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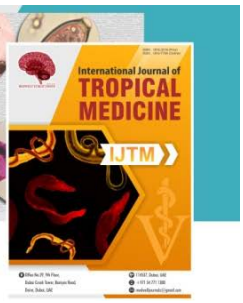

Evaluation of *Zingiber officinale* Effects on Rifampicin Pharmacokinetic Parameters using Animal Model

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Key words: Pharmacokinetics, ginger, rifampicin, concentration, treatment

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Abstract: The effect of ginger extract on the pharmacokinetic parameters of rifampicin and its penetration into the lung was investigated. Albino rats were used, Group A received rifampicin 10 mg kg^{-1} alone orally; Group B and C received 5 and 10 mg kg^{-1} of ginger extract respectively for 10 days and on day 11, 10 mg kg^{-1} rifampicin was given as in Group A. Blood samples were collected at different time and assayed to determine the concentration of rifampicin in the lungs tissues. Treatment with 5 mg kg^{-1} ginger significantly ($p<0.05$) increased T_{max} by 93% and decreased $C_{max,}$ AUC, C_L and $t_{1/2}$ by 18, 13, 72 and 33%, respectively while 10 mg kg^{-1} resulted in a non-significant ($p>0.05$) increase in T_{max} by 7% and decreased $C_{max,}$ AUC, $t_{1/2}$ and C_L by 8, 5, 59 and 77%. Concurrent administration of ginger and rifampicin resulted in significant ($p>0.05$) decrease in the concentration of rifampicin that penetrated the lung from 14.63±4.06 in rifampicin alone to 9.99±4.06 and 9.14±0.97 for 5 and 10 mg kg^{-1} ginger. This study has shown that ginger caused significant change in the pharmacokinetic parameters of rifampicin when taken concurrently and also decreased concentration of rifampicin in the lungs.

INTRODUCTION

Millions of people today use herbal therapies along with prescription and non-prescription medications. Although, considered natural, many of these herbal therapies can interact with other medications, causing either potentially dangerous side effects and/or reduced benefits from the medication. Drug interactions are said to occur when the pharmacokinetics and/or the pharmacodynamics of a drug are altered by the presence of another drug, food, drink or herb[1]. Phamacokinetics is proposed to study the absorption, the distribution, the biotransformations and the elimination of drugs in man and animals. It provides a mathematical basis to assess the time course of drugs and their effects in the body. A single kinetic profile may be summarized by C_{max}, T_{max}, $t_{1/2}$ and AUC and having more than one profile, 8 parameters at least, the mean and standard deviation of these parameters, summarizes the drug kinetics in the whole population[2]. Most often pharmacokinetic interaction results in a change in the plasma concentration of the interacting drug which can lead

to toxicity or sub-therapeutic effect[3]. Understanding of pharmacokinetic and metabolism characteristic of the drug compounds is needed in establishing drug dosage regimen and designing appropriate human clinical trials.

There is an increasing interest and medical need for the improvement of bioavailability of a large number of drugs. Of the promising approaches, the co-administration of therapeutic agents with natural compounds possessing absorption improving activities has gained great interest in oral drug deliver. However, it is paramount to note that co-administration of medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another while interactions between them may increase or decrease the pharmacological or toxicological effects of either component. Consequently, synergistic or antagonistic effects may complicate the dosing of long/short-term medications[4].

Ginger is a flowering plant whose rhizome is widely used as a spice or medicine. It contains gingerol which facilitates better absorption by regulating gastro-intestinal tract function by increasing blood perfusion Ginger has been shown to improve blood circulation and bioavailability of some prescription drugs when concurrently administered or co-formulated[5, 6]. Different studies have shown that ginger also enhance the protective functions of the immune system[7], inhibit airway contraction and help stimulate the secretion of mucus, hence, a drug of choice for cough and sore throat associated with cold possesses anti-inflammatory, analgesic, antioxidant and anti-emetic properties[8, 9]. If consumed in reasonable quantities, ginger is safe with few negative side effects such as heartburn, bloating, gas and belching[10]. Some pharmacokinetic studies have also revealed that ginger interacts with nifedipine[11] and warfarin[12].

