International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

Pharmacokinetics study of ethyl acetate extract of *cylicodiscus gabunensis* (mimosaceae) after oral administration and its effect on the eradication of *shigella dysenteriae* infection on rats

Laure Brigitte Mabeku Kouitcheu [1*], Joseph Lebel Tamesse [2] and Oyono Essame Jean Louis [3]

[1,*]Microbiology and Pharmacology Laboratory, Department of Biochemistry, Faculty of Science, University of Dschang-Cameroon, P.O. Box 67, Dschang, Cameroon.
[2]Department of Biological Sciences Higher Teacher's Training College, University of Yaoundé I, P.O. Box: 47, Yaoundé, Cameroon.
[3]Institute of Medical Research and Medicinal Plants Studies (IMPM), P.O. Box 8013 Yaoundé, Cameroon

***Corresponding author e-mail:** lauremabek@yahoo.fr

ABSTRACT

Cylicodiscus gabunensis (CG) is a tropical plant traditionally known for its medicinal use. The plant has also been investigated for a number of pharmacological activities. However kinetic studies are lacking for this. Hence to better elucidate the effects of the body on this plant preparation, this study was planned. Sixty Wistar albino rats were giving orally a single dose of 500 mg/kg of plant extract. Blood samples were then collected in EDTA coated tubes prior to plant extract administration and at 10, 20, 40, 80, 160, 320, 640, 960 and 1280 minutes after. Plasma obtained was analyzed to measure concentration of plant extract using its anti-shigellosis properties. Various kinetic parameters were then calculated from the plasma concentrations. For the in *vivo* anti-shigellosis activity of the plant extract, a suspension of *Shigella dysenteriae* type I (*sd1*) was orally administered to thirty rats. The diarrheic rats were then divided into the control group and four others received 125, 250, 500 mg/kg of the plant extract and ciprofloxacin (20 mg/kg) respectively for 7 days. The frequency and weight of normal and diarrheic faeces emitted was recorded. The presence of stools containing mucus or blood and the number of *sd1* in faeces were also recorded. The peak plasma levels (81.937 mg/ml) of *CG* were reached at 10.66 h. The concentration declined with a mean elimination half life of 6.61 ± 0.97 h. The $AUC_{0-\infty}$ was 1367.7 mg h/ml. *CG* reduces the frequency faeces released and *sd1* density from 100% (diarrheic rats) to 50.79 and 45.33% (500 mg/kg) respectively. We concluded that CG extract is effectively absorbed though the intestinal wall. The elimination half life suggests that the drug needs to be given orally at the interval of seven hours. These parameters provide a baseline for the further exploration of what the body does to the drug and justify the pharmacodynamic correlation.

Keywords: *Cylicodiscus gabunensis*; Pharmacokinetic parameters; Gastrointestinal infection

INTRODUCTION

For long now, Scientists have been studying the pharmacodynamics of natural products, the action of the drug on the body but less attention has been paid to study the effect of body on herbs. This has been demonstrated by a study indicating only few pharmacokinetic reports on herbal preparations [1]. Pharmacokinetic studies are of prime importance to clinical trials of herbal products in order to qualify them as evidence-based-drugs. Pharmacokinetic parameters are essential to understand the time course of drug after its administration and will aid in determining the optimum dosage schedule for better therapeutic use. Unlike pharmaceuticals, the pharmacokinetics of herbal products, mixture of known and unknown components is always challenging due to their complexity and

unavailability or inadequacy of standards and methods. Moreover, lack of pharmacokinetic studies is a biggest hindrance in the modernization of herbal products as there is no way to establish any bioequivalence between products prepared by modified method and an original method [2]. Different types of marker compounds, characteristics to a particular plant can be utilized to carry it pharmacokinetic study using high performance liquid chromatography (HPLC). Using marker compounds, the pharmacokinetic profiles of few herbal products such as *Ginkgo biloba*, *Allium sativum*, *Ephedra sinica* have been delineated [3, 4, 5]. In order to determine the plasma concentration of chloroquine and study the pharmacokinetic of this antimalarial drug, Kotecka and Reickmann [6] assessed the *in vitro* antimicrobial activity of the plasma obtained from individuals treated with choroquine using the antimicrobial dilution technique and after compared with High Performance Liquid Chromatography values, they recommended the bioassay technique as a useful tool for the determination of drug concentration equivalence in the blood. In this study, the anti-microbial activity of the plasma from animals exposed to a single dose of plant preparation was used to evaluate the quantity of antimicrobial principle in the blood. Two approaches, non-compartment and compartment model are commonly used to evaluate the pharmacokinetic profile of a compound. Compartment model such as one compartment, two- and three-compartment model are associated with more assumptions as compared with non-compartment model. Therefore, we have used non-compartment model in this study to evaluate pharmacokinetics profile of CG extract using trapezoidal rule [7]. *Cylicodiscus gabunensis (CG)* is a tropical plant used traditionally in West and Central Africa regions to cure various ailments such as headache, filariasis, rheumatism and dysentery [8]. The plant has also been investigated for a number of pharmacological activities such as anti diarrhoeal [9], antibacterial [10] and toxicological studies [11]. Given the large diversity of the biological activities of this plant and the paucity of data on its pharmacokinetic profile in the literature, the pharmacokinetic study of CG extract was carried out in rats to delineate their absorption and distribution. After the assessment of the absorption of the active principles of this plant extract, its effect on *Shigella dysenteriae* type I induced diarrhoea in rats was carry out.

