics parameters like peak plasma concentration (C_{max}), and time to reach peak plasma concentration (t_{max}), area under the plasma concentration-time curve (AUC_{0-1}), area under the plasma concentration-time curve from zero to infinity (AUC_{0-∞}), of both the formulations were evaluated and compared. The results evaluated by estimated pharmacokinetic parameters did not find any statistically significant difference between the two formulations. The relative bioavailability of micronized test formulation was found to be 104.62% to that of reference conventional formulation.

Key words: Bioequivalence, domperidone, liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis, pharmacokinetics.

INTRODUCTION

Gastroesophageal reflux disease (GERD) can be defined as one of the most common incident related to upset in...
the gastro intestinal system (Hosseini et al., 2017; Dent et al., 2005). The recent advancement of medical sciences has already explored several options to treat and manage GERD effectively. Among them Acid suppression is one of the best treatment strategy to counter GERD symptoms for this reasons, Acid suppressing agents proton pump inhibitors (PPIs) provides the rapid and smooth symptomatic relief and heals esophagitis in a high proportion of patients (Dent et al., 2005). Domperidone can be considered as one of the most effective antiemetic drug for the treatment of mild to severe GERD symptoms Domperidone has dual mechanism of action. Domperidone can act as prokinetic agent which can stimulate the sphincter muscle of duodenam and easily can induce the effect of prokinetic movement. In addition, domperidone can block D₂ receptor antagonist in chemo trigger receptor zone (CTZ). The problem associated with domperidone for the treatment of GERD is its less bioavailability Domperidone is a water insoluble drug, domperidone is one of the ideal candidate to increase the solubility and as well as bioavailability. Here we developed a sustained release formulation with micronised domperidone. The main objective of our research work is to access the bioavailability of sustained release formulation and compare it with conventional non micronised formulation. Bioequivalent study of water insoluble drug has been executed by several researchers but very few related to micronised formulation has been addressed in a proper scientific way. Thorough literature survey finds that several methods was performed for conducting bioequivalent study but an attempt was made here to perform bioequivalent study of micronised domperidoene formulation as compare to conventional domperidone dosage form (Toyama et al., 2015; Bhadoriya et al., 2018; Blandizzi et al., 2015; Censi et al., 2015; Benet, 2013; Rockville, 2001). The interesting research conclusion which may come out from this study can be described as whether micronised drugs formulated as sustained release matrix tablet dosage form are able to deliver better and sustained bioavailability.

MATERIALS AND METHODS

Chemicals reagents and drug product

Raw domperidone (API) was provided as gift samples as by Kusum Healthcare, Punjab, India. HPLC grade methanol and Ethyl acetate were procured from Merck India Pvt. Ltd. (Mumbai). Milli Q water purification system was installed to acquire High Performance Liquid Chromatography (HPLC) grade water. The human blank plasma sample with EDTA-K₃ anticoagulant was procured from Bioequivalence Study Centre, Jadavpur University, Kolkata, India. Test product: Tablet containing micronized Domperidone 20 mg and Reference product: Domstal, from Torrent Pharmaceutical Ltd, (Torrent House, Ahmedabad, India), containing domperidone 20 mg.

Instrumentation

The LC system was purchased from Shimadzu (Kyoto, Japan). API 2000 triple quadrupole mass spectrometer (MDS Sciex, Canada) with electrospray ionization (ESI) source was used for detection of the compound. Data acquisition was done with Analyst 1.4.1 software. Chromatographic separation was performed on a standard C8 column, 50 mm × 3 mm, 3 μm i.d (Phenomenex, USA).

Products studied

The following test and reference products were used in the present study. Test product: Tablet containing micronized domperidone 20 mg and reference product: Domstal, from Torrent Pharmaceutical Ltd, (Torrent House, Ahmedabad, India), containing domperidone 20 mg.