Rifampicin is a bactericidal agent active against the three populations of M. tuberculosis. It is a potent CYP3A inducer which has been known to markedly decrease plasma concentrations of various drugs which are concomitantly administered during treatment. Suboptimal or failed treatment has been shown to arise from interactions with other drugs and foodstuffs that affect the metabolism of rifampicin[13, 14, 15].

Esimone *et al*.[16] has shown that in Nigeria, most patients on antibiotics therapy also chew ginger habitually or because of its traditionally acclaimed anti-infective properties while Adibe in his work showed that the prevalence of concurrent use of herbal and synthetic medicines in Nsukka, Nigeria is still high. In addition, for the successful treatment of pulmonary tuberculosis, drugs need to penetrate complex lung lesions and permeate the mycobacterial cell wall in order to reach their intracellular targets. There is scarce information on the effect of herbs on the distribution of prescription drugs on target tissues. Therefore, this study was designed to investigate the effect of co-administration of ginger on the pharmacokinetic parameters of rifampicin and penetration profile of rifampicin into the lung tissues.

MATERIALS AND METHODS

Drugand plant material: Rifampicin (Sanofi-Aventis Ltd, Lagos Nigeria) and ginger rhizomes were used. Ginger rhizomes used in this study were obtained locally in Nsukka (Southeast, Nigeria) and were authenticated by Department of Pharmacognosy, University of Nigeria, Nsukka and stored in their herbarium.

Animals: Albino rats (190-300 g) of both sexes were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept under close observation for 1 week before the commencements of the experiment to enable them acclimatize. They were housed according to their sex to avoid conception before the commencement of the experiment. Food and water were provided throughout the period of observation and was withdraw 24 h before the commencement of the experiment. The animals were handled according to our institutional ethical standards on the handling and use of experimental animal.

Extraction of ginger: The rhizomes of ginger were washed, peeled, cut into tiny pieces and then air dried for 10 days. They were pulverized with a milling machine (Pascall Engineering Co. Ltd, England). Exactly 1.5 kg of the powder was extracted with 4 L of 90% methanol using cold maceration method for 48 h. The extracts were filtered and concentrated using rotary evaporator and stored in refrigerator until used.

Preparation of drug sample: We suspended 200 mg of rifampicin in 1 mL of 0.1 M HCl solution (pH-1.0) for oral administration to the rats. While ginger was dissolved in ethanol at a desired concentration.

Beer-lambert's plot: The standard solutions of rifampicin was prepared in the concentration of 1-8 ug mL^{-1} and dissolved in 3 mL plasma. The plasma solutions were further diluted with 10% V/V of methanol and determine the absorbance of the samples using UV spectrophotometer (Sp-6-450 UV/V is PyeUnicam). In the method, protein free samples were prepared by mixing each sample of 5 mL plasma with equal volume of 10% V/V of methanol; the samples were centrifuged and filtered[17]. The absorbances of the filtrates collected were determined at 375 nm using spectrophotometer.

Determination of plasma drug concentrations in rats: Fifteen albino rats were divided into three Groups (A-C). The rats were fasted for 12 h before the experiment. Group A received 10 mg kg^{-1} of rifampicin alone orally; Group B and C received 5 and 10 mg kg^{-1} of ginger extract, respectively for 10 days and on day 11, Rifampicin 10 mg kg^{-1} were given to both groups. Blood samples (0.3 mL) were collected from each group at time 0.5, 1, 2, 4, 8, 12 and 24 h time intervals, respectively[17].

Determination of the effects of ginger on rifampicin penetration into the lungs: After the 24 h, following the oral administration of the drug and ginger in each group, all the animals were sacrificed with chloroform and the lungs collected. Blood was also collected from the lung area and their plasma collected after centrifugation. The lungs were rinsed in non-bacteriostatic saline to eliminate contaminating blood; they were later homogenized individually and centrifuged to collect fluid and stored in a refrigerator until assayed.

