Journal of Complementary and Alternative Medical Research

3(4): 1-8, 2017; Article no.JOCAMR.35960
ISSN: 2456-6276

Effects of *Moringa oleifera* Leaf Powder Suspension on the Pharmacokinetics of Amodiaquine in Rats

Olanrewaju Samuel Olawoye[1], Babatunde Ayodeji Adeagbo[1] and Oluseye Oladotun Bolaji[1*]

[1]Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

This study was conducted in collaboration between all authors. Author OOB conceived and designed the study. Author OSO performed the sample preparation and collection, quantitative analysis of amodiaquine and its metabolites in consultation with authors OOB and BAA. Author BAA drafted the first manuscript, authors OOB and BAA were responsible for the review and editing of the draft manuscript and author OOB revised and approved the final manuscript. All authors contributed to the interpretation of data, revision of the manuscript and gave final approval for the manuscript to be published.

Article Information

DOI:10.9734/JOCAMR/2017/35960
Editor(s):
(1) Bamgboye M. Afolabi, Health, Environment and Development Foundation, Nigeria.
Reviewers:
(1) Voravuth Somsak, Walailak University, Thailand.
(2) Rafael Fernandez Da Silva, FACYT-University of Carabobo, Venezuela.
Complete Peer review History: http://www.sciencedomain.org/review-history/20963

Original Research Article

Received 4th August 2017
Accepted 9th September 2017
Published 13th September 2017

ABSTRACT

Aims: *Moringa oleifera* Lam (Moringaceae) is commonly used as food plant, food supplement and as medicine in most African countries. With increasing acceptance and use of *M. oleifera* as food and medicine, the possibility of co-administration of MO with conventional drugs, especially antimalarials, also increases. Hence, this study investigated the effects of *M. oleifera* leaves (MO) on the pharmacokinetics of amodiaquine (AQ) in male albino Wistar rats.
Methods: 180 male Wistar Albino rats, weighing 180-220 g, randomly divided into 3 groups of 60 rats each (6 rats per each time point) were used for this study. In the control group (CT), a single dose of amodiaquine (10 mg/kg) was administered orally to rats while in the co-administration group (CA), the same dose of AQ was given concurrently with MO. In the third group, the pre-treatment group (PT), each rat received MO for one week and on the 8th day received the MO dose along with AQ (10 mg/kg). Blood samples were collected and the plasma concentrations of AQ and its metabolite, desethylamodiaquine (DEAQ), were determined using a validated HPLC method.

**Corresponding author: E-mail: obolaji@oauife.edu.ng;*

Results: Compared to the CT group, the maximum plasma concentration (C_{max}) of AQ in the CA and PT groups decreased by 28% and 53.42% respectively while for DEAQ, a 59.2% reduction in C_{max} was observed in CA but there was a 36.0% increase in the PT group. The plasma exposure (AUC_{total}) of AQ significantly increased (P <0.001) by about 318.2% and 144.6% in CT and PT respectively, while for DEAQ there was a 28.8% reduction in AUC_{total} in co-administration, but a significant increase (P<0.001) of 242.4% was observed after pre-treatment.
Conclusion: The study established pharmacokinetic interaction between AQ and MO in rats with the effects being more on the absorption of AQ during co-administration buton both absorption and elimination of AQ after pre-treatment.

Keywords: Pharmacokinetics; Moringa oleifera; amodiaquine; desethylamodiaquine.

1. INTRODUCTION

Moringa oleifera Lam. is a fast growing plant that is widely cultivated in tropical regions of the world [1]. All parts of the plants have been identified to have nutritional and medicinal values [2-4] and the leaves have been characterized to contain a desirable nutritional balance, containing vitamins, minerals, amino acids, and fatty acids. Additionally, the leaves are reported to contain various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids [4-6]. Studies on the traditional use of *M. oleifera* have acknowledged the plant to have several pharmacological properties such as antidiabetic, anti-inflammatory, anti-hypertensive, antimicrobial, antioxidant, and diuretic [6-13]. The use of *M. oleifera* in boosting the immune system has also been reported to be prevalent among HIV-positive patients in Zimbabwe. [14]. The plant is currently of commercial value in several countries in Africa, South and Central America and Asia [2] where various formulations of the plants are available over the counter, mainly as food supplements [6].

