
Moshood O. Akinleye¹, Ogochukwu U. Amaeze²*, Omotoke T. Opeodu¹ and Omotunde O. Okubanjo³

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Nigeria.
²Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Lagos, Nigeria.
³Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Nigeria.

ABSTRACT

Aim: Herb-drug interaction is a growing concern due to the increasing consumption of herbal medicines among populations globally. Assessment of potential interaction of herbs with concomitantly administered drugs is thus very necessary to ensure patient safety. The aim of this study was to determine the effect of Ciklavit® on the in vitro release profile of proguanil tablets as indicator of potential in vivo herb-drug interaction.

Methodology: Physicochemical characteristics of proguanil tablets namely hardness, friability, weight uniformity, disintegration and chemical assay were evaluated as described in official
compendia. In vitro dissolution of proguanil tablets was studied alone and with Ciklavit® in three different dissolution media with pH 1.2, 4.5 and 6.8 respectively using USP dissolution apparatus II at 75 rpm. Analysis of proguanil was done using High Performance Liquid Chromatography coupled with a UV detector. Dissolution data was analyzed and percentage proguanil released in all dissolution media determined; dissolution profiles were compared using a model dependent approach – dissolution efficiency.

**Results:** Ciklavit® caused 51.6, 61.0 and 57.1% inhibition of proguanil release in vitro at gastro-enteric simulated pH 1.2, 4.5 and 6.8 respectively; this inhibition was statistically significant \((P < .001)\). Release profiles for proguanil alone and proguanil with Ciklavit® showed difference in dissolution efficiency of 56.2%, 64.8% and 59.2% at pH 1.2, 4.5 and 6.8 respectively.

**Conclusion:** Ciklavit® significantly decreased the dissolution of proguanil tablets at all simulated gastro-enteric pH studied. Further studies are needed to assess herb-drug interaction *in vivo.*

**Keywords:** Ciklavit®; Proguanil; dissolution; sickle cell disease; herb-drug interaction.

1. **INTRODUCTION**

Ciklavit® is a liquid herbal formulation made from the extracts of *Cajanus cajan* seeds, used for the management of sickle cell anaemia/ disease in Nigeria; it is the most prominent and widely used of all drugs prepared from medicinal plants for sickle cell anaemia [1]. Ciklavit® has been shown to possess anti-sickling effects, clinically reduce painful crises and may ameliorate the adverse effects of sickle cell anaemia on the liver [2,3,4].

Sickle cell disease is the most common genetic disorder among Africans and one of the top ten (10) Non-Communicable Diseases (NCDs) in Nigeria which contributes significantly to both child and adult morbidity and mortality. It is also occasionally associated with HIV and viral hepatitris (mainly B and C) infections due to frequent blood transfusions [5]. Nigeria has the largest population of People Living with Sickle Cell Disease (PLWSD) in Africa, with about 150,000 births annually; the prevalence of the sickle trait ranges between 20 – 30%, while the homozygous state is found in about 3% of the population [6,7].

Malaria is the most common precipitating cause of frequent vaso-occlusive crises with consequent fatal complications in people living with sickle cell disease in malaria endemic regions like Nigeria; malaria prevention in sickle cell disease has therefore been considered to be essential in malaria-endemic regions [8]. The recent National Guideline for the management of sickle cell disease in Nigeria recommends the use of Proguanil 100 mg daily for children up to 15 years, and 200 mg daily for adults for malaria prophylaxis [5]. Proguanil is a synthetic biguanide derivative of pyrimidine widely used in chemoprophylaxis of malaria [9,10]. Wide use of proguanil prophylaxis in sickle cell disease in Nigeria has been reported in previous studies [11-13].

Herbal supplements are now commonly used in both developed and developing countries as Complementary or Alternative healthcare in the management of many diseases; they are legally categorized as over-the-counter dietary supplements. In Nigeria and most parts of developing countries, medicinal plants have been used in the treatment of painful crises associated with sickle cell disease especially among the lower socio-economic class who cannot afford the high cost of Western medicine. The goal of traditional/ herbal intervention is to manage symptoms and to limit the number of crises [14]. The perception that herbal medicines/supplements may interact with prescription medicines thereby reducing or enhancing their effects is earning global attention [15,16]. Herbal supplements contain numerous active phytochemicals; the possibility of interactions is thus increased when compared with the likelihood of interactions between two prescription medicines. The common misconception that herbal medicines, being natural are safe to be taken concomitantly with prescription drugs also exists; these herals have however been shown not to be entirely safe, and are associated with diverse adverse effects, toxicity and drug interactions [17,18,19,20]. An overwhelming majority of herbal drug use involves self-medication; herbal products and supplements are commonly taken concomitantly with conventional medicines with little or no information available on potential herb-drug interactions [15].