Analysis of rifampicin: The methods of Wells[18] and Onyishi *et al*.[17] were adopted. Protein free samples were prepared by mixing each sample of 0.5 mL plasma with equal volume of 10% v/v of methanol; the samples were centrifuged and filtered. The absorbencies of the filtrates collected were determined at 375 nm using spectrophotometer (Sp-6-450 UV/Vis PyeUnicam).

Determination of pharmacokinetic parameters: After a single oral administration, different pharmacokinetic parameters were determined using Non-compartmental method as implemented in WinNonLin pharmacokinetic programs (Version 5.0), (Pharsight Corporation, Mountain View California).

Statistical analysis: Results were expressed as mean ± Standard Deviation (SD) and statistical comparisons were made using the students paired t-test. p-value of 0.05 was considered statistically significant.

RESULTS

The wavelength of maximum absorption of rifampicin in 0.5N NaOH was 375 nm Beer-Lambert's regression equation:

$$Y = 0.0303x+0.1286$$

$$R^2 = 0.9477$$

Comparison of mean plasma rifampicin concentration (μg mL^{-1}) at different time intervals following oral administration of rifampicin alone (10 mg kg^{-1}) and in the presence of 5 and 10 mg kg^{-1} ginger extract: Maximum plasma concentration (C_{max}) for rifampicin

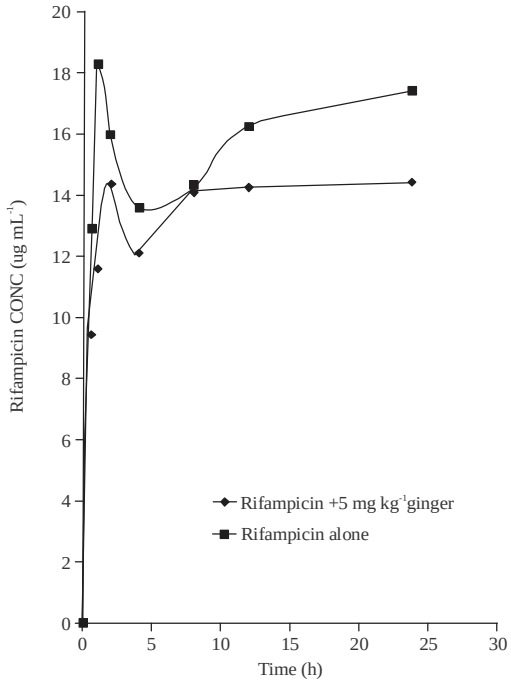

Fig. 1: Concentration-time graphof the effect of ginger (5 mg kg^{-1}) on rifampicin (10 mg kg^{-1}) plasma concentration in rats following a single oral administration of rifampicin

alone (18.24±1.38 μL mL^{-1}) was achieved after 1 h while C_{max} of 14.34±1.27 and 16.27±1.68 μL mL^{-1} were attained after 2 h for rifampicin+5 mg kg^{-1} ginger and rifampicin plus 10 mg kg^{-1} ginger, respectively. The concentrations of 16.80±0.80, 13.57±0.45, 14.29±0.42, 16.26±0.33 and 17.30±1.29 μL mL^{-1} were obtained at time 2, 4, 8, 12 and 24 h for rifampicin alone, respectively, while 12.20±1.24, 14.06±0.62, 14.28±1.27 and 14.43±1.27 μL mL^{-1} were obtained for rifampicin +5 mg kg^{-1} ginger and 13.77±0.64, 14.87±0.82, 15.53± 1.13 and 16.03±1.15 μL mL^{-1} were also obtained for rifampicin plus 10 mg kg^{-1} ginger for time 4, 8, 12 and 24 h, respectively. There was a significant difference ($p<0.05$) between the plasma concentrations obtained at different time interval for rifampicin alone compared to rifampicin with 5 mg kg^{-1} dose of ginger extract while that obtained from 10 mg kg^{-1} was not statistically significant. Figure 1 and 2 display the graphical representations of the different concentrations obtained.