MATERIALS AND METHODS

Plant Material: The stem bark of CG was collected in the morning on Mount Eloumdem, Yaoundé-Cameroon in January 2013. The identification of the plant was done at the National Herbarium Yaoundé (voucher specimen: N° 21574/SRF/CAM). The plant material was then air dried at room temperature and ground into a fine powder.

Preparation of extract: This was carried out by soaking the dried powdered plant (1000 g) in bottle with 14 l of ethyl acetate (EA) and kept for 72 hours. The plant-(EA) mixture was then filtered. The filtrate (extract) was concentrated by evaporating ethyl acetate under reduced pressure using a rotary evaporator. The extract was further concentrated by allowing it to stand overnight in an oven at 30°C. The yield of the extraction was calculated.

Animals: Healthy *Wistar* albino rats weighing 65 - 95 g and 180 – 200 g taken from the animal house of the Institute of Medical Research and Medicinal Plant Studies Yaoundé (IMPM) were housed in standard cage for 7 days to acclimatise them. Standard pellet diet was given and tap water was supplied *ad libitum*. Animals were used for pharmacokinetic studies and for the anti-shigellosis drug assessment of the plant preparation. Animal housing and the bioassay was conducted in accordance with the internationally accepted principles for laboratory animal use and care of the European Community guidelines (EEC Directive of 1986; 86/609/EEC)

Microorganisms: The strain used in this study was *shigella dysenteriae* type 1(*sd1*) isolated from patient suffering from bloody diarrhoea. This strain was supplied by the Centre Pasteur of Yaoundé Cameroon.

Pharmacokinetic studies

In vitro anti-shigellosis activity of the plant extract

Well-diffusion assay: the well diffusion test was performed based on the method described by [12]. 100 µl of the suspension of *sd1*, containing 10^8 CFU/ml of cells prepared with 18 h old culture were plated on Mueller Hinton agar. Wells (6 mm diameter) were punched in the agar and filled with 100 µl of plant extract (320 mg/ml) prepared using vehicle (0.5% v/v Tweens 80 in distilled water). 100 µl of ciprofloxacin (10 mg/ml) were also dropped into the wells to serve as positive control, while 0.5% v/v Tweens 80 in distilled water was used as negative control. The plates were incubated at 37°C for 18-24 h.

After this preliminary screening showing the anti-shigellosis activity of CG extract, 8 serially two-fold decreasing concentrations (1.25, 2.5, 5, 10, 20, 40, 80, 160 and 320 mg/ml) were tested against *sd1* using well diffusion assay as described above . The tests were run in triplicate for each concentration and the

results were expressed as a mean diameter of inhibition zone (DI) for each concentration. Then, the plot of the mean diameter of inhibition as a function of the concentration of the extract was used to establish the linear regression curve.

***Broth microdilution assay*:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were assessed using the broth microdilution method [13]. The test was performed in peptone water supplemented with glucose 1% (w/v) with red phenol as a colour indicator (PPG1%). A suspension of *sd1* was adjusted with a turbidity equivalent to that of a 0.5 McFarland standard and further diluted to obtain final inoculums of 5×10^5 CFU/ml. For the susceptibility test, the 96-well round bottom sterile plates were prepared by dispensing 180 µl of the inoculated broth into each well. A 20 µl aliquot of the plant extract was added. The concentration of extract adopted to evaluate the antimicrobial activity was included from 12.8 to 0.00039 mg/ml. One well was considered as growth control since no extract solution was added. The bacterial growth was indicated by the colour change of the well content from red to yellow. The MIC was defined as the lowest concentration of the extract to inhibit the growth of microorganism. MBC were determined by platting 5 µl sample from red wells on Mueller Hinton agar without extract. The MBC was the concentration at which there was not microbial growth. The extract testing in this study was screening three times against *sd1*.

