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Original Research Article

In vitro study of interaction between quinine and *Garcinia kola*

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Abstract

Purpose: *To investigate the interaction between quinine and Garcinia kola using an in vitro adsorption study.*
Methods: *In vitro interaction between quinine and G. kola was conducted at 37 ± 0.1 °C. Adsorption of quinine (2.5 - 40 µg/ml) to 2.5 % w/v G. kola suspension was studied. Thereafter, quinine desorption process was investigated. The amount of quinine adsorbed and desorbed was quantified using HPLC. A Freundlich isotherm was constructed to describe the resulting data and percentage of quinine desorbed was determined from the desorption data.*
Results: *An adsorption isotherm of the data gave a Freundlich constant (K) of 52.66 µg/g, with a slope of 0.69 indicating a high capacity and affinity of G. kola to adsorb quinine at a concentration smaller than 2.41 µg/g of G. kola. However the adsorptive capacity of G. kola for quinine at 37 ± 0.1 °C appears to be a saturable process as observed from the isotherm. Quinine desorption from G. kola peaked at 1 hour (37.51 %) and decreased to a constant amount (about 35 %) over the remaining sampling time.*
Conclusion: *Quinine is adsorbed on G. kola in vitro. This suggests that concurrent administration of quinine and G. kola should be avoided, to prevent potential drug interaction and decreased drug bioavailability.*

Keywords: *Quinine, Garcinia kola, Adsorption, Desorption, Drug interaction*

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INTRODUCTION

Assessment of the *in vitro* potential of natural products to interact with drugs especially when taken concurrently has important implications for predicting the likelihood of natural product-drug interactions or a possible mechanism of interaction. *Garcinia kola* seed, also commonly called bitter kola, is a natural product relevant in Africa traditional medicine, as well as in cultural and social ceremonies in many parts of West and Central Africa [1]. The seeds or seed extracts (alone or in combination with other phytochemicals) are available as proprietary dietary supplements [2]. Owing to its acclaimed health benefits relevant to the management and chemoprevention of some life-threatening diseases related to the liver and lungs, and as an anti-infective agent, the seeds are usually eaten as snack [1,2].

In spite of being a common masticatory nut, not much has been reported on its possible interaction with prescription drugs in humans. Previous *in vitro* and *in vivo* studies have suggested that *G. kola* seeds or its extracts have

the potential to modulate the activities of some drug metabolising enzymes [3-5]. Some authors have reported that *G. kola* reduced ciprofloxacin absorption *in vitro* [6], and reduced both the C_{max} and AUC when co-administered with ofloxacin in humans [4,5]. Some authors have also reported a herb - drug interaction in which a Kampo preparation which contains various herbal medicines and metal cations such as calcium, magnesium and aluminum caused significant reduction in plasma tetracycline and ciprofloxacin concentrations in healthy volunteers through complex formation [8,9].

A significant pharmacokinetic interaction between quinine (an antimalarial medicine) and *G. kola* in healthy volunteers has earlier been reported and the results were suggestive of interference with quinine absorption since parameters indicative of interference with elimination were not significantly altered [7]. This report, coupled with the fact that knowledge of the mechanism by which food-drug interactions occur may contribute to predicting and preventing unwanted alterations in the pharmacokinetic profile of drugs [10], formed the rationale for the present study which investigated a possible mechanism of interaction between quinine and *G. kola* through *in vitro* interaction experiments.

EXPERIMENTAL

Chemicals and reagents

Quinine sulphate powder was purchased from BDH Chemicals (Poole, UK). HPLC grade acetonitrile, methanol, perchloric acid, hydrochloric acid, potassium phosphate monobasic, and sodium hydroxide were from Sigma-Aldrich (Steinheim, Germany). Fresh *Garcinia kola* seeds were purchased locally from a retail market in Ile-Ife, Nigeria and were identified by Mr G. Ademoriyo, a taxonomist in the Herbarium of the Department of Botany, Obafemi Awolowo University, Nigeria. A voucher specimen (voucher number IFE-17478) was deposited at the Herbarium.

