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Effect of Coconut Milk on Bioavailability of Isoniazide

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Abstract : Isoniazide is an essential component of all anti-tubercular regimens. Carbohydrates reduce absorption of isoniazide. *Cocus nucifera* (Palmae) is a widely grown plant in coastal India, it is rich in carbohydrate, fat, electrolyte specially potassium. Coconut is a cheap and widely available fruit for diarrhoeal dehydration and malnutrition. South East Asia specially India accounts about 2.1 million cases of tuberculosis. As tuberculosis is more prevalent in lower economic and malnutrited people to whom coconut milk is a cheap source of nutrition. The present study was undertaken to show the effect of coconut milk on simultaneous administration with isoniazide. Concurrent administration of coconut milk with isoniazide shows highly significant reduction in C_{max}, T_{max}, AUC (Area under the curve) and AUMC (Area under the first moment curve). Biological half life was increased on simultaneous administration of coconut milk with isoniazide. Results show administration of coconut milk 30 minutes after and before isoniazide reduces the bioavailability up to 63.87 and 80.51%. The study reveals that high carbohydrate and fat containing coconut milk reduces rate and extent of absorption as well as bioavailability of isoniazide.

Keyword : Isoniazide; Coconut milk; Carbohydrate; Bioavailability.

Introduction

The World Health Organization (WHO) in 1993 declared a global emergency against tuberculosis (TB). Despite that call for action and government programs to slow its spread, TB continues to kill millions of people annually. Isoniazide introduced in 1952 is even now the most effective treatment of tuberculosis. Isoniazide is a drug par excellence and an essential component of all anti-tubercular regimens. Isoniazide is completely absorbed orally and penetrates all body tissues, tubercular cavities, placenta and meninges. It is extensively metabolized in liver, most important pathway being acetylation and metabolites are excreted in urine (Barar, 2000).

Presence of food and other substances in the gut may delay absorption of drug by binding or diluting drug, further, food delays gastric

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emptying and thereby delays absorption. Modification of bioavailability of drugs by concurrent administration of food is of clinical significance. Interaction of food with drug alters different steps at the pharmacokinetic level with possible interaction in absorption, distribution, metabolism and excretion, and at the pharmacodynamic level by alteration in the action of the drug. It is necessary to make special recommendations about the time interval between drug and food intake to ensure optimum therapeutic efficacy (Ekmekcoglu, 2000). The most important clinical significance in the history of drug-food interactions occurred in the fifties, the vitamin B_6 deficiency when administering the tuberculostatic isoniazide (Cardona, 2000).

Cocus nucifera, coconut tree (Palmae) is a tropical plant, which grows widely in the coastal region of South India. The aqueous content inside the hard nut of coconut fruit is called coconut water or milk. Coconut water mainly contains carbohydrate, fat, little quantity of protein, electrolyte and vitamin C **(Chuah and Yeoh, 1992; Adams and Bratt, 1992).** Coconut water is a good source of nutrition before and after **diarrhea.** It can also break the cycle of malnutrition and **diarrhea** as it is a good source of potassium (Monaco *et al*., 1982). Coconut water is also reported to have cardiotoxic effect due to high concentration of potassium (Kumar *et al*., 1975).

An estimated, 20 million people are sick worldwide with full-blown TB. More than 95% of TB cases reported in 1990 were in the developing countries, with estimated two thirds in Asia, India accounted for 2.1 million cases (Platt, 1994). Women are at increased risk of progression to disease during their reproductive years. However, in most low-income countries, twice as many men are notified with tuberculosis as women (Connolly and Nunn, 1996). The patients are largely those who are in absolute poverty, socially marginalized, inerant laborers, poorly integrated in the city (Singh *et al*., 2002). Coconut water is a cheap and hugely available food supplement for dehydration therapy and malnutrition (Guthrie *et al*., 1990; Jabre, 1981). However, no detailed reports were found in the Indian context regarding effect of high carbohydrate containing coconut milk on concurrent administration with isoniazide.