Chromatographic conditions

The entire chromatographic analysis was executed at ambient atmospheric temperature with a runtime 5 min. The injection volume was taken as 20 μl. The composition of water: methanol (2:98, v/v) was used as mobile phase containing 0.5% formic acid with a flow rate of 1 ml min⁻¹. The column oven was kept at 23°C. While the temperature of auto sampler was maintained at 10°C. The mass spectra of the compounds were acquired by using Electrospray ionization (ESI) with multiple reactions monitoring (MRM) technique. The entire ionization of the drug was accomplished in positive ionization mode. The important tuning parameters were calculated and optimized by injecting 100 ng mL⁻¹ of standard solution containing all two drugs including internal standard. The validation parameters like sensitivity, accuracy, precision, stability, recovery, reproducibility and system suitability were measured in accordance with the US-FDA bioanalytical method guidelines (Bhadoriya et al., 2018).

Study design

The whole bioequivalent study was executed under fasting conditions as a two-sequence two-period crossover study. The study design was based on free randomization. The drug was administered with single-dose. Between the two periods minimum one week of dosing interval was taken as a washout period (Blandizzi et al., 2015) prior to the study. The experimental protocol was reviewed and approved by Institutional Ethical Committee (ICE) of Jadavpur University, Kolkata, India. The volunteers were enrolled after thorough investigation including medical history, vital parameters of physical examination, laboratory investigation, drug screening, ECG and HIV/hepatitis status. An experience clinical pharmacologist was actively present throughout the study to guide and monitor the entire study.

Drug administration and blood sample collection

12 non-smokers healthy Indian male volunteers were screened for the study. The age of the volunteers were between 19 to 33 years (29 ± 3.49) with a standard body mass index between 19 to 27 (24.66 ± 3.76). The whole study was executed under the regulation and guidance issued by U.S. Food and Drug Administration (FDA) and European Agency for the Evaluation of Medicinal Products (EAMP) from time to time (Censi et al., 2015; Benet, 2013). A pre planned blood sampling schedule was designed to evaluate the rate and extent of absorption in such a manner so that all the important

Total of 13 blood samples were collected from each volunteers at various interval of time including 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 24.0 and 48.0 h in the sterile EDTA added centrifuge tubes. After the centrifugation the plasma was separated entirely and stored at a temperature of -20°C.

Pharmacokinetic analysis

Noncompermental pharmacokinetic model were used here to calculate various pharmacokinetic parameters of domperidone. The peak plasma drug concentration (C_{max}) and time to reach peak plasma concentration (t_{max}) were calculated directly from the results obtained after the analysis of the drug. The elimination half-life (t_{1/2}) was calculated by using the formula of 0.693/K_{e}, where K_{e} is considered as apparent elimination rate constant calculated from the slope of the terminal log linear phase. Area calculation of trapezoidal rule was implemented to find AUC_{0-t}. AUC_{0-\infty} was also estimated according to the following standard formula:

\[ AUC_{0-\infty} = AUC_{0-t} + C_{last} / K_{e}, \]

where C_{last} is the last quantifiable plasma concentration (US Food and Drug Administration, 2017)

Statistical analysis

Pharmacokinetic parameters of each subject were studied thoroughly on the basis of statistical approach. Bioequivalence study parameters like AUC_{0-1}, AUC_{0-\infty} and C_{max} values were compared as primary variables. Statistical tool of analysis of variance (ANOVA), including treatment, period and subject were applied for these primary parameters and also for log-transformed values of these parameters. The statistical approach of bioequivalence analysis was done according to guidance of Committee for Proprietary Medicinal Products (Censi et al., 2015). The experimental test formulation was considered to be bioequivalent to reference formulation when 90% confidence interval (CI) for the ratio between each pharmacokinetic parameters of test and reference was found to be within the fixed equivalence range of 80-125% (Nation and Sansom, 1994).