The plant is thought to have gained popularity in poor countries of the world because of its claimed nutritional and medicinal value which is said to be unparalleled by other herbs**.** In the midst of the uses of *M. oleifera,* either as food or medicine, there is an increasing possibility that it can be taken with drugs, thus exposing such people to herb/drug interactions. In fact, *in vitro* studies have shown that leaf extracts of *M. oleifera* inhibited CYP3A4 enzymes which metabolize a large proportion of drugs [10,15]. As earlier indicated, *M. oleifera*is widely used in developing countries for a lot of indications and also as food supplements thus suggesting the possibility of its administration with conventional drugs. Therefore, it is of great interest to evaluate the interaction of *M. Oleifera* with conventional drugs, especially antimalarials, since its use has been widely accepted in most malaria endemic regions. One of such antimalarial of interest is amodiaquine (AQ), a first line drug in the WHO-recommended Artemisinin-based Combination Therapy(ACT) [16]. A recommended combination of AQ with artesunate is very popular in most developing countries, including Nigeria, possibly due to its ready availability and lower cost compared to other ACT drugs. This justifies the choice of AQ in this study.

AQ is rapidly absorbed and extensively metabolized mainly to desethylamodiaquine (DEAQ) which has a long elimination half-life and is probably responsible for the long antimalarial action of AQ [17]. The metabolic pathway for the conversion of AQ to DEAQ is via the cytochrome P450 isoenzyme, CYP 2C8 [18]. Since this isoenzyme metabolizes many clinically-used drugs, a study of the interaction of AQ with drugs or herbal preparations is very important.

Although there is evidence to support the health benefits and nutritional values of Moringa, little is known of its possible pharmacodynamic and pharmacokinetic effects on drugs. Therefore, this study investigated the effect of *M. oleifera* powdered leaves suspension on the pharmacokinetics of AQ using rats as an animal model as a prelude to the study in humans.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Amodiaquine hydrochloride dihydrate (Sigma, USA); Desethylamodiaqine (TLC Pharma, Canada); Hydroxychloroquine sulphate (AK Scientific Inc, USA); HPLC grade Methanol (Scharlau, Spain); Analytical grade Diethyl ether (Lobal Chemie, India); Triethylamine (Scharlau, Spain); Orthophosphoric acid (Aldrich, Germany). ASAQ™ Tablets (Sanofi-Aventis, Morocco. BN: 3MA121; NAFDAC No: A4-3357 with MAS codes).

2.2 Preparation of Suspension of Powdered Leaves of *Moringa oleifera*

The leaves of *Moringa oleifera* L. (Moringaceae) were collected from a cultivated garden and authenticated at the Herbarium Unit of the Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife (voucher identification number: IFE-17,420). The leaves were air dried at room temperature and thereafter, powdered and stored in a glass bottle at room temperature until ready for use. Just immediately before use, a 40 mg/mL suspension of the powdered *M. oleifera* leaves (MO) was prepared by mixing 20 mL potable water with 2 g of powdered leaves and finally making up the volume to 50 mL with more water.

2.3 Study Design

One hundred and eighty male Wistar Albino rats, weighing 180-220 g, obtained from the animal house of the Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife and fed on standard pelleted diet were used for this study. For the determination of the sample size (http://www.biomath.info/power/ttest.html), an assumption of a 1:1 ratio between each treatment arm and the control group was used. For each time point a minimum power of 80% to detect changes in PK data was assumed with a 10% standard deviation and paired two-tailed t-test with significance level of $P \leq 0.05$. This gave a minimum of 6 animals per time point and 60 rats each for each of the treatment arms, giving a total of 180 rats. The rats were housed under a 12 h light/dark cycle while ensuring that they had free access to food and potable water for at least one week before use.