Administration of plant preparation and collection of plasma: In the previous study, we evaluated the toxicity profile of the ethyl acetate (EA) extract of the bark of *Cylicodiscus gabunensis* (CG) in rats [11]. The rats were administered graded doses (0.75, 1.5, 3 and 6 g/kg p.o.) of the extract daily for 6 weeks. The results suggested that the margin of safety of the extract is high at dosages used clinically since no substantial toxic effect occurred in animals at 0.75 g/kg. Therefore, the dosage of 0.5 g/kg was administrated orally to rats to study the pharmacokinetic profile of CG extract.

For the determination of plasma concentration equivalent, 60 Wistar albino rats weighing 180 – 200 g were used. They were divided into ten groups of six animals each, one group per one time point. After overnight fasting the rats, they received orally a single dose of the plant extract (500 mg/kg). Rats from each groups were ether-anaesthetised and blood samples were collected by cardiac puncture in EDTA coated tubes before (0 h: pre dose group); and at 10, 20, 40, 80, 160, 320, 640, 960 and 1280 minutes after dosing. The tubes containing blood were centrifuged at 3000 rpm for 15 min to get plasma which was stored frozen.

In vitro assessment of the anti-shigellosis activity of the plasma: The plasma concentration of CG extract was determined by study the anti-shigellosis activity of plasma obtain from rats treated with CG. The plasma was aseptically diluted two-fold (1:1 to 1:64) using sterile normal saline. The anti-shigellosis activity of each dilution was assessed by well diffusion method as outlined in the early experimental section. Each diffusion test was performed in triplicate and the mean diameter of inhibition (DI) was calculated. The highest dilution that yielded a visible zone of inhibition was considered as the maximum inhibitory dilution (MID). The active principle concentration was then estimated from the linear regression equation as the concentration corresponding to the DI obtained with the MID.

Pharmacokinetic analysis [14, 15, 16]: The plasma concentration of CG extract at different time point were expressed as mean + SD, and the mean concentration-time curve was plotted. Non compartmental pharmacokinetic analysis of concentration time data was performed. Maximum observed plasma concentration (C max) and the time to reach C max (T max) were determined by visual inspection of concentration versus time curves.

Area under the concentration versus time curve through C_{last}, the last measurable concentration (AUC_{last}) was calculated by linear trapezoidal method. Area under the concentration versus time curve, extrapolated to infinity ($AUC_{0-\infty}$) was calculated according to the following equation: $AUC_{0-\infty} = AUC_{last} + C_{last} / Kel$.

The apparent elimination rate constant K_{el} (h^{-1}) was calculated using fitting mean data at three terminal point of the plasma concentration profile with a log-linear regression equation using the least-squares method. The half–life $T_{1/2}$ was calculated as $0.693/K_{el}$.

In vivo anti-shigellosis activity of the plant extract

The action of drug depends on the rate and extends at which drug reaches at the site (s) of action. The pharmacokinetic study of *CG* extract was a prerequisite to understand whether it may be beneficial for its safe and effective use. After making sure that *CG* extract cross the physiological barrier we therefore study its effect on the shigellosis induce in rats.

Induction of shigellosis on rats: Thirty Wistar albino rats weighing 65 - 95 g were kept singly in metabolic cages for 7 days of observation before diarrheal induction. Diarrhoea was induced in rats using *sd1* strains. The turbidity of the *sd1* inoculum was matched spectrophotometrically at 450 nm to 4 McFarland standards. After verifying that the rats were not *sd1* carriers, they were orally administrated with 1 ml of the saline diluted inoculum.

Anti-shigellosis drug assessment: Diarrheic rats were randomly divided into 5 groups of six animals. The first group (diarrheic control) received the vehicle of the extract (0.5% Tween 80 in distilled water); the second group received the antibiotic ciprofloxacin (20 mg/kg of body weight) and the remaining three groups were given orally 125, 250, and 500 mg/kg of EA extract of *CG*. When diarrhoea appeared, animal were administrated the anti-diarrheic drugs daily by the oral route for 7 consecutive days.

During all the experiment period, animals were observed daily for behavioural changes and mortality patterns once before, during and up to 7 days after induction. The stools of each animal were also collected daily using a white cloth fixed under the bars supporting the animals in the metabolic cage. The daily frequency and weight of normal and diarrheic faeces were determined at each experimental period. The presence of stools containing mucus or blood was also noted daily at each experimental period. Enumeration of *sd1* in faeces was performed before diarrheal induction to make sure that the rats are not *sd1* carriers, after the appearance of diarrhoea and once per 2 days during the 7 days of treatment. For this purpose, 0.5 mg of faeces was homogenized in 4.5 ml sterile saline; serial of dilution were made and 50 µl of each dilution was seeded over salmonella-shigella agar. After 24 hours of incubation at 37 °C, the number of CFU was determined.