HPLC analysis of quinine

Quinine analysis was performed using a validated HPLC method as previously reported [7]. The HPLC equipment (Agilent 1200 series, Agilent Technologies, Santa Clara, California) was fitted with an isocratic pump (model G1341A) coupled to a variable wavelength detector [Agilent Technologies; standard version (model G1341B)]. Mobile phase consisted of methanol: acetonitrile: 0.02 M potassium dihydrogen phosphate (volume ratio 15:15:70), and 74 mmol/L perchloric acid (0.64 ml), adjusted to pH 2.6 with 10 M NaOH. It was pumped through the column (Eclipse XDB-C18 reverse phase; 5µm particle size and 150 x 4.6 mm, i.d., Agilent Technologies, Santa Clara, California) at a flow rate of 1.6 ml/min at ambient temperature while monitoring the effluent at 254 nm. Quinine stock (1 mg/ml) was prepared by dissolving 50 mg of the pure quinine powder in 50 ml of 0.1 M HCl. Further dilutions were made to obtain calibration concentration ranging from 2.5 - 80 µg/ml. A volume of 20 µl of each calibration concentration was injected in triplicate, and the mean of the peak area was used to generate a calibration curve.

Preparation of *G. kola* suspension

The husks of *G. kola* seeds (50 g) were removed and the seeds were sliced into small bits, oven-dried at 40 ^{o}C for about 24 h and subsequently blended into coarse powder in an electric blender. Thereafter, the powder was transferred into a beaker and a small quantity of 0.02 M KH_2PO_4 (pH 5.8) buffer solution was added to make a paste which was later transferred into a 2 L volumetric flask and made up to 2 L with the same buffer solution resulting in 2.5 % w/v *G. kola* suspension.

Determination of equilibration time for adsorption of quinine on *G. kola*

Previous methods [11-13] on adsorption of drug by antacids and *G. kola* were adapted. Stock solution of quinine (8 ml) containing 1 mg/ml of quinine was pipetted into a 100 ml volumetric flask and made up to mark with the 2.5 % w/v suspension of *G. kola*. This was mixed in a vortex mixer (Parloworld Scientific Ltd, Straffordshire, UK) and incubated in a water-bath with a shaker at 37 ± 0.1 °C. Thereafter, the suspension (5 ml) was taken at 0.25, 0.5, 1, 2, 3, 4, 5, 6 and 8 h and centrifuged at 3000 g for 5 min. The concentrations of quinine in the supernatants were then determined using HPLC. From the peak area obtained at the various time points, the corresponding concentrations of the drug in the supernatants were estimated from a calibration curve. Subsequently adsorption equilibration time was determined from a plot of the concentration of the quinine in supernatant against time.

Adsorption of quinine on *G. kola*

Duplicate series of 100 ml flasks containing aliquots (0.25, 0.5, 1.0, 2.0 and 4.0 ml) of 1

mg/ml quinine solution were prepared. *G. kola* suspension (2.5 % w/v) in 0.02 M KH_2PO_4 buffer was added to the respective flask to a final volume of 100 ml mixture. All flasks were incubated in a thermostated water bath with shaker at 37 ± 0.1 °C for 5 h (equilibration experiment showed that after 5 h, no further adsorption changes occurred in the quinine - *G. kola* suspension system). Thereafter, 5 ml was taken from each of the flasks, centrifuged and the concentration of quinine in the respective supernatant determined using HPLC. The amount of quinine adsorbed was determined by subtracting the amount of quinine in supernatant from the initial amount of quinine in the mixture. Adsorption percentage (%) was derived from the difference of the initial concentration of quinine (C_o) and equilibrium concentration of quinine in the supernatant (C_e) using the equation:

$$\text{Adsorption \%} = (C_o - C_e)/C_o \times 100\ \% \quad \ldots\ldots\ldots (1)$$