The rats were randomly divided into 3 groups of 60 rats each i.e. Control (CT), Co-administration (CA) and Pre-treatment (PT) groups. Each group was further subdivided into 10 subgroups such that each sub-group represents a time point. Each rat in the CT group after an overnight fast received once daily dose of AQ solution at 10 mg/kg body weight (recommended daily therapeutic dose) using a metal feeding cannula while in the CA group, each rat received a combined single oral dose of AQ (10 mg/kg/) and MO suspension (200 mg/kg). Rats in the PT group were pre-treated orally with 200 mg/kg MO suspension once daily for 7 days and on the 8th day, after an overnight fast, the 200 mg/kg dose of MO suspension was mixed with 10 mg/kg of AQ solution and administered to the rats.

Blood samples were collected by cardiac puncture of anesthetized rats (diethyl ether) at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 h with disposable hypodermic syringes, into heparinised tubes. An average of 3-4 mL of blood was collected from the heart, after opening the chest of the anesthetized rats, and the animals were humanely disposed of, thereafter. Plasma was obtained by centrifugation at 3000 g for 10 min and stored at -20ºC until analysis.

2.4 HPLC Analysis

Plasma concentrations of AQ and desethylamodiaquine DEAQ were simultaneously determined using a slightly-modified validated HPLC method [19]. For sample preparation, 10 µl of a 1 ng/mL hydroxychloroquine solution (internal standard) was added to 500 µl of plasma in an extraction tube and made up to 2 mL with distilled water and vortex-mixed for 1 min. Thereafter, 2 mL of diethyl ether was added to the mixture, vortexed for 2 min, and then centrifuged at 3000 g for 10 min. The organic layer was transferred to a clean tube and the extraction was repeated. The combined organic phases were allowed to dry in a fume cupboard and the residue was reconstituted in 500 µl of the mobile phase followed by vortex-mixing. A 20 µl aliquot of the supernatant was subjected to HPLC analysis. The average recovery for AQ and DEAQ were 58.32% and 42.72%, respectively, while the lowest limit of quantitation (LLOQ) was 1 ng/mL for both AQ and DEAQ. The accuracy, measured at three concentration levels, ranged from 92.27 to 101.53% for AQ and from 95.9 to 97.85% for DEAQ. Precision was likewise acceptable (between 6.7% and 11.2% for AQ and between 6.8% and 12.2% for DEAQ). The assay was sufficiently robust, there was no influence of plasma matrix from different rats on the outcome of the assay and stability of the samples under the assay conditions was assured.

2.5 Pharmacokinetic Analysis

The data were analyzed based on a non-compartmental pharmacokinetic model using Kinetica™ Version 4.1, (Inna Phase Corporation, Philadelphia, USA) pharmacokinetics fitting software to calculate the model-independent pharmacokinetic parameters. The parameters determined were the area under the plasma concentration–time curve (AUC), the clearance (CL/F) the half-life (T½), maximum plasma

concentrations (Cmax) and time to achieve maximum plasma concentration (T_{max}).

2.6 Statistical Analysis

The pharmacokinetic parameters were compared between the three groups using a paired two samples t-test and a two-tailed significance level of *P* ≤0.05 (GraphPad Prism 4.0 software; San Diego, CA, USA).

