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Original Research Article

Effect of Ciklavit® - (A Poly-Herbal Formulation) on the Pharmacokinetics of Proguanil in Healthy Nigerian Volunteers

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ABSTRACT

The therapeutic efficacy of herbal medicines in the treatment of human diseases or disorders has gained more attention over the years. A survey established that approximately 15 % of patients receiving orthodox medicine also take herbal products, and among these, potential adverse herb-to-drug interactions were observed in 40 % of patients. Recently, there have been reports of herb-drug interactions which have impacted on wellness of users resulting in toxicity or loss of therapeutic efficacy of either the herb or the orthodox drug when co-administered. These reports make herb-drug interactions a research area of public interest. This study was embarked upon to evaluate the effect of Ciklavit® used for the management of sickle cell anaemia on the pharmacokinetic profile of proguanil tablets in healthy Nigerian volunteers. Twelve healthy participants were recruited and completed the study after signing a consent form. The study was performed using a single dose, two-way randomized crossover design. The participants were randomly grouped into control and treatment. The treatment group were administered proguanil tablets with Ciklavit® while the control group took proguanil with water only. The blood and urine samples collected at different time interval for 24 hours were analyzed using High Performance Liquid Chromatography. The results obtained, demonstrated that co-administration of Ciklavit® with proguanil altered pharmacokinetic parameters of proguanil but the difference in values were found to be statistically insignificant ($p>0.05$). The study demonstrated that there are no significant pharmacokinetic interactions between proguanil and Ciklavit® hence the two medicinal agents can be co-administered.

Keywords: Herb–Drug interactions, Pharmacokinetics, Ciklavit®, Proguanil, Sickle cell, Biological fluids

Introduction

The use of herbal formulated medicinal products either in form of nutritional supplements or therapeutic agents in both developing and developed countries is on the increase now and may escalate in the future. These naturally occurring agents co-exist with conventional medicine hence the tendency and possibility of concurrent use is high. Beneficial effects and adverse herb-drug interactions have been reported as consequences of herbs being taken together with the orthodox medicine.[1,2] The safety of this practice has been a major public concern more so as it is desirable that drugs are rationally prescribed and dispensed. However, the world continues to be confronted by longstanding, emerging and remerging infectious diseases threat. These threats differ widely in terms of severity and probability.[3]

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Malaria, according to World Health Organization (WHO), is considered and declared as a life- threatening disease caused by *Plasmodium* parasites that are transmitted to people through bite of infected female Anopheles mosquito called malaria vectors. It is common in countries with tropical climates. There are five major parasite species that causes malaria in humans and two of these species – *P. falciparum* and *P. vivax* pose the greatest threat. In Africa, malaria contributes extensively to the early mortality in patients with Sickle cell disease (SCD).[4] Proguanil is a synthetic biguanide derivative of pyrimidine widely used as chemoprophylactic medicinal agent for malaria in people living with sickle cell disease (PLWSCD).[5] The drug could be used alone or in combination with chloroquine or atovaquone. In-fact, a fixed-dose combination tablet of two antimalarial agents such as proguanil-atovaquone is highly effective for the prevention of *Plasmodium falciparum* malaria. In combination with proguanil, the ability of atovaquone to inhibit parasitic mitochondrial electron transport is markedly enhanced and its tolerability has been proven.[6,7] Malaria and Sickle cell have been a common associated disease. Sickle cell anaemia is a major component within haemolytic anaemias; occurs more often among people from parts of the world where malaria is common. According to Centers for Disease Control and Prevention (CDC),[8] Sickle Cell Disease affects millions of people throughout the world. About 1 of every 365 black or African-American births; 1 in 13 Black or African-American babies is born with Sickle Cell Trait. It is crucial that in countries with high prevalence of malaria patients with Sickle cell anaemia (SCA), particularly children, be protected from malaria by appropriate prophylaxis.[9] High level of childhood mortality has been associated with the co-morbidity.[10] The prevalence of this scourge led