An adsorption isotherm was also generated. The adsorption isotherm of quinine on *G. kola* at 37 ± 0.1 °C expressed by a double logarithm plot based on Freundlich model was derived from the equation:

$$\log x/m = \log K + 1/n\ (\log C_e) \quad \ldots\ldots\ldots\ldots\ldots (2)$$

where x is the mass of the quinine adsorbed by m grams of *G. kola* (adsorbent), K is the Freundlich constant representing the quinine adsorbed per gram of the *G. kola* at a µg/ml quinine concentration, log K is the intercept, 1/n is the slope which represents the amount of quinine adsorbed for a given concentration change and C_e is as previously defined.

Desorption experiments

Elution of the adsorbed quinine by *G. kola* was performed by preparing a concentration of 80 µg/ml solution of quinine in 2.5 % w/v *G. kola* suspension (100ml). This was equilibrated in a thermostated water bath at 37 ± 0.1 °C for 5 h. Thereafter, the suspension was centrifuged, and the concentration of quinine in the supernatant measured using HPLC. Quinine concentration in the sediment was calculated from a difference in the initial concentration of quinine (80 µg/ml) and the concentration in the supernatant. The sediment was then dispersed in 100 ml of 0.1 M HCl; the flasks were shaken and aliquots of 5 ml were removed at 0, 0.25, 0.5, 1, 2, 4, 6, and 8 h and centrifuged. The amounts of quinine in the new supernatants were determined from the calibration curve, and the percentage of quinine desorbed was calculated as the ratio of the concentration of quinine in the supernatant and the concentration of quinine in the sediment multiplied by 100.

RESULTS

Adsorption and desorption of quinine on *G. kola*

The standard curve gave a coefficient of determination (R^2) of 0.9999 for quinine in 0.1 M HCl at concentrations between 1.25 and 80 µg/ml with the regression equation:

$$y = 41.628x - 10.858.$$

A plot of the concentration of quinine in the supernatant against time (Figure 1) clearly revealed that the adsorption equilibration time of quinine in the quinine - *G. kola* suspension was between the 4th and 5th hour.

Quinine was adsorbed on *G. kola* and the amount adsorbed increased with increasing concentration of quinine (Table 1). At a concentration of 10 µg/ml of quinine, the highest percentage of quinine adsorbed by *G. kola* was 63.66 %.

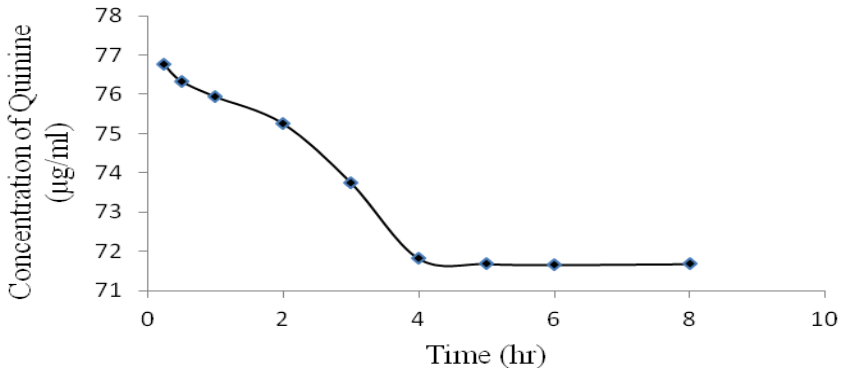

Figure 1: Equilibration time curve for the adsorption of quinine on 2.5 % *G. kola* suspension