3. RESULTS

The plasma concentration–time curves of AQ in the CT, CA and PT groups are as shown in Fig. 1. AQ was rapidly absorbed in the rats with a T_{max} of 0.5 h but there was an 8-fold increase in T_{max} in the CA and PT groups. The half-life (T½) of AQ also increased from 0.58 h to 1.82 h and 2.1 h in both CA and PT, respectively (Table 1). However, there was a significant reduction in the Cmax of AQ in both CA and PT when compared with CT. In the CA group, the Cmax was reduced by 29%, while in PT, it was reduced by 53% with the reduction in both groups being significantly different (P=0.001) (Table 1). Also, the AUC_{total} of AQ increased significantly (*P*=0.001) by about 318.2% and 144.6% in both CA and PT, respectively, while its clearance was reduced by 76.3% and 59.2% in CA and PT, respectively (Table 1). The plasma concentration–time curves of DEAQ after AQ administration in the three groups are as shown in Fig. 2. Compared to CT, the Cmax of DEAQ was significantly decreased in CA by 59.2% whereas a significant increase of 36.0% was observed with PT (P<0.05). In the CA group, there was a 28.8% reduction in the AUC_{total} of DEAQ but, in the PT group, the AUC_{total} increased significantly (P<0.05) by 242.4%. The clearance of DEAQ increased by 13.3% in CA but reduced significantly by 70.9% in PT. Evaluation of the metabolic ratio (MR) revealed a reduction in the CA group but showed a 40% increase in the PT group, when compared with that of the CT group (Table 1).

4. DISCUSSION

Drug-herb interactions resulting from the concurrent use ofherbal drugs with prescription and over-the-counter drugsmay cause adverse reactions such as toxicity andtreatment failure.Notable herb–drug interactions include inhibition or induction of cytochrome P450 (CYP) enzymes, uridine 5′-diphospho (UDP)-glucuronosyltransferase enzymes, and drug transporters [20]. Although, *M.oleifera* leaves seems to be safe and well tolerated as detailed in literature, there is the possibility that its consumption with conventional prescription or over the counter drugs may have significant clinical effects.

Table 1. Pharmacokinetic parameters of AQ and DEAQ in rat after administration of a single dose of AQ alone (CT) or co-administration (CA) and pre-treatment with MO (PT)

Parameter	CT	CA	PT
AQ			
Cmax (ng/mL)	7.32 ± 0.16	5.20 ± 0.06*	3.41 ± 0.05*
T max (h)	0.5	4*	4*
AUC (ng/mL*h)	8.47 ± 0.22	35.42 ± 0.24*	20.72 ± 0.48*
T½ (h)	0.58 ± 0.03	1.82 ± 0.05*	2.10 ± 0.10*
CL/F (L/h)	238.83 ± 6.58	56.50 ± 0.39*	97.46 ± 2.44*
Vd (L)	270.85 ± 6.07	308.72 ± 2.04*	592.82 ± 9.10*
DEAQ			
Cmax(ng/mL)	9.71 ± 0.52	4.83 ± 0.08*	13.21 ± 0.15*
T max (h)	3.67 ± 0.18	3.67 ± 0.18*	8 ± 0.00*
AUC (ng/mL*h)	59.59 ± 1.58	42.41 ± 0.51*	204.04 ± 4.82*
T½ (h)	1.82 ± 0.08	3.77 ± 0.12*	8.07 ± 0.32*
CL/F (L/h)	33.99 ± 0.96	47.27 ± 0.57*	9.90 ± 0.23*
Vd (L)	169.27 ± 6.36	349.31 ± 4.26*	142.74 ± 1.33*
Metabolic ratio $\left(\frac{AUC_{DEAQ}}{AUC_{AQ}}\right)$	7.05 ± 0.14	1.20 ± 1.67	9.85 ± 1.1

** Each value represents the mean ± SD of six rats.*

** Significantly different with P ≤ 0.05 versus AQ alone (control group).*

AUC - area under time–concentration curve, C_{max}- maximum concentration, T_{max} - time to reach maximum concentration, T½ elimination half-life, CL/F- clearance, Vd – Volume of distribution