to the search of herbal medicine as alternative and complementary treatment. Ciklavit herbal formulation is a product of well researched herbal medicine development that has been reported and analyzed to have anti-sickling properties and to enhance the well-being of sicklers.[11] The claims have been attributed to *Cajanus cajan*, reported to preserve and make red blood cells steady leading to significant reduction in the usual hyper bilirubinaemia seen in sickle cell disease. Ciklavit® is also indicated in the reduction of pain threshold common with PLWSCD. The use of proguanil and Ciklavit medicinal agents is thus prevalent in medical practice in Nigeria and Africa at large. It is a common parlance in medical research that when two or more medicinal agents are going to be used concomitantly, there is an urgent need to investigate the potential for any interaction most especially herb-drug interactions.

Thus, the objective of this study was to investigate the potential pharmacokinetic interaction of co-administration of single oral dose of Proguanil on multiple doses of Ciklavit for the purpose of assessing the safety of the combination.

Materials and Methods

Chemicals (All reagents and chemicals were of analytical grade)

Reagent used were Diethyl ether (J.T Baker, USA), Acetonitrile, HPLC grade (Emsure ®ACS, Merck, Germany), Methanol HPLC grade (LichroSolv®, Merck, Germany), Ammonium acetate (J.T Baker, USA), Perchloric acid (Riedel–de Haen® Honeywell, UK), Sodium Hydroxide (Loba Chemie pvt. Ltd®, India), Ethyl rubbing Spirit (Nino pharm®, Nigeria), Micro filter syringe, 0.45µm.

Experimental drugs

Proguanil Tablets (Paludrine®, Manufacturing Date: 02-2021, Expiry date: 01-2023 NAFDAC Number: 04-0364, Lot Number: 1490527, Manufacturer: Alliance); Ciklavit® (Manufacturing Date: 08-2020, Expiry Date: 02-2023, NAFDAC number, Lot No: 00836018A, Manufacturer: Neimeth Plc), Proguanil and pyrimethamine standards were donations from Drug Quality Control Laboratory of Lagos State Government.

Participants and ethical clearance

Twelve healthy Nigerian volunteers between the ages of 23 and 45 years and weighing between 57 kg and 95 kg were recruited for this research after they have shown interest and understanding of the research process by signing a consent form and having met both inclusion and exclusion criteria. None had history of liver disease or cardiac disease and all normal physical examinations were carried out. The participants were instructed to abstain from other medications, alcohol, bitter kola and other herb products one-week prior to and during the period of the study. Ethical clearance for the study was obtained from the Health Research Ethnics Committee (HREC) of College of Medicine, University of Lagos, Nigeria (CMUL/HREC/O3/21/836).

Drug administration

The study was performed using a single dose, two-way randomized crossover design. The participants were divided into two groups, A (control) and B (treatment), six in each group. After 12 hours overnight fasting time has been observed, group A were orally administered 200 mg proguanil tablet with 200 mL of table water on an empty stomach and food intake was not allowed for a period of 4 hours following drug administration. Group B were placed on 30 mL single daily dose of Ciklavit® for 4 days prior to proguanil drug administration. On the 5th day after overnight fast, they were administered 200 mg proguanil tablets with 30 mL Ciklavit. A 2-week washout period was observed for proper elimination of the drug components from the body compartment(s) of each participant and the drug administration procedure was interchanged between the two groups. All participants were encouraged to take 400 mL of water every hour to induce diuresis.

Sample collection

A blank sample of blood and urine was collected from each participant before drug administration. After drug administration, blood samples of 5 mL were collected from the participant's forearm veins using a needle and syringe at various time intervals; 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h into heparinized bottles and mixed and centrifuged at 4000 rpm for 4 minutes. The plasma layer was aspirated into a sterile plastic sample bottle. The plasma samples were then stored in the freezer at -20 ^{o}C until analysis. Total urine voided was collected from each participant at 0-2, 2-4, 4-6, 6-8, 8-10 and 12-24 hours, total volume measured, pH taken and 5 mL aliquot was kept for high performance liquid chromatographic (HPLC) analysis.