Table 1: Adsorption data of quinine on 2.5 % *G. kola* suspension

C_o (µg/ml)	(µg/ml) (C_e)	Conc. of quinine adsorbed (µg/ml)	X (µg/100ml)	x/m (µg/g)	Log C_e (µg/ml)	Log x/m (µg/g)	Quinine adsorbed (%)
2.5	1.60	0.90	89.63	35.94	0.21	1.56	35.85
5	2.25	2.75	275.25	110.63	0.35	2.04	55.05
10	3.63	6.37	636.64	257.23	0.56	2.41	63.66
20	11.73	8.27	827.09	337.59	1.07	2.53	41.35
40	30.33	9.67	966.81	402.84	1.48	2.61	24.17

C_o = Concentration of quinine before adsorption; C_e = concentration of quinine in supernatant after adsorption. x = Amount of Quinine adsorbed; m = 2.5g of G. kola suspension/100 ml of suspension; x/m = maximum adsorption capacity in µg/g

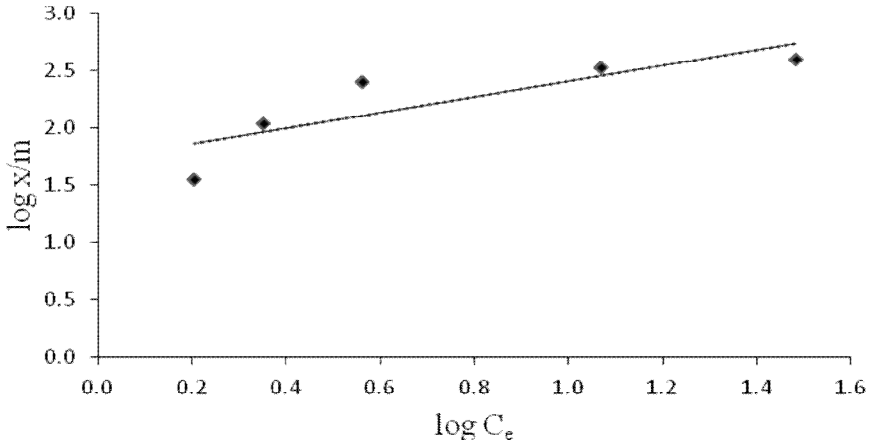

Figure 2: Freundlich adsorption Isotherm for adsorption of quinine on 2.5 % *G. kola* suspension

The adsorption isotherm of quinine onto *G. kola* at 37.1 ^{o}C is shown in Figure 2. The Freundlich constant (K) and the slope (1/n) which represents the amount of quinine adsorbed for a given concentration change were 52.66 µg/g and 0.69 respectively.

Desorption data of quinine on 2.5 % *G. kola* is presented in Table 2.The amount of quinine eluted increased from 30.12 % at 0.25 h and peaked to 37.51 % at 1 h. Thereafter, the amount desorbed decreased from the 2 h sample and remained relatively constant (35.77 to 35.426 %) through the remaining sample time point.

Table 2: Desorption data forquinine from 2.5 % *G. kola* suspension

Time (h)	Quinine desorbed (%)
0.25	30.12
0.5	33.21
1	37.51
2	36.02
4	35.77
6	35.46
8	35.43

DISCUSSION

Interaction of quinine with *G. kola* has been demonstrated in this *in vitro* study to occur as a result of capacity-limited adsorption of the drug unto *G. kola*. Numerous factors may have aided *G. kola* in its capacity to absorb quinine. In the first place, *G. kola* is known to contain flavonoids [14] which have functional groups that may favour complex formation with some compounds [15]. Secondly, *G. kola* also contains trace elements or minerals such as calcium, aluminum, magnesium, potassium, sodium, zinc and copper [16], some of which are known to cause drug interactions through chelate formation [8,17]. It is known that quinine may also act as a ligand by forming a stable five-membered ring with some metals through the quinuclidinic nitrogen and the hydroxyl oxygen, or by binding through the quinolinic aromatic nitrogen [18].