Effects of 5 mg kg^{-1} ginger on pharmacokinetics parameters of rifampicin: The plasma concentration-time curve of rifampicin was used to estimate the pharmacokinetic parameters. The pretreatment with 5 mg kg^{-1} of ginger resulted in a significant decrease in rifampicin C_{max} ($p<0.05$) from 18.60±0.42 to 15.35±

0.38 µL mL^{-1} with no significant ($p>0.05$) difference in time to reach the maximum concentration (T_{max}) when compared with the group that received rifampicin alone. Pretreatment with the same concentration of ginger also resulted in significant decrease ($p<0.05$) in Area Under Curve (AUC) from 377.23±2.77 to 327.04±4.79 µL mL h^{-1} and half-life ($t_{½}$) from 9.53±2.80 to 2.63±3.97 h while there was significant increase ($p<0.05$) in clearance (C_L) and last measurable drug plasma concentration (C_{Last}) ($p<0.05$). The Area Under Moment Curve (AUMC), volume of distribution (V_d) and Mean Resident Time (MRT) showed no significant difference ($p>0.05$). The percentage difference and pharmacokinetic parameters obtained with 5 mg kg^{-1} ginger are shown in Table 1.

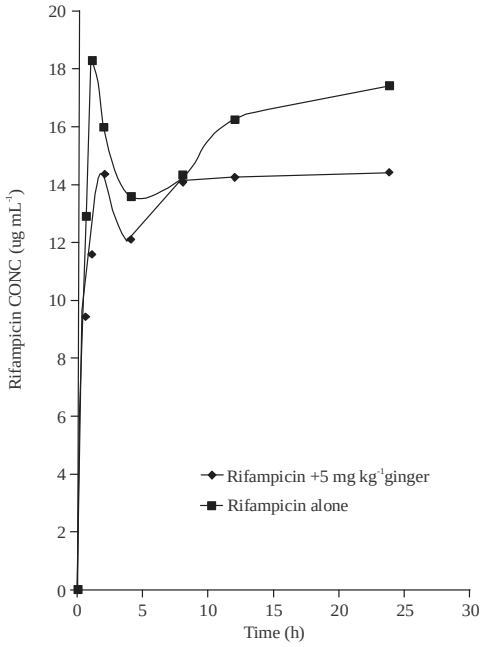

Fig. 2: Concentration-time graph of the effect of ginger (10 mg kg^{-1}) on rifampicin (10 mg kg^{-1}) plasma concentration in rats following a single oral administration of rifampicin

Effect of 10 mg kg^{-1} of Ginger on Pharmacokinetics parameters of rifampicin: Pretreatment with 10 mg kg^{-1} of ginger resulted in a non-significant decrease ($p>0.05$) in rifampicin C_{max} from 18.60±0.42-17.15±0.25 µg mL^{-1} and AUC from 377.23±2.77-360.13±2.93 µg mL h^{-1}. There was no significant difference ($p>0.05$) in T_{max} (5.60±4.60 h) and V_d (0.63±0.04 mL kg^{-1}) of rifampicin alone and T_{max} (6.00±2.45 h) and V_d (0.64±0.06 mL kg^{-1}) obtained with 10 mg kg^{-1} ginger. There was no significant difference between the AUMC when rifampicin was administered alone (4739±71.56 µg mL h) and that obtained when in 10 mg kg^{-1} ginger pretreated group (4473±64.02 µg mL h^{-1}). In the same way, MRT in the presence and absence of 10 mg kg^{-1} ginger (12.28±0.13 and 12.57±0.12 h, respectively) showed no significant difference. However, $t_{½}$ and C_L showed a significant difference ($p<0.05$) in the presence and absence of 10 mg kg ginger. The pharmacokinetic parameters of rifampicin following 10 mg kg^{-1} ginger pretreatment in albino rats are presented in Table 1.

Effect of ginger on rifampicin penetration into the lung: Analysis of the result showed that the concentration of rifampicin when administered alone was 14.63±4.06 µL mL^{-1} while when rifampicin plus 5 mg kg^{-1} was administered, the concentration of rifampicin in the lung decreased significantly ($p<0.05$) to 9.99±4.06 µL mL. When the dose of ginger was doubled to 10 mg kg^{-1} a non-significant decrease in the concentration of rifampicin from 9.99±4.06-9.14±0.97 µL mL^{-1} was obtained. A graphical representation of the effect of ginger on rifampicin penetration into the lung is shown in Fig. 3.