Statistical analysis: The results are expressed as means ± standard deviation. Bacterial densities were expressed in log10 before analysis. Data were statistically evaluated using the analysis of variance following by the paired t-student's test. The differences between groups were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The result of the in *vitro* anti-shigellosis activity of the plant extract using Well-diffusion assay is shown in Table 1. As the known standard drug which showed an inhibition diameter of lysis of 35±1 mm at 10 mg/ml, the extract of *CG* was active against *sd1* in a concentration dependent manner. The inhibition diameter began at 10 mg/ml and increased progressively up to 27.33 mm at 320 mg/ml of extract. These values were used to plot zone of lysis versus extract concentration curve. The linear regression equation of the plot was $Y= 0.064 X + 7.142$ ($R^2= 0.9836$) and CMI and CMB values were 100 and 800 µg/ µl respectively.

The different values of lysis zone obtain at each time point was used to calculate the plasma concentration of extract using the linear regression equation of the plot determined above ($Y= 0.064 X + 7.142$). The plasma concentration versus time profile of *CG* is presented in Figure 1. Plasma concentration versus time was analysed and the pharmacokinetics parameters were shown in Table 2. Following 500 mg/kg oral administration of *CG*, the compound showed a peak concentration (Cmax) of 81.937 mg/ml after 10.66 h of dose (absorption phase). The compound appeared in the blood as early as 40 min post oral treatment with C max occurring at 10.66 h post dose (Table 2). The results also indicated that *CG* extract exhibited a $T_{1/2}$ of 6.61 ± 0.97 h with a K_{el} of 0.106 ± 0.015 h^{-1}. The (AUC_{0-t}), ($AUC_{0-\infty}$) values were respectively 1119.672 mg.h/ml and 1367.7 mg.h/ml.

The pharmacokinetic study was planned in rats with single oral dose of 500 mg/kg *CG*. In the best of our knowledge, this is the first report on pharmacokinetic studies of *CG*. Since there is no published study on pharmacokinetics of *CG*, comparison is not possible.
A delay between dose administration and the detection of drug in the circulation is seen frequently after oral dosing, and is usually caused by delayed gastric emptying. In addition, drug may be metabolized by enzymes and microbial flora and the fraction of drug which is absorbed may be metabolized by enzymes in intestinal wall and in liver. If there is extensive metabolism at one or more of these sites, only a fraction of the administered oral dose may reach the general circulation [17].

According to the data finding, only a limited amount of the parent compound reached the general circulation. The maximal concentration (Cmax) of *CG* extract in rat's plasma was 81.937 mg/ml which represented 16.38 % of the administered dose. This showed that *CG* is poorly absorbed by the intestine. This may be due to the fact that it might have been metabolized by the gut wall and liver on one hand and on the other, may be due to its highest binding activity in organs and low blood distribution [17]. The

low bioavailability of extract can be justifying by the structural complexity of their component. Taking into account the fact that ethyl acetate is the solvent of extraction of plant material use; *CG* extract is a mixture of both polar and non-polar plant's compounds. The non-polar components of *CG* extract dissolve and diffuse freely across lipid membranes contrary to the polar one which stagnates in the lumen gut. The determination of the partition coefficient of the extract may help us to make a precise explanation. However, the particle size of the compounds present in the extract may also justify this low rate absorption.

Elimination half-life of nearly seven hours is indicative that *CG* needs to be given orally at the frequency of seven hourly intervals. Kinetically *CG* will attain the steady state plasma concentration after approximately 35 hours [14].

The area under the curve of plasma concentration of the extract showed that drug exposure is long, which implies the need of both the control of dose quantity and dosing interval.

The *in vivo* anti-shigellosis study has showed changes in animals behavioural 3 hours after the induction. This changes included prostration, slow response to external stimuli and rapid breathing. The first diarrheic stool was emitted within 24 hours after the induction. Diarrheic stools were soft or liquid, containing mucus and smooth or not. During the 7 days of treatment, 83.33 and 33.33 % of deaths were registered respectively in diarrheic control animals and those receiving 125 mg/kg of extract (Table 3).
CG extract markedly reduced the frequency and weight of faeces emitted (Table 4 and 5). The weight of the defecation significantly reduced from 3.15 (control group) to 1.69 g (125 mg/kg) – 1.49 g (500 mg/kg). The weight of defecation at the highest dose of *CG* was the same as the one obtain with the standard drug ciprofloxacin.