Drug analysis

The plasma and urine samples were analyzed for proguanil using a validated HPLC method described by Ebeshi *et al*.[12]. Briefly, the method involved sample treatment by the basification of the plasma with NaOH followed by extraction with diethyl ether and pooled extract was evaporated to dryness. The residue was reconstituted in 100 µL of methanol; vortex-mixed and 20 µL was injected onto the HPLC system. Chromatographic analysis was done using Agilent Technologies® HPLC 1200 series, Binary pump, micro-vacuum Degasser, Standard and Preparative Autosampler, Thermostated Column Compartment, Diode Array and multiple Detector with ChemStation software. The Column used was Agilent® Eclipse XDB-C18, 4.6 x 150 mm, 5 µm diameter particle size. A mobile phase consisting of methanol: acetonitrile: 0.5 % ammonium acetate (40:5:55) containing 75 mM/L perchloric acid was pumped through the column at a flow rate of 1.0 mL/min. The pH of the mobile phase was 2.2, and the chromatogram was run at ambient temperature. The plasma and urine samples were analyzed using the HPLC chromatographic conditions.

Data and statistical analysis

Plasma data analysis

Non compartmental analysis using Phoenix WinNonlin® PK/PD modeling Tool version 2.1 pharmacokinetic software was used to characterize the concentration-time profile of Proguanil for each participant. Observed values of maximum plasma concentration (Cmax) and time of maximum concentration (Tmax) were taken from the actual data points. The Area under the plasma concentration-time curve (AUC_{0-24}) and the area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was determined using the linear trapezoidal method. The elimination rate constant (Kel) was calculated from the least-squares regression slope of the terminal plasma concentration. The elimination half-life ($t_{1/2}$) was determined from the expression 0.693 over elimination rate constant. The Volume of distribution (Vd), Clearance (Cl), and mean residence time (MRT) were also obtained from the software modelling.

Urine data analysis

The data obtained from the HPLC system were subjected to Microsoft excel application for pharmacokinetic parameters calculation which include Ke (Excretion constant), Kel (Elimination constant), Xu (Cumulative amount excreted), and $T_{1/2}$ (half-life). The graphs showing amount excreted and cumulative amount excreted were plotted against time.

Statistical analysis

The pharmacokinetic parameters such as K_{el}, K_e, Xu, $t_{1/2}$, T_{max}, C_{max}, Vd, Cl and MRT obtained from plasma and urine were expressed as mean and standard deviation. The student t-test were conducted to determine the level of variation between the control and treated groups. The data were considered statistically significant if p-value was less than or equal to 0.05.

Results and Discussion

Plasma analysis

Twelve volunteers (male) gave written informed consent to participate in the study. They all met all the inclusion criteria and completed the study. Mean ± SD of height, weight and age were 1.76 ± 0.1 meter, 69.1 ± 14.2 kg and 35.5 ± 7.3 years respectively. The study drugs were well tolerated, and no one reported any adverse effects. Figure 1 and 2 represent the HPLC chromatograms obtained from plasma analysis of blank and Ciklavit in combination with proguanil samples while figure 3 and 4 depict the chromatogram obtained from urine sample of a participant at 8-10 h who was placed on proguanil alone and proguanil

with Ciklavit respectively. The chromatograms indicate no interference from any other components in the Ciklavit and biological matrix.

The mean and standard deviation of the plasma concentration versus time for participants who ingested proguanil alone, and with Ciklavit® are presented in Figure 5. Of a great note was a sharp rise in concentrations of proguanil at half hour in participants that ingested proguanil with water which pervaded all participants.

Summary of the derived mean pharmacokinetics parameters of proguanil in the plasma following administration of 200 mg single oral doses of PG with water and PG with Ciklavit is presented in Table 1. Despite large variations in the pharmacokinetic parameters when compared statistically using student's t test, the differences were found to be statistical insignificant ($p > 0.05$).

The mean excretion rate of proguanil versus time of participants that administered proguanil with water and Ciklavit are presented in figure 6. The maximum urinary excretion rate for proguanil alone and with Ciklavit were 1.73 and 1.37 mgh^{-1} respectively. The maximum urinary excretion rate of proguanil with Ciklavit was reduced by 21 % when compared proguanil alone. This indicates that the rate is faster in water then when taken with Ciklavit®.