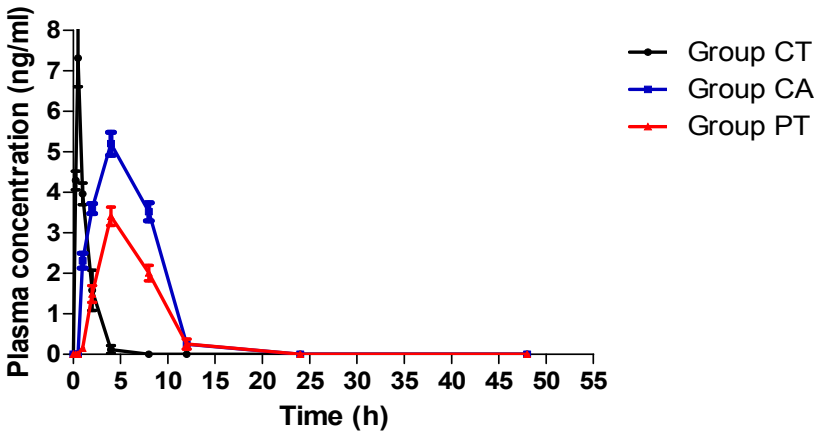

Fig. 1. Plasma concentration-time curve of amodiaquine (AQ) in rat after a single dose of AQ alone (Group CT), co-administration (CA) with MO and pre-treatment (PT) with MO for seven consecutive days (n = 6). Data are expressed as mean ± S.D

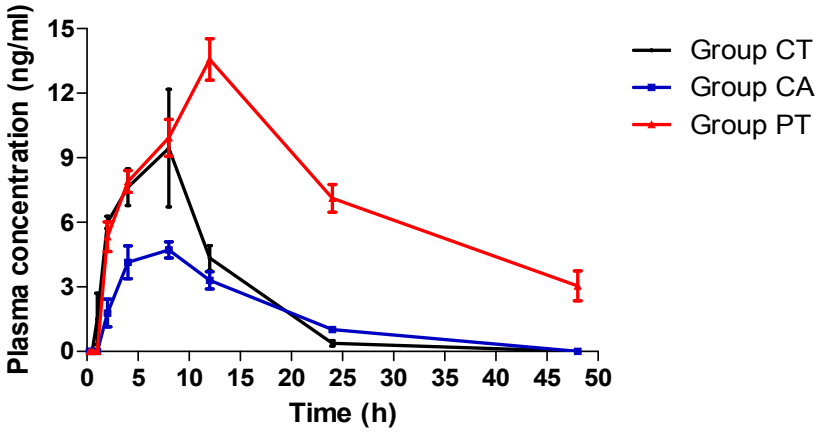

Fig. 2. Plasma concentration-time curve of desethylamodiaquine (DEAQ) in rat after a single dose of AQ alone (Group CT), co-administration (CA) with MO and Pre-treatment (PT) with MO for seven consecutive days (n = 6). Data are expressed as mean ± S.D

In this study, we report for the first time, the pharmacokinetics of AQ in rats and also the effects of concurrent intake of *M. oleifera* leaves powder suspension on its pharmacokinetics. AQ was rapidly absorbed within 30 minutes and, similar to reports in humans [21], it was also rapidly cleared from the body as the drug was not detectable in plasma 5 h after drug administration.However, there was a significant delay in its absorption following concurrent (CA) and pre-treatment (PT) administration of MO. Delay in drug absorption has generally been attributed, in part, to delayed gastric emptying or decreased intestinal motility resulting from the presence of other substances, apart from the drug, in the stomach. Such other substances include food, other drugs or herbs [22]. The delay in the rate of absorption of AQ, observed in the present study, could probably be due to effect of the high fiber content of the MO, which could form complex with AQ, thereby reducing its absorption. Moreover, antispasmodic activity of 4-[α-(L-rhamnosyloxy) benzyl]-o-methyl thiocarbamate,a compound that has been previously isolated from*M.oleifera* has been reported [22-24], therefore, there is a possibility that this could causea delay in gastric emptying resulting from reduced intestinal motility. The delay in gastric emptying of a drug is known to lead to prolonged release of low concentrations of the drug into the small intestinewhich could account for the observed decrease in the Cmax of AQ following its co-administration with MO. Other possible reasons that may account for the