Compared with control group, treatment with each dose of the plant extract also reduced significantly the percentage of diarrheic stools from the 3rd to the end of the treatment (Fig. 2). Like diarrheic stool, mucus contains stool also reduced with the treatment. At the end of the treatment the record of mucus containing stool was null at 250 and 500 mg/kg doses (Fig. 3).

In the stools of diarrheic control rats, *sd1* density increased significantly from the first to the last day of the treatment compared with the value administered. Compared with the diarrheic control group, the antibiotic ciprofloxacin significantly reduced the density of *sd1* in the stool. Similar to ciprofloxacin, *CG* inhibit the bacterial growth in a dose dependent manner. *CG* extract at the dose 125 mg/kg inhibited bacterial growth by the 3rd day of treatment. This reduction was significant at the dose of 250 and 500 mg/kg of the extract. The percentage of reduction of *sd1* density was respectively 48.28 (250 mg/kg) and 45.33 % (500 mg/kg) compared to the control group (Fig 4).

In *vivo* anti-shigellosis study, diarrhoea went along with increase in faeces frequency and weight ($p < 0.05$), increase in the percentage of diarrheic and mucus contains stool ($p < 0.05$), increase in bacterial population in stool ($p < 0.05$). Shiga toxin is responsible for most of the pathology of Shigellosis. Shiga toxin binds to receptors on intestinal epithelial cells and blocks absorption of electrolytes and nutrients from the intestinal lumen. This enterotoxicity leads to diarrhea, dysentery, dehydration, loss of electrolytes, and impaired nutrient absorption [18]. Exposures of wistar rats to p.o. doses of *CG* reduced the density of *sd1* in the stool and the typical shigellosis symptoms, along with frequent soft or liquid stools, frequent mucous and smooth stools.

Earlier studies showed that anti-dysenteric and anti-diarrhoeal properties of medicinal plants were due to tannins [19, 20]. Denatured proteins form protein tannates make the intestinal mucosa more resistant and hence, reduce secretion [21]. This could also be the mechanism of action of *CG* extract since phytochemical screening reveal that tannins is it major substance.

CONCLUSION

CG extract is effectively absorbed though the intestinal wall. The elimination half life suggests that the drug needs to be given orally at the interval of seven hours. These parameters provide a baseline for the further exploration of what the body does to the drug and justify the pharmacodynamic correlation. The kinetic data after oral administration of *CG* needs to be compared with intravenous *CG* along with determination of urinary concentration of its metabolites in order to assess the pharmacokinetic parameters including oral bioavailability and renal clearance.

Table 1: Anti-shigellosis activity of ethyl acetate extract of CG stem bark and ciprofloxacin using *Well diffusion assay***.**

Drugs concentration tested (mg/ml)	Inhibition diameter (mm)	
	Extract	Ciprofloxacin
0.078	NT	8 ± 0.00
0.156	NT	10 ± 1.00
0.312	NT	15 ±1.73
0.625	NT	20 ± 0.00
1.25	6 ± 0.0	26 ± 1.00
2.5	6±0.0	29 ± 1.73
5	6±0.8	34 ± 0.0
10	7±0.5	35 ± 1.00
20	9±0.8	NT
40	11±0.9	NT
80	13 ±0.1	NT
160	17.33±0.1	NT
320	27.33±0.6	NT

Each value represents the mean of three assay ± standard deviation

Table 2: pharmacokinetic parameters of CG extract after oral administration of 500 mg/kg to rats.

Cmax (mg/ml)	Tmax (h)	Kel (1/h)	T1/2 (h)	AUC_{0-t} (mg.h/ml)	$AUC_{0-\infty}$ (mg.h/ml)
81.937	10.66	0.106	6.53	1119.672	1367.700

Table 3: Effect of *Cylicodiscus gabunensis* and ciprofloxacin treatment on death rate of *Shigella dysenteriae type 1* diarrheic rats.