RESULTS

The developed bioanalytical method used for the estimation of domperidone in biological matrix was found to be accurate and sensitive. The peaks of domperidone and Internal standard both were found to be well resolved. No interference was observed in the chromatogram of blank plasma sample during. During LC-MS/MS analysis (Figure 1). The retention time (RT) of domperidone and Internal Standard was found to be at 1.67 and 2.03 min respectively. The lower limit of quantification (LLOQ) for domperidone in plasma was noted as 3.33 ng /ml. The peak area ratio (domperidone: Internal standard) between concentration and was found to be linear within the range of 3.33 ng/ml to 500 ng/ml \( (r^2=0.9998) \). Stability, absolute recovery, within-day and between-day, precision and accuracy was estimated for three different quality control points at low, medium, and high levels (5, 50 and 80 ng/ml). Mean drug plasma concentration at various interval of time after oral
Table 1. Mean (±SD, n = 12) pharmacokinetic parameters of 20 mg domperidone tablet for test and reference preparation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>Reference</th>
<th>90% CI (Log-transformed data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-t} (ng. h /ml)</td>
<td>510.55±24.25</td>
<td>488.05±33.52</td>
<td>0.99007 - 1.00296</td>
</tr>
<tr>
<td>AUC_{0-∞} (ng. h /ml)</td>
<td>563.69±31.25</td>
<td>545.98±36.95</td>
<td>0.98942 - 1.00080</td>
</tr>
<tr>
<td>C_{max} (ng/ml)</td>
<td>78.32±53.30</td>
<td>73.17±51.55</td>
<td>0.99527 - 1.00836</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>0.800±0.150</td>
<td>0.798±0.04</td>
<td></td>
</tr>
<tr>
<td>K_{e} (h⁻¹)</td>
<td>0.15±0.008</td>
<td>0.17±0.004</td>
<td></td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>4.62±0.306</td>
<td>4.081±0.295</td>
<td></td>
</tr>
</tbody>
</table>

AUC_{0-t}, AUC_{0-∞}, C_{max}, t_{max}, K_{e} and t_{1/2} are the area under the plasma concentration-time curve upto 48h, area under the plasma concentration-time curve upto infinity, maximum plasma concentration, time to reach maximum plasma concentration, elimination rate constant, and half-life of a drug, respectively.

Figure 2. Mean (± SD, n=12) plasma concentration-time profiles after administration of test and reference preparations in healthy Indian subjects [- - -] is test formulation graph and - - - is reference formulation graph obtained by plotting time (h) on X-axis and plasma concentration (ng/ml) on Y-axis.

administration of reference and test products to healthy volunteers are depicted in Table 1. The comparison of all the major pharmacokinetic parameters for the drugs including ratios of C_{max}, AUC_{0-t}, and AUC_{0-∞} were obtained within the range of 0.80-1.25 at 90% confidence interval.

**DISCUSSION**

The above described bioanalytical method used for estimation of domperidone in plasma matrix was found to be very simple, robust, accurate and sensitive. The entire therapeutic window was covered by the linearity range achieved for this assay (3.33 to 500 ng/ml). The peak of drug domperidone and Internal Standard were well resolved as shown in Figure 2. Throughout the whole experimental study, domperidone was found to be stable in biological matrixes. Final mean recovery of three different quality control sample for three freeze and thaw cycles was found to be 87.60% and coefficient of variation (CV) was noted as 4.26%.

The elimination half-life (t_{1/2}) of domperidone in various formulations was found to be in the range 4.21 to 5.02 h. For this reason, one-week wash out period was sufficient
between the two phases. Peak drug plasma concentration ($t_{\text{max}}$) was observed at 0.8 h after drug administration, and the last samples were sufficient for calculating at least 80% of $\text{AUC}_{0\rightarrow\infty}$. After oral administration of reference drug the peak plasma concentration $C_{\text{max}}$ was found to be $73.17\pm21.55$ ng/ml at the time of $0.798\pm0.04$ h ($t_{\text{max}}$). For the test preparation peak plasma concentration ($C_{\text{max}}$) was found to be $78.32\pm23.30$ ng/ml at the time of $0.800\pm0.150$ ($t_{\text{max}}$). $\text{AUC}_{0\rightarrow1}$ of the test and reference were found to be $510.55\pm24.25$ ng h/ml versus $488.05\pm33.52$ ng h /ml respectively and $\text{AUC}_{0\rightarrow\infty}$ of the test and reference were found to be $563.69\pm31.25$ ng h /ml versus $545.98\pm36.95$ ng h /ml respectively. On the basis of calculation of comparison of the $\text{AUC}_{0\rightarrow1}$ for domperidone after single dose administration, the relative bioavailability of the test preparation was 105% to that of reference preparation.

The objective of the bioequivalence study is to confirm interchangeability between a test (innovator sample) and a generic drug (reference) formulation on the basis of efficacy and safety. When a pharmacological effect of certain drug is difficult to estimate, the plasma levels of a drug may be utilized as an indicator of clinical activity. For this reasons, domperidone plasma concentration obtained in this bioequivalent study suggest an equal clinical efficacy of the two brands tested and provide pharmacokinetic data from Indian healthy volunteers.