decrease in Cmax include formation of a chemical complex which may make the drug unavailable for absorption, an increase in the concentration of ionized drug resulting from a reduction of gastrointestinal pH, pre-systemic metabolism, degradation of the drug in the stomach with a subsequent reduction in the amount of drug absorbed, increased viscosity in the gastro-intestinal tract due to the high fibre content of MO or induction of the intestinal efflux transporter, P-glycoprotein (Pg-P).However, the pH of the suspension of MO, as used in this study, was not different from that of water hence the possibility of a drastic change in the pH of the gastrointestinal tract is remote. Also, AQ is not a known substrate of Pg-P nor is its major metabolising enzyme located in the gastrointestinal tract. Moreover, it is unlikely that any of these reasons would lead to an increase in the extent of absorption (AUC) of AQ in both the CA and PT groups, as observed in the study.

The systemic exposureof AQ increased significantly by about four-foldin CA and by two and a half-fold in the PT group. This is possibly because the delayed absorption led to a prolonged exposure of AQ to the absorptive surface area of the gastrointestinal tract and a resultant reduction in its systemic clearance, as observed in both the CA and PT groups, which eventually increased its systemic exposure. However, the higher clearance and lower bioavailability of AQ in PT than in CA is an indication of a possible induction of metabolizing enzymes as a result of prolonged use of MO prior to administration of AQ.

The conversion of AQ to its active metabolite, DEAQ, was significantly reduced in CA possibly as a result of the decreased absorption of AQ during this phase. However, significant increase in the Cmax of DEAQ in the PT group suggests an induction or increase in the levels of the metabolising enzyme responsible for the biotransformation of AQ to DEAQ.Although there was an insignificant decrease in the AUCtotal of DEAQ after co-administration (CA) the situation following pre-treatment with MO was different.The observation of a significant 242% increase in the AUC supports the earlier suggestion of an induction of the enzyme responsible for the metabolism of AQ to produce more of DEAQ.In humans, the metabolism of AQ is mediated by CYP2C8 and although the metabolism of AQ in rats has not been investigatedit is known that rats also express CYPs of the 2C family[25].

AQ and DEAQ are both potent antimalarials and although AQ is threefold more potent than DEAQ it is rapidly cleared from the systemic circulation. This makes DEAQ stay longer in the circulation and be responsible for the observed antimalarial activity[17]. Therefore, concurrent administration of MO with AQ, either concurrently or after pre-treatment, will affect the antimalarial activity of AQ.

The plasma half-life of AQ and DEAQ increased significantly in both the CA and PT groups. Drugs with elongated T½ are slowly eliminated from the plasma either by biotransformation or excretion, thus prolonging their therapeutic actions. Concomitant administration of MO with AQ may, therefore, lead to prolonged effects of AQ and DEAQ.Increased systemic exposures of AQ and/or DEAQ, as observed in the study, may indicate increased antimalarial activity during therapy with MO. Similar enhancement of systemic exposure of drugs by a mixture of dried fruits of pepper (*Piper nigrium* and *Piper longum*) and their active ingredient, piperine, have been reported [26-28]. However, increased exposure to AQ and its metabolites may also lead to toxicity. In humans, the clinical use of AQ has been associated with organ damage, including hepatotoxicity. This has been attributed to the bioactivation of AQ to a chemically-reactive haptenate protein called amodiaquine-quinoneimine which reacts with cell components or produce an antigen which invokes immune response from the body [29-31]. Similarly, AQ-induced hepatotoxicity and reproductive toxicity have been reported in rats, often in a concentration-dependent manner [32-34]. Therefore, continuous exposure, to this compound, due to increased concentrations of AQ and DEAQ, may lead to organ damage [35].

5. CONCLUSION

This study investigated, for the first time, the effect of concurrent intake of MO on the disposition of AQ in the rat. MO significantly delayed the absorption of AQ and consequently increased its exposure and that of its active metabolite, DEAQ. This indicates the possibility of an increase in the antimalarial activity of AQ in the presence of MO during malaria therapy. Similar studies in humans are required to assess the clinical implications of this finding.