Day after induction	Number of death				
	Diarrheic control	Ciprofloxacin (20 mg/kg)	C.G (125 mg/kg)	C.G (250 mg/kg)	C.G (500 mg/kg)
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	1	0	0	0	0
5	2	0	2	0	0
6	2	0	0	0	0
7	0	0	0	0	0

Table 4: Total stool frequency before and during treatment of *Shigella dysenteriae* type I diarrheic rats with ethyl acetate extract of *Cylicodiscus gabunensis* and ciprofloxacin**.**

Days of treatment	j-1	j0	j1	j2	j3	j4	j5	j6	j7
vehicle	18.0±1.72	22.5±0.70	27.0±1.41	31.5±2.77	32.0±0.70	31.5±0.70	34.5±2.53	33.0±1.94	35.5±1.62
CG (125mg/kg)	18.83±1.72	22.0±1.33	26.0±0.00	25.5±0.69*	25.0±0.48*	27.0±0.59*	24.5±2.94*	26.0±0.70*	26.5±0.52*
CG (250mg/kg)	18.16±0.98	21.5±2.12	24.0±1.17	26.0±0.00*	24.0±2.82*	21.0±1.41*	18.0±1.41**	17.0±0.82**	16.0±0.82**
CG (500mg/kg)	17.4±1.67	23.0±1.41	24.0±0.41	24.5±0.82*	22.5±0.70*	23.0±1.41*	18.33±2.0**	15.66±1.73**	16.0±0.48**
Ciprof(20mg/kg)	18.4±2.16	21.5±0.57	24.0±0.98	20.0±0.70**	18.0±0.20**	16.0±1.41**	15.0±0.0**	16.33±2.12**	15.0±1.41**

CG: *Cylicodiscus gabunensis* , Ciprof : ciprofloxacin . Each data column represents the mean ± S.E.M. (n = 6). Data column of the same day with superscript * are significantly different compared with diarrheic control (* $p < 0.05$; ** $p < 0.01$).

Table 5: Total stool weight before and during treatment of *Shigella dysenteriae* type I diarrheic rats with ethyl acetate extract of *Cylicodiscus gabunensis* and ciprofloxacin.

Days of treatment	j-1	j0	j1	j2	j3	j4	j5	j6	j7
vehicle	1.73±0.44	2.22±0.43	2.77±0.67	2.79±0.70	2.97±0.59	3.05±0.15	3.13±0.07	3.42±0.04	3.15±0.06
CG (125mg/kg)	1.67±0.61	2.40±0.60	2.73±0.19	2.76±0.28	2.76±0.35	2.81±0.33	2.57±0.59*	2.085±0.07*	1.69±0.14**
CG (250mg/kg)	1.53±0.34	2.215±0.33	2.46±0.29	2.11±0.05	2.44±0.66	2.17±0.07*	1.765±0.14**	1.60±0.04**	1.66±0.07**
CG (500mg/kg)	1.17±0.28	2.24±0.60	2.34±0.60	2.1±0.61	2.15±0.02	2.08±0.01*	1.60±0.48**	1.58±0.34**	1.49±0.01**
Ciprof (20 mg/kg)	1.8±0.25	2.17±0.61	2.01±0.53	2.00±0.19*	1.99±0.43**	1.64±0.04**	1.46±0.04**	1.63±0.53**	1.49±0.14**

CG: *Cylicodiscus gabunensis* , Ciprof : ciprofloxacin

Each data column represents the mean ± S.E.M. (n = 6). Data column of the same day with superscript * are significantly different compared with diarrheic control (* p< 0.05; **p < 0.01).

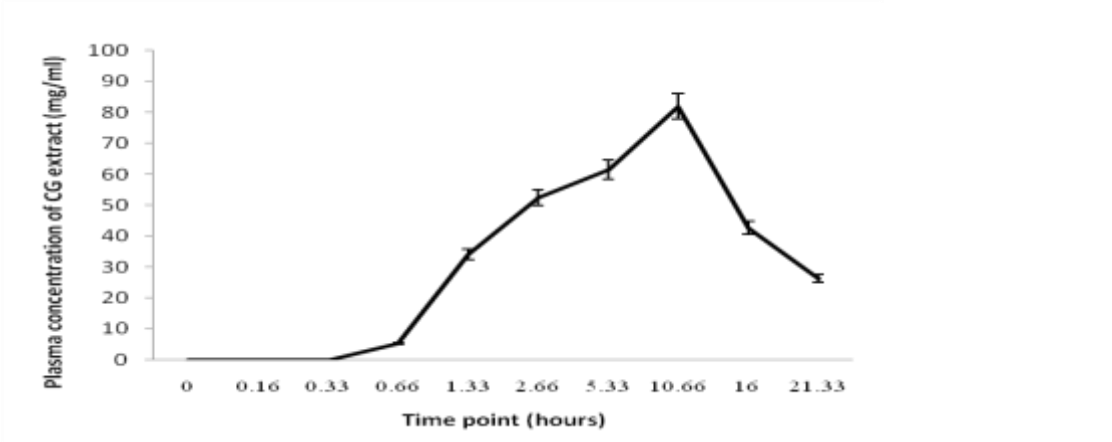

Figure 1: Plasma concentration equivalent of CG extract as a function of time, after a single oral dose of 500 to rats (each point is means of six rats ± SD).