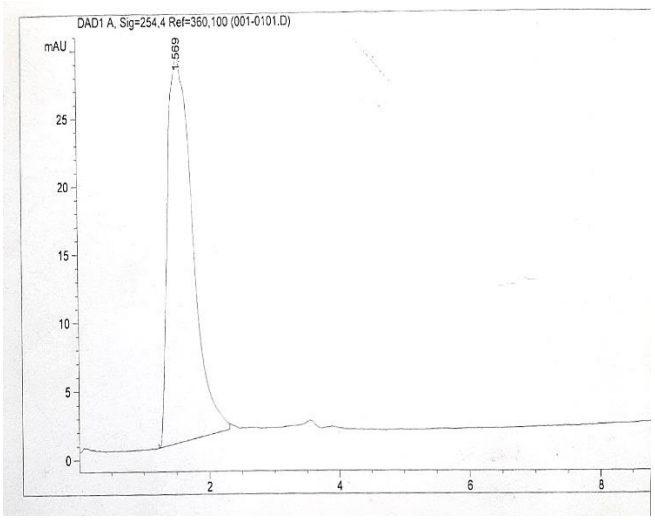

Figure 1: Chromatogram of blank urine sample from one of the participants

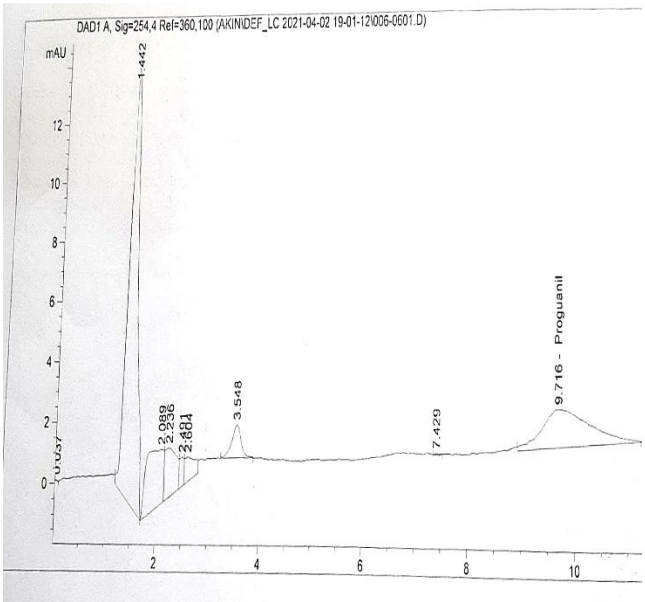

Figure 2: Chromatogram of proguanil extracted from plasma sample obtained 3 h following a 200 mg single oral dose of Proguanil taken with Ciklavit

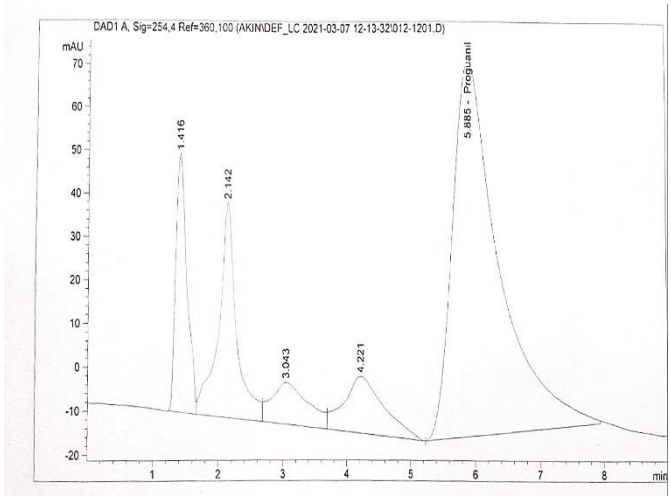

Figure 3: Chromatogram of proguanil extracted from urine sample obtained 8-10 h following a 200 mg single oral dose of Proguanil alone