CONSENT

It is not applicable.

COMPLIANCE WITH ETHICAL STANDARDS

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed and in agreement with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, as adopted by our institution. The experimental protocol was approved by the Health Research Ethical Committee of the Institute of Public Health, Obafemi Awolowo University, Ile-Ife on 11thMarch 2015, with the assigned number IPH/OAU/12/333.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nambiar VS, Guin P, Parnami S, Daniel M. Impact of antioxidants from drumstick leaves on the lipid profile of hyperlipidemics. J Herb Med Toxicol. 2010;4:165-172.
2. Thurber MD, Fahey JW. Adoption of *Moringa oleifera* to combat under-nutrition viewed through the lens of the "Diffusion of Innovations" theory. Ecol Food Nut. 2009;48(3):212-225.
3. Mbikay M. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A Review. Front Pharmacol. 2012;3:24.
4. Abdull R, Ahmad F, Ibrahim MD, Kntayya SB. Health benefits of *Moringa oleifera.* Asian Pac J Cancer Prev. 2014;15:8571-8576.
5. Moyo B, Masika PJ, Hugo A, Muchenje V. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. African J Biotech. 2011;10:12925-12933
6. Godinez-Oviedo A, Guemes-Vera, Acevedo-Sandoval OA. Nutritional and phytochemical composition of *Moringa oleifera* Lam and its potential use as nutraceutical plant: A review. Pak J Nutr. 2016;15:397-405.
7. Kumari D. Hypoglycemic effect of *Moringa oleifera* and Azadirachta indica in type-2 diabetes. Bioscan. 2010;5:211-214.
8. Anwa F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytother Res. 2007;21: 17-25.
9. Ndong M, Uehara M, Katsumata S, Suzuki K.. Effects of oral administration of *Moringa oleifera* Lam on glucose tolerance in Goto-Kakizaki and Wistar rats. J Clin Biochem Nutr. 2007;40:229-233.
10. Monera TG, Wolfe AR, Maponga CC, Benet LZ, Guglielmo J. *Moringa oleifera* leaf extracts inhibit 6-beta-hydroxylation of testosterone by CYP3A4. J Infect Dev Ctries. 2008;2:379-383.
11. Sujatha BK, Patel P. *Moringa oleifera* – Nature's Gold. Imperial J Interdisciplinary Res. 2017;3(5):1175-1179.
12. Brilhante RSN, Sales JA, Pereira VS, Castelo-Branco DSM, Cordeiro RA, Sampaio CM, et al. Research advances on the multiple uses of *Moringa oleifera*: A sustainable alternative for socially neglected population, Asian Pac J Trop Med; 2017. Available:http://dx.doi.org/10.1016/j.apjtm.2017.07.002 (Accessed online on 12th August, 2017)
13. Biel W, Jaroszewska A, Łysoń E. Nutritional quality and safety of moringa (*Moringa oleifera* Lam., 1785) leaves as an alternative source of protein andminerals. J. Elem. 2017;22(2):569-579
14. Monera TG, Maponga CC. Prevalence and patterns of *Moringa oleifera* use among HIV-positive patients in Zimbabwe: A cross-sectional survey. J Public Health Afr. 2012;3:22-24.
15. Wrighton SA, Stevens JC. The human hepatic cytochromes P450 involved in drug metabolism. Crit Rev Toxicol. 1992;22:1-21.
16. World Health Organization. WHO model list of essential medicines, 17th list, 2011;13-14.
17. Mariga ST, Gil JP, Sisowath C, Wernsdorfer WH, Bjorkman A. Synergism between amodiaquine and its major metabolite, desethylamodiaquine, against Plasmodium falciparum in vitro. Antimicrob Agents Chemother. 2004;48:4089-4096.
18. Li XQ, Bjorkman A, Andersson TB, Ridderstrom M, Masimirembwa CM. Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: A new high affinity and turnover enzyme-specific probe substrate. J Pharmacol Exp Ther. 2002;300:399-407.
19. Adedeji ON, Bolaji OO, Falade CO, Osonuga OA, Ademowo OG. Validation and pharmacokinetic application of a high-performance liquid chromatographic

technique for determining the concentrations of amodiaquine and its metabolite in plasma of patients treated with oral fixed-dose amodiaquine-artesunate combination in areas of malaria endemicity. Antimicrob Agents Chemother. 2015;59:5114-5122.