Table 1: Pharmacokinetic parameters of rifampicin (10 mg kg^{-1}) administered orally alone and rifampicin plus 5 and 10 mg kg^{-1} of Ginger in rats. (Mean±SEM) (n = 5)

Parameters	RIF alone	RIF+5 mg kg^{-1} GIN (% change in parenthesis)	RIF +10 mg kg^{-1} GIN (% change in parenthesis)
T_{max} (h)	5.60±4.60	10.80±3.66(93)*	6.00±2.45(7)
C_{max}(µg mL^{-1})	18.60±0.42	15.35±0.38(-18)*	17.15±0.25(-8)
C_{last} (µg mL^{-1})	17.30±0.51	13.51±0.38(-22)*	15.83±0.59(-8)
AUC (µg mL h^{-1})	377.23±2.77	327.04±4.79(-13)*	360.13±2.93(-5)
$t_{½}$ (h)	9.53±2.80	2.63±3.97(-72)*	3.86±4.90(-59)*
V_d (mL kg^{-1})	0.63±0.04	0.69±0.08(9)	0.64±0.06(2)
C_L (mL kg h^{-1})	0.009±0.02	0.006±0.02(-33)*	0.002±0.02(-77)*
AUMC (µg mL h^{-2})	4739±71.56	10471±74.47(120)	4473±64.02(-6)
MRT (h)	12.57±0.12	12.28±0.13(-2)	12.42±0.11(-1)

SEM is standard error of mean; n is number of animal per group, *$p<0.05$ significantly different compared to the parameter of RIF alone; The negative sign implies decrease in parameter measured; RIF means Rifampicin; GIN means Ginger; T_{max} is time taken for drugs to attain maximal plasma concentration; $C_{max}$is maximal drug plasma concentration; C_{last} is the measurable drug plasma concentration; AUC is area under concentration time curve from the time of dosing to the time of the last observation; AUMC is area under moment curve from the time of dosing to the time of last measurable concentration MRT is mean residence time $t_{½}$ is terminal half-life; V_d is volume of distribution based on the terminal phase; C_L is total body clearance

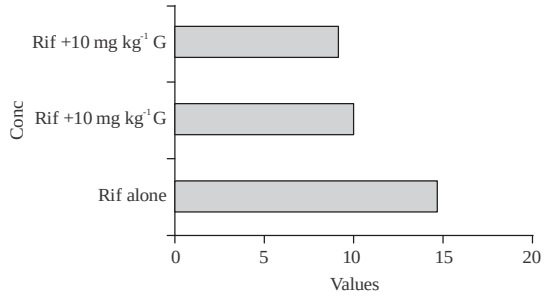

Fig. 3: Effects of ginger extracts on the rifampicin penetration into the lungs. RIF means Rifampicin, G means Ginger, Conc means Concentration

DISCUSSION

Drug interaction is one critical factor in determining the best drug or dose for individual patient while evaluation of the concurrent use of herbal therapies among patients receiving prescription drugs is of great importance because of the potential for herb-drug interaction and the level of reliance patients have on herbal drugs as natural medicines. Though ginger has been found to be very effective in the management of respiratory problems with few negative side effects if consumed in reasonable quantities[19] and is on the Food and Drug Agency's list "generally recognized as safe" list it, however, interacts with some medications when administered con currently[20].