Drug dissolution under physiological conditions is a pre-requisite to drug absorption and clinical
response for almost all oral drugs. Any disruption in the dissolution process could affect the amount of active drug in the systemic circulation and consequently, alter the clinical effects. In vitro drug dissolution measures the extent and rate of drug release from a drug product into solution, and is vital in predicting in vivo performance of a drug product [21,22].

In view of the increasing use of herbal supplements among patients globally, it becomes pertinent to evaluate the effects of such herbal use on concomitantly administered orthodox drugs. This study therefore was aimed at determining the effect of concomitant / co-administration of Ciklavit® and proguanil tablets in vitro using their dissolution profiles.

2. MATERIALS AND METHODS

2.1 Materials

Proguanil standard was obtained as a gift from Lagos State Drug Quality Control Laboratory, Lagos, Nigeria. The internal standard, Pyrimethamine was offered by Swiss Pharma Limited, Dopemu, Lagos. Proguanil tablets (Paludrine®) and Ciklavit® were purchased from a registered community pharmacy outlet in Lagos, Nigeria.

Acetonitrile HPLC grade, methanol HPLC grade, glacial acetic acid HPLC grade, concentrated hydrochloric acid (Sigma-Aldrich®), sodium hydroxide pellets, potassium dihydrogen orthophosphate (JT Baker® USA), ammonium acetate (Lab Tech Chemicals), perchloric acid (SCP, England), sodium acetate trihydrate were all obtained locally from reputable vendors.

2.2 Methods

2.2.1 Physicochemical characterization of proguanil tablets and Ciklavit®

Physicochemical parameters – weight uniformity, hardness, friability and disintegration of proguanil tablets were assessed as specified by the British Pharmacopoeia [23].

The colour of Ciklavit® was determined by visual inspection; taste and pH were also assessed.

2.2.2 Preparation of dissolution media

The dissolution media: acetate buffer (pH 4.5) and phosphate buffer (pH 6.8) were prepared as specified by the BP (2012); pH 1.2 was prepared using 0.1M hydrochloric acid.

To prepare the dissolution media for the dissolution of proguanil tablets in the presence of Ciklavit®, 300 mL of Ciklavit® was measured into a 1000 mL measuring cylinder and made up to volume with the prepared dissolution media respectively.

2.2.3 Preparation of proguanil and pyrimethamine standard stock, working solutions and calibrators

10 mg of proguanil powder was weighed into a 10 mL volumetric flask, dissolved thoroughly with 5 mL distilled water and made up to mL volume with more water to obtain a concentration of 1 mg/ mL proguanil standard stock solution. A working concentration of 100 µg/ mL was achieved by doing a 1 in 10 dilution of the stock solution. Similarly, pyrimethamine 1 mg/ mL (stock solution) and 100 µg/ mL (working concentration) were prepared in methanol. For assay of proguanil tablets, a one-point assay method was adopted by preparing standard concentration of 40 µg/ mL in internal standard using water as diluent.

Gradient calibration concentrations (10 – 60 µg/mL) were prepared with 10 µg/mL internal standard using distilled water (for assay of drug content of proguanil) and in 0.1 N HCl (pH 1.2), acetate buffer (pH 4.5) and phosphate buffer (pH 6.8) respectively to obtain the calibration curve which was used for the quantification of the dissolution samples.

2.2.4 Assay of drug content of proguanil tablets

Twenty tablets from the same batch of Proguanil tablets were randomly selected, weighed, their mean weight determined and pulverized. An accurately weighed amount of the powder equivalent to 10 mg proguanil was transferred into a 100 mL volumetric flask. This was dissolved with about 50 ml of distilled water and sonicated for 5 min. The solution was made up to volume with distilled water and filtered through a 0.45 µm syringe filter. 40 µg/ mL proguanil solution was prepared from the sample solution in 10 µg/mL internal standard and samples were prepared in replicates. 20 µL sample was injected into the HPLC equipment. The peak area obtained from the sample solution was divided by that of the standard and multiplied by 100 to obtain the percentage purity of the tablets.
2.2.5 In vitro dissolution test

In vitro dissolution was carried out using tablet dissolution test USP Apparatus 2 (paddle). Dissolution testing was done in two parts, proguanil tablets alone and proguanil tablets with Ciklavit®. Six dosage units of proguanil tablets were evaluated in 900 mL of each of the dissolution media, temperature was maintained at 37 ± 0.5°C at a fixed speed of 75 rpm. 5 mL of dissolution samples were simultaneously withdrawn at predetermined sampling time of 5, 10, 15, 30, 45, and 60 minutes respectively, and replaced with fresh 5 mL of appropriate medium in order to maintain sink condition. The withdrawn samples were filtered using 0.45 µm Millipore filters and analyzed using an Agilent® HPLC-UV machine. The percentage of proguanil released at the different sampling times was determined.