From a plot of the adsorption isotherm, 0.69 and 52.66 µg/g were obtained for slope (1/n) and K, respectively. The slope of the adsorption isotherm is dependent on the linearity of the isotherm, usually varies between 0 and 1 [11]

and provides information on the adsorption intensity between quinine and *G. kola*. These isotherm values decrease with increasing intensity of drug adsorption, while large values suggest that the adsorbent has a high capacity for the adsorbate [19,20]. In the present study, the Freundlich linear isotherm profile suggests a slope indicative of weak intensity in interaction between quinine and *G. kola*. Although a close observation of the isotherm plot showed initial linearity at lower values of log x/m suggestive of intense interaction at concentrations less than 2.41 µg quinine per gram of *G. kola* (Table 1), the observed K for the overall data, however, indicates a mild adsorption capacity of the *G. kola* for quinine [11]. Desorption data (Table 2) showed that no appreciable change occurred after the second hour during which the elution of quinine attained equilibrium in the system. On the basis of these results and what is known about the constituents of *G. kola* [8,17,18], it appears that the adsorption of quinine on *G. kola* may have occurred by physical adsorption and perhaps by some degree of chemisorption.

The findings from this *in vitro* study corroborate our earlier *in vivo* report of a pharmacokinetic drug interaction between quinine and *G. kola* when ingested concurrently. In this study, we aimed at using the same *G. kola* concentration as in our *in vivo* study since one goal of an *in vitro* study is to simulate experimental conditions as close as possible to what is obtained *in vivo*. In order to obtain an *in vitro* experimental condition close to the earlier *in vivo* study in fasting healthy volunteers, the estimated volume of intestinal fluid and the amount of *G. kola* used in the *in vivo* study [7] were also put into consideration in determining concentration of *G. kola* used. About 12.5 g of *G. kola* was used in our human studies. It has been reported that the average volume of intestinal fluid in a fasted individual is about 500 ml [21,22]. Thus 2.5 % w/v of *G. kola* suspension was employed so as to simulate the dose used in the *in vivo* study. In the *in vivo* study, the time lag required to reach maximum plasma concentration (T_{max}) increased by about 48 %, with a significant reduction in maximum plasma concentration (C_{max}) and a non-significant decrease in area under the plasma concentration-time curve, AUC [7]. These *in vivo* findings were suggestive of interferences with the absorption of quinine. Indeed, the results of this present adsorption-desorption experiments make it apparent that quinine is adsorbed on *G. kola*. Since *G. kola* is a popular snack in the tropics, especially in West African region where malaria is also endemic, the interaction between quinine and *G. kola* may have decrease bioavailability of quinine and compromise its anti-malarial efficacy.

CONCLUSION

The results of this *in vitro* study clearly demonstrate that quinine is adsorbed on *G. kola* and it may be necessary to avoid concurrent administrations of quinine and *G. kola*.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Iwu MM. Handbook of African medicinal plants, Boca Raton, Florida: CRC Press INC; 1993.p 223-224.*
2. *Farombi EO. Bitter Kola (Garcinia kola) Seeds and Hepatoprotection. In: Preedy, VR; Watson, RR; Patel, VB, editors. Nuts and Seeds in Health and Disease Prevention. 1st ed. Califonia: Elsevier Inc: 2011.p. 221-228.*
3. *Farombi EO. Mechanisms for the hepatoprotective action of kolaviron: Studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. Pharmacol Res 2000; 42: 75-80.*
4. *Esimone CO, Nwafor SV, Okoli CO, Chah KF, Uzuegbu DB, Chibudu C, Eche MA, Adikwu, MU. In vivo evaluation of interaction between aqueous seed extract of Garcinia kola Heckel and ciprofloxacin hydrochloride. Am J Ther 2000a; 9: 275-280.*
5. *Esimone CO, Nworu CS, Adikwu MU, Odimegwu DC, Ezugwu CO. The effect of a new adaptogen, Garcinia kola seed, on the bioavailability of ofloxacin in humans. Sci Res Essay 2007; 2: 482-485.*
6. *Esimone CO, Adikwu MU, Ibezim EC, Ofoefule SI, Nwafor SV, Onuoha OM. Potential application of Garcinia kola seed as a natural antidote in the management of ciprofloxacin hydrochloride poison: an in vitro study. Acta Pharm 2002b; 52: 219-226.*