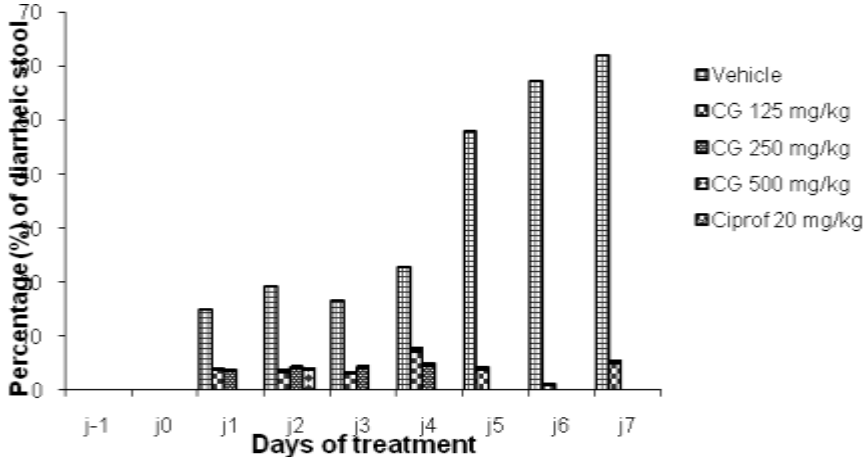

Figure 2: Effect of ethyl acetate extract of *Cylicodiscus gabunensis* (CG) and ciprofloxacin (Ciprof) administration on the percentage (%) of diarrheic stool of *shigella dysenteriae* type 1 diarrheic rats.

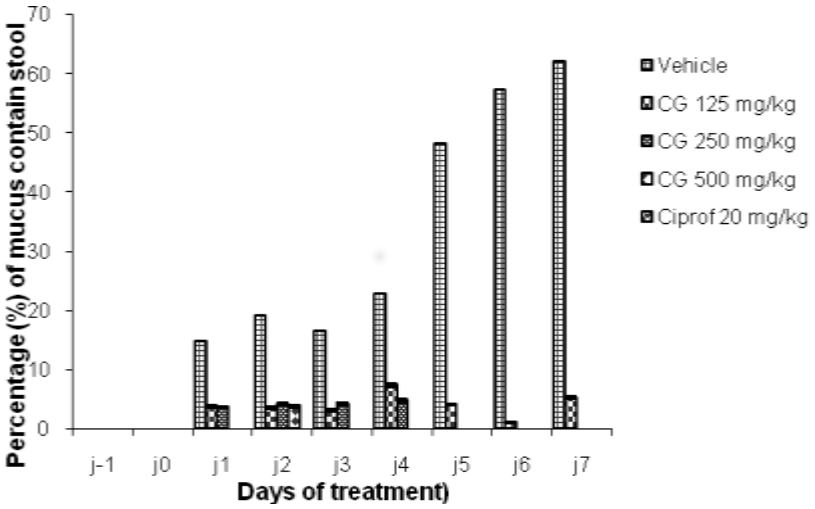

Figure 3: Effect of ethyl acetate extract of *Cylicodiscus gabunensis* (CG) and ciprofloxacin (Ciprof) administration on the percentage (%) of mucus contains stool of *shigella dysenteriae* type 1 diarrheic rats.