The above procedure was repeated in the presence of Ciklavit® in the three different media.

2.2.6 Chromatographic conditions

The chromatographic procedure was carried out using an Agilent™ 1260 Infinity series with a reverse phase Zobrax Eclipse XDB C-18 (150 mm X 4.6 mm, 5.0 µm) column, quaternary pump with auto sampler injector set at 20 µL. The mobile phase consists of methanol, acetonitrile (0.5%) and ammonium acetate in the ratio 40:5:55, containing perchloric acid (75 mM/ L). The mobile phase was filtered using filtration unit coupled with suction pump. This is a slight modification of a previously reported method [24].

2.2.7 Statistical analysis

Results were expressed as mean ± SD. Unpaired t-test was used to evaluate the percentage release of proguanil in the presence and absence of Ciklavit® using GraphPad Prism 6.0 software (La Jolla, CA). Dissolution profiles were analyzed using dissolution efficiencies (DE), calculated using the area under the dissolution curve up to a certain time t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time as shown in Equation 1.

\[
DE = \left\{ \int_{t_1}^{t_2} y \cdot dt / y_{100} \cdot (t_2 - t_1) \right\} \times 100
\]

where y is the percentage of drug dissolved at time t.

The integral of the numerator which is the area under the curve was calculated using the trapezoidal method shown Equation 2.

\[
AUC = \sum_{i=1}^{i=n} (t_i - t_{i-1}) (y_{i} + y_{i-1}) / 2
\]

The percentage drug inhibition was calculated using Equation 3.

\[
\% \text{ drug inhibition} = \frac{\% Q_{\text{max}} \text{ (drug alone)} - \% Q_{\text{max}} \text{ (drug/herb)}}{\% Q_{\text{max}} \text{ (drug alone)}} \times 100
\]

where % Q_{\text{max}} (drug alone) is the maximum drug release alone, while % Q_{\text{max}} (drug/herb) is the maximum drug release in the presence of Ciklavit®.

3. RESULTS AND DISCUSSION

From the results obtained, proguanil tablets passed all the physicochemical parameters assessed as specified by official compendia. Ciklavit® decreased the release of proguanil tablets at all gastro-enteric simulated pH studied, with percentage release inhibition of 51.6%, 61.0% and 57.1% at pH 1.2, 4.5 and 6.8 respectively. Statistical evaluation of the percentage release of proguanil alone and with Ciklavit® using student’s t-test also showed a significant difference (P < .001).

Model dependent evaluation of the dissolution profiles of proguanil tablets alone and with Ciklavit® in the three media showed a difference in Dissolution efficiency > 10%.

3.1 Discussion

A significant number of herbal medicines have been shown to be efficacious and culturally accepted by diverse populations; however, they are not totally without unwanted/ adverse effects. One important safety concern of herbal medicines use is risk of interaction with synthetic drugs which often leads to toxicity or loss of efficacy. Recently, there have been numerous reports of herb-drug interaction (HDIs) mediated by different mechanisms, making it a subject
area of particular interest. This study therefore sought to predict the possibility of interaction between the herbal formulation Ciklavit® and proguanil often used concomitantly, employing in vitro dissolution tests.

Dissolution of a drug in solid dosage form into an aqueous medium is absolutely crucial to its absorption with impact on its bioavailability and hence, therapeutic efficacy [25]. Due to the critical nature of release of drug from the dosage form and dissolution under physiological conditions, in vitro dissolution are often used to predict in vivo drug performance [26]. Physicochemical parameters such as friability and hardness assure drug product quality; and were thus assessed prior to carrying out dissolution studies. All the parameters evaluated as shown in Table 1 were within compendia specifications for immediate release solid oral dosage forms. Table 2 shows the physical properties of the poly-herbal formulation Ciklavit®. The pH was found to be is 2.93; this acidity invariably could be attributed to its constituents, chiefly extracts of Cajanus cajan, proteins (essential amino acids), vitamins such as ascorbic acid and minerals such as zinc.