**Conclusion**

The 90% CI of $C_{\text{max}}$, $\text{AUC}_{0\rightarrow1}$, and $\text{AUC}_{0\rightarrow\infty}$ of domperidone of these two preparations was found to be in acceptable range as mentioned earlier. There was no statistically significant difference for the treatment values. Both formulations were equal in terms of rate and extent of absorption. Consequently bioequivalence between two formulations can be concluded.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

The authors are thankful to Department of Science and Technology (DST), New Delhi, India for providing us the necessary instrumental facilities for carrying out the study.

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**Synthesis, characterization, and anticancer activity against human breast cancer cell-line T47D studies of metal ion Cu(II) complex with 2,4,5-triphenylimidazole ligand**

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The complex of metal ion Cu(II) with the ligand 2,4,5-trifienilimidazol has been successfully synthesized with mole ratio of metal and ligand 1:2 in N,N-dimethylformamide as a solvent. Complex synthesis results obtained green light crystalline solid. Complex absorbs UV-Vis light at 529 nm. Fourier transform infrared (FTIR) characterization results indicate occurrence of bonding of metals and ligand that is Cu-N in region 422.38 cm⁻¹. Results of elemental analyzer and Atomic Absorption Spectroscopy (AAS) analysis show the complex formed has the formula [Cu(L)₂(H₂O)₂]Cl₂. The molecular formula is also supported by the Thermal Gravimetric Analyzer data. Thermal Gravimetric Analyzer (TGA) analysis results showed that there was no water in the crystalline complex compounds. The cytotoxicity test complex compounds made by the method of 3-(4,5-dimetiltiazol-2-yl)-2,5-difeniltetrazolium bromide (MTT) and the IC₅₀ value of complex obtained 72.139 μg/ml.

**Key words:** Copper(II), 2,4,5-trifenilimidazol, complex compound, characterization, anticancer.
compounds, in the hope of improving pharmacological properties, reducing side-effects, and obtaining different drug-specific targets (Qiao et al., 2011). Some of the transition metals used in the synthesis of anticancer complex compounds include Co(II), Ni(II), Cu(II), Pd(II), Ru(II) and Pt(II) (Budzisz et al., 2009; Ali et al., 2013). Copper(II) metal is an essential element and plays an important role in the biological system of the human body, as a constituent of redox and hemocyanin enzymes (Linder and Maryam, 1996). As previously reported, complexes synthesized from Cu(II) metal ions with 2-(4-thiazolyl)benzimidazole and 2-(2-pyridyl)benzimidazole ligands have a cytotoxicity to liver cancer cells (hepatocellular carcinoma) (Devereux et al., 2007).

Ligands commonly used and continuously developed in studies of anticancer drug compounds have bound atoms of nitrogen and oxygen atoms, including derivatives of imidazole, benzamide, pyridine, and pyrazole (Goncalves et al., 2013; El Boraey, 2012; Tiwari et al., 2011; Budzisz et al., 2009). N-containing aromatic ligands such as pyridine, imidazole, and their derivatives (which are as electron donors similar to purine and pyrimidine bases) have been reported to possess in vitro anticancer properties such as Cisplatin (Deegan et al., 2006). The imidazole-based complex compounds showed anticancer activity against SK-MEL-31 skin cancer cells and tongue cancer cells CAL-27. The imidazole-based compounds also show cytotoxic effects on HepG2 liver cancer cells and A-498 bowel cancer, MCF-7 breast cancer, cervical cancer HeLa, and HL-60 blood cancers (Devereux et al., 2004; Bhat et al., 2011). Therefore, in this study, synthesized complex compounds of Cu (II) metal ion with 2,4,5-trifenylimidazole ligand and tested anticancer activity by MTT method in vitro assay against breast cancer cell T74D.