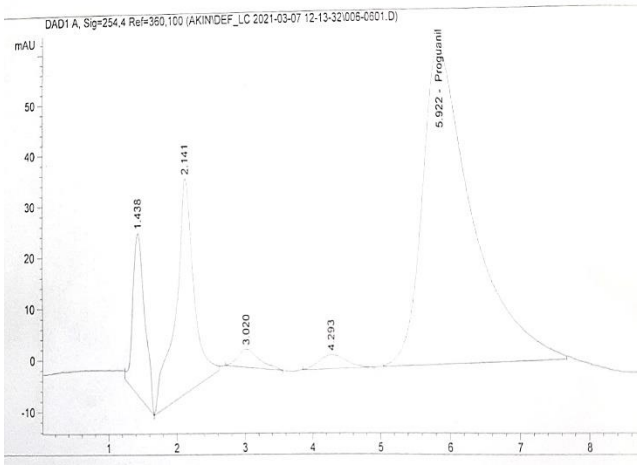

Figure 4: Chromatogram of proguanil extracted from urine sample obtained 8-10 h following a 200 mg single oral dose of Proguanil taken with Ciklavit

Urine analysis

The mean cumulative amount of proguanil taken with water and concomitant administration with Ciklavit® versus time are presented Figure 7. The study shows that the maximum cumulative amount of proguanil excreted was reduced by 14.7 % when concomitantly administered with Ciklavit®.

Impact of Ciklavit® on plasma pharmacokinetic data of proguanil

The pharmacokinetic parameters obtained as a result of concomitant use of Ciklavit® with Proguanil indicate altered values as seen in the time taken to attain peak plasma concentration (Tmax) which was found to be lowered for proguanil alone and higher for proguanil with Ciklavit. The result indicates a 68.4 % increase in the Tmax of proguanil due to Ciklavit interference although the difference was not statistically significant ($p = 0.43$). The Cmax of proguanil was decreased by 74% in the presence of Ciklavit® which is in consonant with the earlier interaction report of Akinleye *et al.*,[5] who reported a reduction in amount of proguanil dissolved *in-vitro*. The biologic impact is a reduced bioavailability and possible delayed onset of pharmacological action. Surprisingly however, the decrease was statistically insignificant ($p = 0.20$). Similar result was obtained with the total systemic exposure as measured by Area under the plasma concentration-time curve to last measurement and to infinity (AUC). The AUCs were insignificantly reduced in the presence of concomitant Ciklavit® and proguanil oral administration. The half-life of proguanil was reduced by 46.2 % as

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impacted by Ciklavit®. The volume of distribution, Clearance and mean residence time were also altered insignificantly in the presence of Ciklavit® herbal mixture.

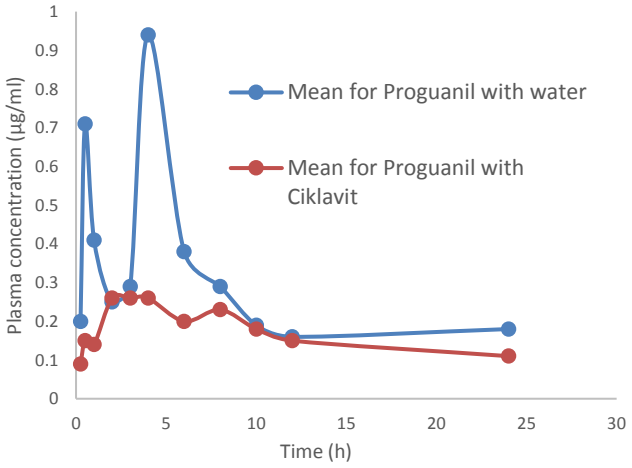

Figure 5: Mean plasma concentration versus time profiles of 200 mg single dose of proguanil and following concurrent administrations with Ciklavit®.