Statistical analysis of dissolution data showing percentage of proguanil released and dissolution efficiency (DE) as represented in Table 3 showed that the dissolution of proguanil tablets were in consonance with BP specifications at all three simulated gastro-enteric pH (1.2; 4.5 and 6.8), as percentage proguanil release was above 90% at 30 minutes. Assessment of the effect of Ciklavit® on the in vitro release of proguanil at the simulated gastro-enteric pH revealed a statistically significant ($P < .001$) inhibition of proguanil release. This was evidenced by the decreased percentage release of proguanil compared to percentage release of proguanil alone. Since a drug has to go into solution before it can be absorbed, this decreased release of proguanil implies reduced drug moiety available for absorption to elicit therapeutic response. Further evaluation of the dissolution profiles of proguanil alone and with Ciklavit® (Figs. 1 – 3) using dissolution efficiency (DE) – a model dependent approach – showed difference in DE of 56.2%, 64.8% and 59.2% in pH 1.2, 4.5 and 6.8 respectively. This again shows a disparity in the release characteristics of proguanil alone and with Ciklavit®, as a difference in DE of less than 10% is assumed to indicate bioequivalence [27,28], where two profiles of different brands are evaluated. In this case however, the higher differences indicate significant decrease in the dissolution of proguanil when administered with Ciklavit®.

Decrease in the dissolution of proguanil observed in this study could invariably hamper bioavailability, with resultant sub-therapeutic concentrations of the drug in systemic circulation. In real in vivo studies, however, other factors such as presence of villi and lipid permeability play a vital role in absorption. The low dissolution observed in the presence of Ciklavit® could eventually lead to therapeutic failure of proguanil for chemoprophylaxis of malaria in Sickle cell disease. With compromised proguanil prophylaxis, the risk of vaso-occlusive crises precipitated by malaria would be increased in SCD patients, with consequent lethal implications.

Drug interactions involving changes in absorption, distribution, metabolism or excretion of a drug by a concomitantly administered drug or herb are classified as pharmacokinetic interactions. Altered pharmacokinetics often inevitably leads to significant changes in response to drugs. For instance, long-term treatment with St John’s wort reduced the plasma levels of co-administered cyclosporin, amitriptyline, digoxin, indinavir, nevirapine, oral contraceptives, warfarin, theophylline or simvastatin [29]. The Chinese herbal product Xaia chai hu tang, when taken with prednisolone, caused a decrease in the plasma concentration of prednisolone. Decreased concentrations of phenytoin when combined with Ayurvedic syrup shankhpushpi have also been observed [30]. Gingko biloba decreased the plasma concentration of omeprazole, ritonavir and tolbutamide [31]. Yoyo cleanser bitters – a Nigerian liquid herbal medication has also been shown to have variable effects on the dissolution of lisinopril at different simulated gastro-enteric pH studied [25].

The decreased dissolution of proguanil in the presence of Ciklavit® observed in this study alludes to the potential of drug interaction when these two medications are used concomitantly. Caution should thus be applied in using these two drugs together, healthcare practitioners should be well informed about potential herb-drug interactions and patients advised accordingly.
Table 1. Physicochemical characteristics of proguanil tablets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation/Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniformity of weight (% deviation), n=20</td>
<td>1.70 ± 0.30</td>
</tr>
<tr>
<td>Hardness (kg/F), n=10</td>
<td>4.07 ± 0.21</td>
</tr>
<tr>
<td>Friability (%), n=10</td>
<td>0.44 ± 0.12</td>
</tr>
<tr>
<td>Disintegration time (mins), n=6</td>
<td>1.54 ± 0.36</td>
</tr>
<tr>
<td>Assay (%), n=20</td>
<td>98.9 ± 1.50</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. Limits for hardness, friability, disintegration time and assay are 4-7kgF, <1%, <15% and 95-105% (BP).

Table 2. Physical properties of Ciklavit®

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Brownish black</td>
</tr>
<tr>
<td>Taste</td>
<td>Bitter</td>
</tr>
<tr>
<td>pH</td>
<td>2.93</td>
</tr>
</tbody>
</table>

Table 3. Statistical analysis of dissolution data showing dissolution efficiency (DE)

<table>
<thead>
<tr>
<th>Media</th>
<th>Proguanil alone</th>
<th>Proguanil + Ciklavit®</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 1.2</td>
<td>90.9</td>
<td>39.8</td>
</tr>
<tr>
<td>pH 4.5</td>
<td>87.2</td>
<td>30.7</td>
</tr>
<tr>
<td>pH 6.8</td>
<td>86.6</td>
<td>35.3</td>
</tr>
</tbody>
</table>

Fig. 1. Dissolution profile of proguanil tablets alone and with Ciklavit® at pH 1.2

Fig. 2. Dissolution profile of proguanil tablets alone and with Ciklavit® at pH 4.5
4. CONCLUSION

Ciklavit® significantly decreased the dissolution of proguanil at all simulated gastro-enteric pH studied; further studies are needed to assess herb-drug interaction in vivo. Due to the clinical significance of drug interactions with herbs, it is important to identify drugs and compounds in use that may interact with herbal medicines. Timely identification of such drugs using proper in vitro and in vivo approaches may have important implications for patient safety and drug development.

CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

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