20. Jeong HU, Lee JY, Kwon S, Kim JH, Kim YM, Hong W, et al. Metabolism-mediated drug interaction potential of HS-23, a new herbal drug for the treatment of sepsis in human hepatocytes and liver microsomes. Arch Pharm Res. 2015;38:171-177.
21. Winstanley P, Edwards G, Orme M, Breckenridge A. The disposition of amodiaquine in man after oral administration. Br J Clin Pharmacol. 1987;23:1-7.
22. Caceres A, Saravia A, Rizzo S, Zabala L, De Leon E, Nave F. Pharmacologic properties of *Moringa oleifera*. 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol. 1992;36:233-237.
23. Gilani AH, Aftab K, Suria A, Siddiqui S, Salem R, Siddiqui BS, Faizi S. Pharmacological studies on hypotensive and spasmolytic activities of pure compounds from *Moringa oleifera*. Phytother Res. 1994;8:87-91.
24. Dangi SY, Jolly CI, Narayanan S. Antihypertensive activity of total alkaloids from the leaves of *Moringa oleifera*. Pharm Biol. 2002;40:144-148.
25. Kim SH, Kim K, Um S, Oh Y, Chung M, Oh H et al. Changes in the pharmacokinetics of rosiglitazone, a CYP2C8 substrate, when co-administered with amlodipine in rats. Biomol Ther. 2009;17:299-304.
26. Atal CK, Zutshi U, Rao PG. Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs. J Ethnopharmacol. 1981;4:229-232.
27. Bano G, Amla V, Raina RK, Zutshi U, Chopra CL. The effect of piperine on pharmacokinetics of phenytoin in healthy volunteers. Planta Med. 1987;53:568-569.
28. Bano G, Raina RK, Zutshi U, Bedi KL, Johri RK, Sharma SC. Effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. Eur J Clin Pharmacol. 1991;41:615-617.
29. Larrey D, Castot A, Pessayre D, Merigot P, Machayekhy JP, Feldmann G, et al. Amodiaquine- induced hepatitis. A report of seven cases. Ann Int Med. 1986;104:801-803.
30. Winstanley PA, Coleman JW, Maggs JL, Breckenridge AM, Park BK. The toxicity of amodiaquine and its principal metabolites towards mononuclear leucocytes and granulocyte/monocyte colony forming units. Br J Clin Pharmacol. 1990;29:479-485.
31. Taylor WRJ, White NJ. Antimalarial drug toxicity: A review. Drug Saf. 2004;27:25-61.
32. Heidari R, Babaei H, Eghbal MA. Amodiaquine-induced toxicity in isolated rat hepatocytes and the cytoprotective effects of taurine and/or N-acetyl cysteine. Res Pharm Sci. 2014;9:97–105.
33. Niu Y, Wei B, Chen B, Li-Hua Xu L, Jing X, et al. Amodiaquine-induced reproductive toxicity in adult male rats. Mol Reprod Dev. 2016;83:174-182.
34. Jamshidzadeh A, Heidari R, Abazari F, Ramezani M, Khodaei F, Ommati FM, et al. Antimalarial drugs-induced hepatic injury in rats and the protective role of carnosine. Pharm Sci. 2016;22:170-180.
35. Winstanley PA, Ward S, Snow R, Breckenridge A. Therapy of falciparum malaria in sub-Sahara Africa: From molecule to policy. Clin Microbiol Rev. 2004;17:612-637.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20963