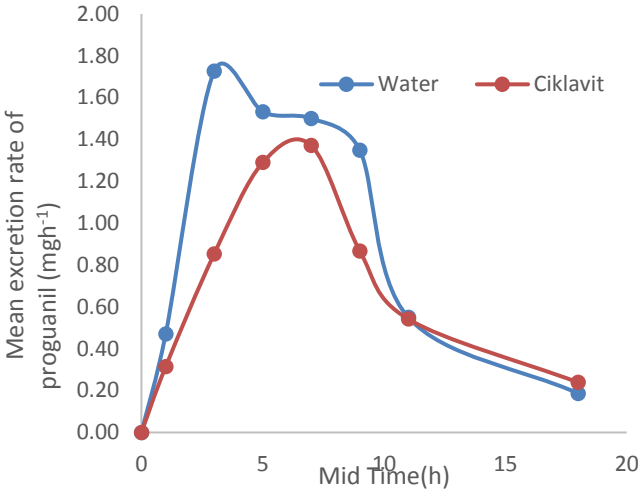

Figure 6: A graph of mean excretion rate of PG versus time of participants that ingested PG with water and Ciklavit®.

Table 1: Summary of the mean pharmacokinetics parameters of proguanil in the plasma following administration of 200 mg single oral doses of PG with water and PG with Ciklavit®

Pharmacokinetic parameters	Proguanil with water Mean ± SD	Proguanil with Ciklavit® Mean ± SD	p-value
T_{max} (h)	3.92 ± 2.40	6.60 ± 5.00	0.43
C_{max} (ng/L)	2093.42 ± 1520.40	534.99 ± 310.50	0.20
AUC_{last} (ng mL^{-1} h)	5658.56 ± 2579.40	4870.69 ± 1995.00	0.59
K_{el} (h^{-1})	0.05 ± 0.00	0.04 ± 0.00	0.57
$t_{1/2}$ (h)	16.97 ± 8.30	9.13 ± 6.30	0.64
$AUC_{0-\infty}$ (ng mL^{-1} h)	9370.57 ± 3731.50	7262.04 ± 2227.40	0.94
Vd (ml)	494.76 ± 249.80	569.63 ± 376.50	0.94
Cl (ml/min)	24.02 ± 8.50	26.08 ± 11.20	0.74
MRT (h)	8.80 ± 1.50	10.74 ± 1.80	0.08

Values are expressed as mean ± Standard deviation (SD), Cmax= maximum drug concentration, Tmax = time to maximum concentration, $t_{1/2}$ = elimination half-life, Kel = elimination rate constant, AUC_{last} Area under the plasma concentration-time curve from time zero to time of last measurable concentration, $AUC_{0-\infty}$ = area under plasma concentration-time curve from zero to infinity, Vd = apparent volume of distribution, Cl = Clearance, MRT = mean residence time

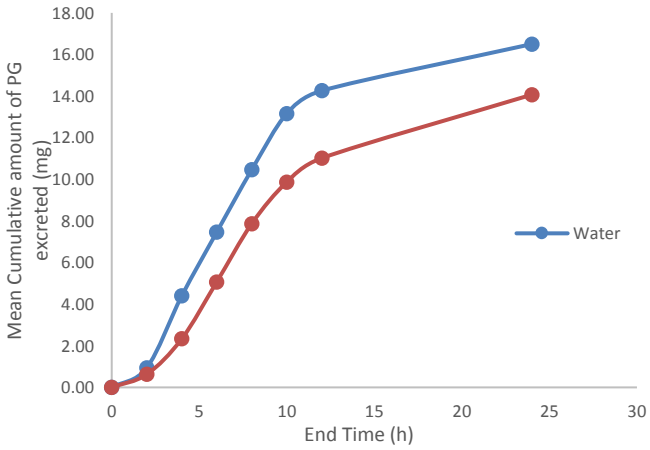

Figure 7: A graph of mean cumulative amount of proguanil excreted versus time, of participants that administered proguanil with water and Ciklavit®.

Impact of Ciklavit® on proguanil pharmacokinetic urinary data

The maximum urinary excretion rates are important indicators of the rate of drug absorption and excretion. The maximum urinary excretion rate of PG was reduced by 21 % due to the effect of Ciklavit on proguanil urinary excretion. This is an indication of reduction in the clearance of proguanil which could also influence the therapeutic effectiveness including likely induction of toxicity.