Concomitant administration of rifampicin and ginger produced some changes in pharmacokinetic profile of rifampicin. From our results,concurrent administration of 5 mg kg^{-1} ginger and rifampicin significantly ($p<0.05$) decreased the C_{max}, AUC, C_L and $t_{1/2}$ of rifampicin. The decrease in C_{max} and AUC implies a decreasein the absorption of rifampicin, since, C_{max} and AUC are variables used to measure the extent of absorption. Inaddition, AUC reflects the actual body exposure to the drug after administration of a dose of a drug, hence, decrease in AUC also imply decreased body exposure to drug with a consequent reduction in rate and extent of absorption. Decreased C_{max} and AUC observed in this study is not in agreement with previous works[5, 6] that suggests that ginger enhances the bioavailability of some prescription drugs. Even though the mechanism of action behind this observation is not known, a possible mechanism might be as a result of upregulation of hepatic cytochrome P450 enzymes by rifampicin which is the most powerful known inducer of the hepaticcytochrome P450 enzyme system, including is oenzymes CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, CYP3A5 and CYP3A7, increasing the rate of metabolism of many other drugs that are cleared by the liver through these enzymes. Hence, it may have enhanced the metabolism of ginger thereby preventing it from eliciting its acclaimed bioenhancing effect. Furthermore, rifampicin also causes its own metabolism into inactive metabolite a consequent decrease in its plasma concentrations through the same process[13, 21].

Ginger (5 mg kg^{-1}) increased the T_{max} of rifampicin by almost twice which is significantly different at $p<0.05$ from that gotten with drug alone. The increase observed is indicative that ginger delayed the time for achieving rifampicin maximum concentration. The implication of this result is that it will be difficult to achieve an immediate therapeutic effect in a patient taking rifampicin and ginger together. There was no appreciable change in the volume of distribution (V_d) and Mean Resident Time (MRT) of rifampicin.

When the dose of ginger was increased to 10 mg kg^{-1} a lesser activity was observed compared to the lower dose. There was a non-statistically significant decrease ($p>0.05$) in $C_{max,}$ AUC and a statistically significant decrease ($p<0.05$) in the $t_{1/2}$ and C_L obtained compared to the corresponding values for rifampicin alone.In pharmacokinetic study, the maintenance dose-rate is determined by the clearance while the uctuations within a dosing interval are determined by the half-life. The significant change in $t_{1/2}$ and C_L suggests that there should be adjustment in the dosage of rifampicin for maximum therapeutic efficacy.

As was obtained with 5 mg kg^{-1} ginger, there was no much difference in the V_d and MRT obtained with 10 mg kg^{-1} compared to drug alone. The effect of ginger on the pharmacokinetic parameters of rifampicin was not dose dependent as 5 mg kg^{-1} ginger elicited more significant changes in the pharmacokinetic parameters.

For the successful treatment of pulmonary tuberculosis, drugs need to penetrate complex lung lesions and permeate the mycobacterial cell wall in order to reach their intracellular targets. However, most currently used anti-tuberculosis drugs were introduced into clinical use without considering the pharmacokinetic and pharmacodynamic properties that influence drug distribution and this has contributed to the long duration and limited success of current therapies. Thus, in this study, the effect of ginger on the penetration ability of rifampicin was investigated.

It was observed that concentrations of rifampicin alone and rifampicin+ginger were higher in plasma than in the lung. This concurs with previously published studies which had indicated that drug concentrations in these remote target sites can be substantially lower than plasma concentrations and could also be different for different drugs[22, 23]. When both 5 and 10 mg kg^{-1} ginger were administered concurrently with rifampicin, the concentration of rifampicin obtained in the

lungs reduced significantly at $p<0.05$ compared to that obtained with drug alone. This indicated that ginger hinders the smooth penetration of rifampicin into the lungs.

The alteration in the pharmacokinetic parameters of rifampicin by gingeris quite significant, hence, their use requires therapeutic caution as it could lead to rifampicin failure in critical circumstances like lung diseases.

CONCLUSION

Our work has demonstrated that ginger causes significant change in the pharmacokinetic parameters of rifampicin which is evident by decrease in bioavailability of rifampicin both in the plasma and in the lung. The interaction between the two substances reduces the penetration of rifampicin into the lung. By implication concurrent administration of ginger and rifampicin will alter the therapeutic efficacy of rifampicin resulting in failure to achieve therapeutic goal. Therefore, patients are advised against concurrent intake of both substance, however, if there arise a need for both to be administered together, there should be dosage adjustment in duration, frequency and concentration of rifampicin administration.

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