7. Igbinoba SI, Onyeji CO, AkanmuMA, Soyinka JO, Pullela SS, Cook JM, Nathaniel TI. Effect of dehusked Garcinia kola seed on the overall pharmacokinetics of quinine in healthy human volunteers. J Clin Pharmacol 2015; 55: 348-354.
8. Ohnishi M, Hitoshi K, Katoh M, Nadai M, Abe F, ShunsukeKurono S, Saito H, Haniuda, M, Hasegawa T. Effect of a Kampo Preparation, Byakkokaninjinto, on Pharmacokinetics of Ciprofloxacin and Tetracycline. Biol Pharm Bull 2009; 32: 1080-1084.
9. Hitoshi K, Katoh M, Tanaka Y, Kurono S, Hotta K, Saito H, Hasegawa T, Nadai M. 2012. Mechanism of drug interaction between a Kampo medicine, byakkokaninjinto, and tetracycline in rats. J Infect Chemother2012; 18: 75-82
10. Englund G, Hallberg P, Artursson P, Michaëlsson K, Melhus H. 2004. Association between the number of co-administered P-glycoprotein inhibitors and serum digoxin levels in patients on therapeutic drug monitoring. BMC Med 2004; 2: 8.
11. Al-Gohary OM, Hosny EA. Effect of antacid magaldrate oral suspension on in-vitro and in-vivo availability of indomethacin in dogs. Pharm ActaHelv 1997; 72: 81-86.
12. Babalola CP, Ajayi O, Kotila O, Ameh S. In-vitro interaction studies of lumefantrine with antacids. Pharmacie Globale (IJCP) 2012; 1: 1-5.
13. Nnadi CO, Nwodo NJ, Agbo MO. Adsorption thermodynamics of phenobarbital sodium on powdered Garcinia kola seeds IJCR 2014; 6: 4980-4985
14. Iwu MM, Igboko OA, Okunji CO, Tempesta MS. Antidiabetic and aldose reductase activities of biflavanones of Garcinia kola. J Pharm Pharmacol 1990; 42: 290-292.
15. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity and cancer. Pharmacol Rev 1991; 52: 673-751.
16. Adesuyi AO, Elumm IK, Adaramola FB, Nwokocha AGM. Nutritional and Phytochemical Screening of Garcinia kola. Adv J Sci Technol 2012; 4: 9-14.
17. Kivisto KT, Ojala-Karlsson P, Neuvonen PJ. Inhibition of Norfloxacin absorption by dairy products. Antimicrob Agents Chemother 1992; 36:489-491.
18. Rey NA, dos Santos KC, Maria ABC, Menezes MABC, Mangrich AS, Pereira-Maia EC. Two Different Modes for Copper (II) Ion Coordination to Quinine-Type Ligands. J Braz Chem Soc 2006; 17: 497-504.
19. Abe I, Kamaya H, Ueda I. Adsorption of local anesthetics on activated carbon: Freundlich adsorption isotherm. J Pharm Sci 1990; 19: 354-358.
20. Foo KY, Hameed BH. Insights into the modeling of adsorption isotherm systems. Chem Eng J 2010; 156: 2-10
21. Jantratid E, Dressman J. Biorelevant Dissolution Media Simulating the Proximal Human Gastrointestinal Tract: An Update, In: Dressman, J, Kraemer, J, Eds. Pharmaceutical Dissolution Testing. Dissolution Technologies, Germany. 2009; pp 21-25.
22. Custodio JM, Chi-Yuan Wu C, Benet LZ. Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. Adv Drug Deliver Rev 2008; 60: 717-733.