The USP XXIII (United States Pharmacopoeia) takes cognizance of the effect of food and in the guide lines set for conducting single dose study on modified release dosage forms, that it should be tested both in fasting condition and concomitantly after administration of a high fat meal having potential for causing maximum perturbation. The objective of such studies as mentioned in USP XXIII are to determine whether there is a need for labeling instructions describing special conditions for administration of a particular drug with respect to meal and secondarily to provide information concerning the pattern of absorption. Hence, the present study was taken up with a view to build up a database regarding high carbohydrate and fat containing coconut milk on co-administration of isoniazide.

Methods and Materials

Drug products and conditions

Isoniazide used for the study was a gift sample from M/s Ipca Laboratories Pvt. Ltd., Ratlam (India). A dose of 50 mg/kg was selected for the study (Singh *et al*., 2002). The drug was given orally to the animals in distilled water.

Preparation of coconut milk

Mature coconut fruits were collected from local market and botanical identity confirmed. Fibrous mesocarp was removed to expose the hard nut, carefully cut open at one end to collect the water content within the endosperm. The endosperm was separately cut into small pieces, mechanically grinded along with coconut water twice in a mixer. The milk then strained with muslin cloth, finally first and second filtrate mixed to get freshly prepared coconut milk. Dose chosen was 25 ml, P.O (*per oral*) irrespective of body weight.

Animals

The study was carried out in 18 healthy laboratory bred New Zealand male rabbits (1.3-1.8 kg), maintained under standard laboratory conditions at 22± 2°C, relative humidity 50±10% and photoperiod (12-h dark and light). Animals were housed in stainless steel metabolic cages and provided with commercial pellet diet (Hindustan Lever Ltd., India) and water was provided *ad libitum.* Animals were fasted over night before the experiment allowing

free access to drinking water. Necessary approval was obtained from Institutional Animal Ethical Committee for conducting this study.

Study design :

Animals were divided into three groups of 6 rabbits in each. .Isoniazide was administered at a dose of 50 mg/kg P.O. (*per oral*). Animals of group I received isoniazide under fasted condition. Animals of group II receives drug 30 minutes after administering coconut milk and group III animals were given isoniazide 30 minutes before coconut milk administration. A cross over design was utilized in the study between Group II and III followed by a wash out period of one week.

Sampling and study procedure :

Blood samples were collected from ear vein of rabbits into heparinized glass tubes. Blood sampling was carried out at 0, 15, 30, 45, 60, 120, 180, 240, 300, 420, 540 and 720 minutes, respectively. Plasma was separated immediately by centrifugation at 3000 RPM for 10 minutes using a cooling centrifuge (4° C; Remi, India). The clear supernatant (plasma) was stored in different labeled eppendorf tubes at -20° C until the time of analysis. A wash out period of two weeks was allowed between the two cross over study periods. Plasma concentration of isoniazide was estimated by reported UV spectrophotometric method with slight modification (Gowda *et al*., 2002).

Data analysis :

Plasma concentration time data for isoniazide was generated by assuming first order absorption and one compartment model with first order elimination. The maximum plasma concentration (C_{max}) and time of its occurrence (T_{max}) was computed directly from the plasma concentration Vs time plot. The elimination rate constant was calculated as Kel = 2.303 × slope from the terminal phase of the log plasma concentration Vs time profile by least square regression analysis. The elimination half life estimated as $t_{1/2}$ = 0.693/Kel. The area under the plasma concentration time curve from 0-t (270 mins) (AUC_{0-t}), from 0-∞ ($AUC_{0-}\infty$), area under first moment curve from 0-t (270 mins) ($AUMC_{0-t}$), from 0-∞ ($AUMC_{0-}\infty$) and mean residence time (MRT) was calculated using trapezoidal rule (Gibaldi *et al*., 1982). All the pharmacokinetic parameters were subjected to statistical analysis using

ANOVA (Analysis of variance) at 95% confidence level and the pooled t-test using computer programme in **BASICS.** Relative availability is calculated (Monaco *et al*., 1982) using the formula, Relative availability = AUC test × 100/ AUC reference. Here reference is the bioavailability of the isoniazide alone group.