MATERIALS AND METHODS

The materials used in this study were copper(II) chloride dihydrate (CuCl2·2H2O) (Merck 99.0%), N,N-dimethylformamide (DMF) (Merck 99.8%), dimethyl sulfoxide (DMSO) (Merck 99.8%), 2,4,5-trifenylimidazole (Sigma-Alrich 98%), methanol (Sigma-Alrich 98%), breast cancer cell T74D (CVCL_0553), RPMI 1640 Medium (Gibco), Phosphate-buffered saline 1X (PBS 1X) (Gibco), and Thiazoyl blue tetrazolium bromide (MTT) (Bio Basic).

**Determination of maximum wavelength of [Cu(L)2(H2O)2]Cl2**

The wavelength of the Cu(II) complex compound with 2,4,5-trifenylimidazole was determined by continuous variation method. Continuous variation begins with a 1 mole molecular synthesis of CuCl2·2H2O and 2,4,5-trifenylimidazole, 1 mole with a volume ratio of 0:10, 1:9, 3:7, 5:5, 7:3, 9:1, and 10:1. Each of these solutions is in DMF and heated 3 h at 120°C. The formed solid is decanted and dried. The solid was dissolved in DMSO and measured the maximum wavelength (λmax) with a UV-Vis spectrophotometer, then graphed between absorbance as ordinate and mole fraction of metal as absciss.

**Synthesis and characterization of [Cu(L)2(H2O)2]Cl2**

The synthesis of these complex compounds was performed by the mole of metal and ligand 1:2. Watched the copper(II) chloride dihydrate and 2,4,5-trifenylimidazole in DMF (Han et al., 2012). The complex solution was put into a vial, distilled for 30 min and heated at 120°C for 3 h. The mixture was then cooled to room temperature in vial covered with aluminium foil which has been given several small holes and left for 7 days to form solids and every day was washed with methanol to remove impurities contained in the mixture. The formed solid is decanted and dried. Subsequently, was characterized by UV-Vis Spectrophotometer, FTIR Spectroscopy, Atomic Absorption Spectroscopy (AAS), Thermal Gravimetric Analyzer (TGA), and CHN analyzer.

**Anticancer activity**

The breast cancer cell T74D with a density of 5 x 10⁵ cells/well was distributed into 96 wells plate, incubated for 24 h at a 37°C CO2 to attach. The medium was then replaced with fresh complete medium containing DMSO 0.1% (control), compound complex at concentration of 50, 25, 12.5, 6.25, 3.13, and 1.56 μg/mL, and incubated for 20 h (37°C/CO2). Then each well was added 100 μl RPMI containing MTT reagent and the plates incubated for an additional 4 h. Living cells react with MTT to form formazan crystals (Mosmann, 1983). After 4 h, the medium containing MTT was discarded and then added 50 μl DMSO solutions to dissolve the formazan crystals, homogenized on top of shaker for 10 min, then read with Microplate reader at wavelength 595 nm.

**RESULTS AND DISCUSSION**

**Maximum wavelength of [Cu(L)2(H2O)2]Cl2**

The solids of CuCl2·2H2O and 2,4,5-trifenylimidazole with a 1:2 mole ratio reacted with DMF solution and heated for 3 h at 120°C. The complexes obtained are light green solids (72.127% of yield) and maximum wavelength complex with UV-VIS Spectrophotometer at 529 nm as shown in Figure 1. The result of 10x magnification photograph shows that the obtained solid is in the form of a needle as shown in Figure 2.

**Analysis of functional groups with FTIR Spectroscopy**

Characterization using FTIR spectroscopy was used to determine the presence of functional groups in complex and new bonds formed between metal and ligand. Comparison of FTIR spectrum of ligand and complex is as shown in Figure 3. Based on the obtained data, the appearance of a new peak at a wavelength 422.38 cm⁻¹ can be seen. The peak is observed as a vibration of metals and ligands. This is in line with previous research showing that new peaks of
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Maximum wavelength spectrum of CuCl$_2$.2H$_2$O (blue light), [Cu(L)$_2$(H$_2$O)$_2$].Cl$_2$ (red), and 2,4,5-triphenylimidazole ligand (green light). There was a maximum wavelength shift from the 2,4,5-triphenylimidazole ligand to the [Cu(L)$_2$(H$_2$O)$_2$].Cl$_2$ complex of 243 nm to 529 nm, and the maximum wavelength of CuCl$_2$.2H$_2$O at 338 nm. This shows that the complex [Cu(L)$_2$(H$_2$O)$_2$].Cl$_2$ has been formed.