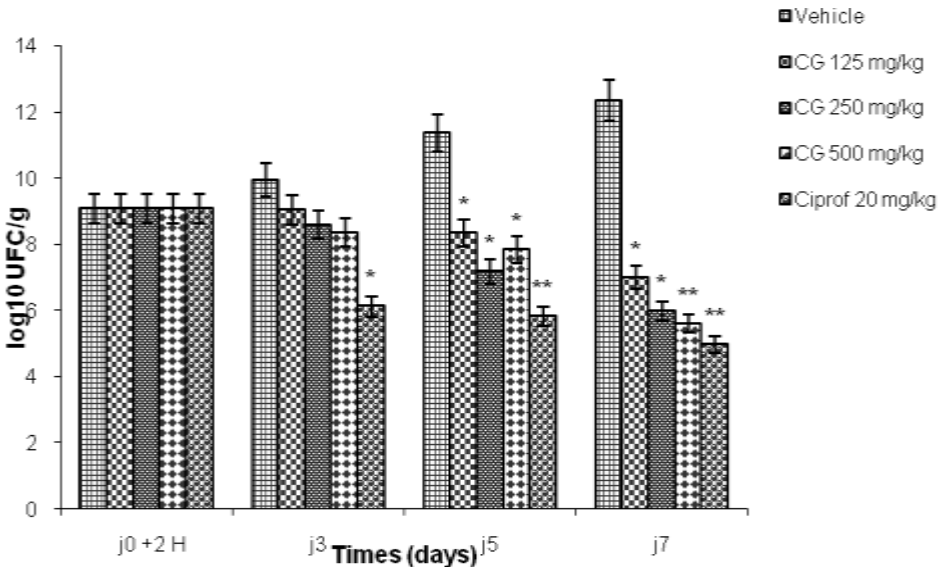

Figure 4: *Shigella dysenteriae* type I density in stool (log_{10}UFC/g) over 7 days of treatment with ethyl acetate extract of *Cylicodiscus gabunensis* (CG) and ciprofloxacin (Ciprof). Each data column represents the mean ± S.E.M. (n = 6). Data column of the same day with superscript * are significantly different compared with diarrheic control (* $p < 0.05$; ** $p < 0.01$).

REFERENCES

1. De Smet PA, Brauwers JR. Clinical pharmacokinetics, 1997; 32: 427-436.
2. Handa SS. Medicinal plants-properties in Indian medicines diverse studies and implications. In: Handa SS and Kakul K (eds.). Supplement to cultivation and utilization of medicinal plants, Regional Research Laboratories, Jammu Tawi, Indian: 1996, pp. 33-51.
3. Gurley BJ, Gardner SE, White IM, Wang PL. Therapeutic drug monitoring, 1998 ; 20 (4): 439-445.
4. Muari P, Simonetti P, Gardana C. Rapid communication in Mass Spectrometry, 2001 ; 15: 929-934.
5. Kodera Y, Suzuki A, Imada O. Journal of agricultural and food chemistry, 2002; 50 (3): 622-632.
6. Kotecka MB and Rieckmann KH. Am J Trop Med Hyg, 1993; 49(4): 460-464.
7. Daltonand IT and Meyer MC. Bioavailability of drugs and bioequivalence. In: Swarbrick J and Boylon JC (eds.). Encyclopedia of pharmaceutical Technology, Informa Healthcare. 2nd ed., New York; USA, 2002, vol 1, pp. 124-135.
8. Tane P, Bergquest KE, Tene M, Ngadjui BT, Ayafor JF, Sterner O. Tetrahedron, 1995; 51(16): 11595-11600.
9. Kouitcheu Mabeku LB, Penlap Beng V, Kouam J, Ngadjui BT, Fomum ZT, Etoa FX. African Journal of Biotechnology, 2006 ; 5: 1062-1066.
10. Kouitcheu Mabeku LB, Kouam J, Penlap Beng V, Ngadjui BT, Forum ZT, Etoa FX. Afr J Trad CAM, 2007; 4 (1): 87-93.
11. Kouitcheu Mabeku LB, Penlap Beng V, Kouam J, Essame Oyono, Etoa FX. Journal of Ethnopharmacology, 2007; 11: 598-606.
12. Saenz Y, Zarazaga M, Lantero M, Gastanares JM, Baquero F, Torres C. Antimicrob agents chemother, 2000 ; 44: 267-271.
13. NCCLS. Methods for determining bactericidal activity of antimicrobial agents. Approved guideline M26. National Committee for Clinical Laboratory Standards, Wayne, Pa: 1999.
14. Ritschel WA. In: The handbook of basic pharmacokinetics. 2nd ed., Drug Intelligence Publications, Inc: 1980.
15. Rowland M and Tozer TN. Clinical Pharmacokinetics – Concepts and Applications. 3rd ed., Williams and Wilkins: 1995.
16. Gibalidi M and Perrier D. In: Pharmacokinetics, Marcel Dekker, Inc. New York and Basel: 1975.
17. Rang HP, Dale MM, Ritter JM and Moore PK. Pharmacology Churchill. 5th ed., Livingstone; Edinburgh U K: 2003.
18. Niyogi S. The journal of microbiology, 2005; 43: 133–43.
19. Galvez J, Zarzuelo A, Crespo ME, Lorente MD, Ocete MA, Jimenez J. Planta Medica, 1993 ; 59: 333- 336.
20. Longanga OA, Vercruysse A, Foriers A. J Ethnopharmacol, 2000 ; 71: 411- 423.
21. Tripathi KD. Essentials of Medical Pharmacology. Jaypee Brothers Medicals Publishers (P), New Delhi: 1994, pp. 775.