The cumulative amount of drug excreted in the urine is related directly to the total amount of drug absorbed which is equivalent to the area under the curve of PG in plasma. There was a drop in the cumulative amount of proguanil excreted as impacted by Ciklavit® co-administration. It is the amount of proguanil absorbed that will definitely go through the process of excretion. The reduction in AUC as reflected in drug plasma analysis ultimately will scale down amount of proguanil to be excreted. So, the result of plasma is in congruence with that of urine analysis. By influence, less of proguanil is absorbed into the circulatory system in the presence of Ciklavit® than when administered alone.

Impact of Flavonoids on P-glycoprotein transporting enzymes (P-gp)

P-glycoprotein (P-gp) is an efflux transporter that influences the pharmacokinetics (PK) of various compounds.[13] Flavonoids (poly-

phenolic compounds) found in fruits, plant-derived beverages even in *Cajanus cajan*[14] have been proven to modulate the activity of cytochrome P450 and P-glycoprotein which ultimately influence the bioavailability of orally administered drugs.[15,16]

Proguanil is a substrate for P-glycoprotein (P-gp);[17] the 14.7 % reduction in the cumulative amount of drug excreted in the urine when proguanil is administered with Ciklavit and the 68.4% increase in Tmax by Ciklavit may be attributable to the modulation of intestinal P-glycoprotein by Ciklavit. The phytochemicals in Ciklavit influenced the activity of this drug transporter hence the elongation of time taken for maximum concentration of proguanil to be achieved. In the work of Soyinka *et al*.,[18] co-administration of proguanil and efavirenz was found to have resulted to significant increase in C(max), T(max), AUC(T) and elimination half-life (T(1/2beta)) of proguanil compared with values for proguanil alone. Study on *Cajanus cajan* seed shows that Ciklavit® contains phenylalanine, carjaminose, and hydroxybenzoic acid as active constituents and are thought to be the reason for its anti-sickling effect.[19] Study also found that flavonoids such as morin and silymarin inhibit P-gp ATPase activity by inhibiting azidopine binding to P-gp. The findings of this study indicate a potential for significant flavonoid-drug interactions with P-gp substrates.[20] In a similar study conducted by Martins *et al*.,[21] when Yoyo bitters was co-administered with ciprofloxacin, the pharmacokinetics parameters of ciprofloxacin were altered but without any significant adverse clinical effect.

Summary of the Pharmacokinetic data obtained from urine for proguanil in the presence of water and Ciklavit from the study are presented in Table 2. The result show altered pharmacokinetic parameters such as elimination rate constant, excretion rate constant and cumulative amount excreted in the presence of Ciklavit but the differences were statistically insignificant as the p-value were above 0.05.

Table 2: Summary of the Pharmacokinetic data obtained from urine for PG in the presence of water and Ciklavit® from the study

Parameters	PG with water	PG with Ciklavit	P –Value
Ke (h^{-1})	0.0109 ± 0.0042	0.0090 ± 0.0020	0.40
Kel (h^{-1})	0.264 ± 0.122	0.208 ± 0.033	0.34
$T_{1/2}$ (h)	3.4 ± 2.3	3.4 ± 0.57	0.99
Ẍu (mg)	16.5 ± 4.11	14.07 ± 4.36	0.37

Values are expressed as mean ± standard deviation; K_e = Excretion rate constant; K_{el} = Elimination rate constant; $t_{1/2}$ = Half-life; Xu = Cumulative amount excreted, PG= Proguanil

Conclusion

The study has demonstrated that the co-administration of Ciklavit with proguanil altered pharmacokinetic parameters but the values were found to be statistically insignificant ($p > 0.05$). Therefore, Ciklavit and proguanil can be given at the same time to sickle cell patients but there is the need to confirm the originality of the herbal mixture before use because of the scourge of fake and substandard products and also for pharmacovigilance concerns.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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