Figure 2. The compounds of [Cu(L)$_2$(H$_2$O)$_2$].Cl$_2$ (right); 10x magnification (left).

Elementals analysis

Determination of the molecular formula of the complex can be obtained through the theoretical calculation approach of the composition of the formation of the complex. In this case, a complex formed of one metal and two ligands with the formula molecule [(H$_2$O)$_2$Cu-(L)$_2$(Cl)$_2$].Cl$_2$ can be seen. The addition of Cl outside the complex serves to neutralize the complex charge, so the amount of Cl will adjust to its charge. The theoretical
approach is done by calculating the percentages of Cu, C, H, and N on the complex. Theoretically, the complex formed has the formula \([\text{Cu}(L)_2(H_2O)_2]Cl_2\) where two ligands bind one Cu metal according to continuous variation.

The result of the analysis shows that the synthesized complex contains Cu elements of 8.334%, C element of 66.2751%, H element 4.7284% and N element equal to 7.3861%. Based on the data, the percentage level of each element in theory from some complex structures that may be formed was calculated. The result of the calculation of elemental content theoretically approaching experimental measurement result is the prediction result of molecule formula of complex compound. Matches show results appropriate for the complex formula \([\text{Cu}(L)_2(H_2O)_2]Cl_2\). These results indicate that the 2,4,5-triphenylimidazole ligand only binds one Cu. The presence of a steric hindrance causes the 2,4,5-triphenylimidazole ligand to be very difficult to bind metals and can only bind one metal. This shows that H bound to N is not all replaced by Cu.

**Thermal gravimetric analysis (TGA)**

In the determination of complex molecular formulas, thermo gravimeters can provide specific information of a complex that decompose when it is heated. The complex thermo gravimetric analysis of Cu(II)-2,4,5-triphenylimidazole was carried out at 25 to 600°C with a complex sample weight of 6.3670 mg. Based on the complex TGA curve of Figure 4, it can be seen that there is one stage of decomposition in the complex. Weight loss of 86.8791% occurring at 255.33 to 355.83°C indicates a complex decomposition consisting of 2 ligand molecules 2,4,5-triphenylimidazole, 2 molecules H_2O and 1 molecule Cl_2. This result corresponds to the theoretical weight that in weight reduction of 86.8791% is decomposition \(((C_{21}H_{16}N_2)(H_2O)_2Cl_2)\). The residue of 13.1209% (theoretically 8.333%) can be predicted as Cu, as in the previous study that CuO is the final residue of the complex \([\text{Cu}(6\text{-hydroxypicolinate})_2(3\text{-picolinate})_2]\) (Kukovec et al., 2012). Weight loss does not occur at temperatures of 75 to 147°C. This means the complex does not contain crystalline water (Tamaekong et al., 2014).

**Anticancer activity**

In this research, cytotoxicity test was done by MTT method using T74D breast cancer cell. This test is used to determine the cytotoxic effect of tested compound. The classification of toxicity level of the extract based on IC\(_{50}\), which is very high category (highly toxic) if it can kill 50% of cells at concentrations of 1 to 10 μg/ml, medium category (medium toxic) at concentrations of 10 to 100 μg/ml, and low toxic concentration of 100 to 1000 μg/ml.
Based on the calculation, IC50 value for complex [Cu(L2)(H2O)2]Cl2 was obtained at 72.139 μg/ml. Based on the IC50, the [Cu(L2)(H2O)2]Cl2 complex belongs to the category of medium toxicity compound (medium toxic). This is in consistent with the previous research in which the CuL complex (L = 3-(1,3-dioxoisouindolin-2-yl)-2,6-dioxopiperidine-1-carbothioate) kills more cancer cells than its free ligands (Ali et al., 2013). The result of IC50 of [Cu(L2)(H2O)2]Cl2 complex in this study was greater than in previous study, where the MTT assay was performed on the complex [Cu(TBZH)(BZA)].0.5TBZH.H2O wherein the TBZH ligand was 2-(4′-thiazole)benzimidazole and the BZA ligand was benzoic acid, IC50 of 32 μM (Devereux et al., 2007).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Figure 4. Thermal gravimetric analysis curve of [Cu(L2)(H2O)2]Cl2.


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