Results :

The mean plasma concentration of isoniazide with standard deviation obtained at various time intervals on administration of single oral dose of 50 mg/kg under different conditions are graphically presented in Figure 1. Mean pharmacokinetic parameters of isoniazide are summarized in table I.

Table I. Mean plasma concentration of isoniazide achieved under various conditions of drug administration

Sl. No.	Time in minutes	Mean plasma concentration of isoniazide (µg/ml)		
		Fasting condition	30 min after coconut milk	30 min before coconut milk
1	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2	15	0.054 ± 0.002	0.020 ± 0.002	0.057 ± 0.008
3	30	0.672 ± 0.005	0.069 ± 0.003	0.814 ± 0.002
4	45	1.860 ± 0.013	0.385 ± 0.008	1.552±0.030
5	60	3.054 ± 0.042	1.455 ± 0.085	1.950 ± 0.080
6	120	4.691 ± 0.055	2.615 ± 0.020	2.837 ± 0.056
7	180	5.052 ± 0.091	3.285 ± 0.060	3.765 ± 0.074
8	240	3.971 ± 0.063	3.621 ± 0.075	4.110 ± 0.036
9	300	3.185 ± 0.070	2.510 ± 0.082	3.221± 0.058
10	420	2.121 ± 0.082	2.042 ± 0.047	2.342 ± 0.041
11	540	1.255 ± 0.067	0.905 ± 0.055	1.188 ± 0.091
12	720	0.820 ± 0.035	0.413 ± 0.032	1.004 ± 0.072

Table II. Mean pharmacokinetic parameters of isoniazide achieved under various conditions of drug administration

Sl. No.	Parameters	Fasting condition	30 min after coconut milk	30 min before coconut milk	ANOVA
1	Peak plasma conc (C_{max}) µg/ml	5.062 ± 0.04	3.654 ± 0.01***	4.225 ± 0.02***	716.45
2	Time required to reach C_{max} (T_{max}) min	178.50 ± 2.53	273.42 ± 3.34***	239.62 ± 2.76***	275.84
3	Elimination rate constant (kel)	0.0062 ± 0.003	0.0108 ± 0.007	0.0084 ± 0.002	Ns
4	Biological half life ($t_{1/2}$) min	111.77 ± 3.21	64.17 ± 1.98***	82.50 ± 2.06***	93.634
5	AUC_{0-t} µg/ml/min	1999.09 ± 11.53	1276.88 ± 28.91***	1609.51 ± 16.73***	313.95
6	$AUC_{0-\infty}$ µg/ml/min	2131.34 ± 5.76	1315.12 ± 10.82***	1729.03 ± 12.14***	1678.90
7	$AUMC_{0-t}$ µg/ml/min^2	618962.84 ± 371.51	429434.54 ± 254.04***	550958.62 ± 347.30***	85579.00
8	$AUMC_{0-\infty}$ µg/ml/min^2	714188.64 ± 587.92	456967.87 ± 762.78***	637015.76 ± 539.72***	42885.00
9	MRT_{0-t} min	306.62 ± 5.36	336.32 ± 7.11*	342.31 ± 7.83**	7.795
10	$MRT_{0-\infty}$ min	335.08 ± 6.25	347.47 ± 8.59^{ns}	368.42 ± 7.66*	4.967
11	Relative bioavailability %	—	63.87	80.51	—

Data are expressed as mean ± S.D.

ns - Non significant

* - $p < 0.05$

** - $p < 0.01$

*** - $p < 0.001$

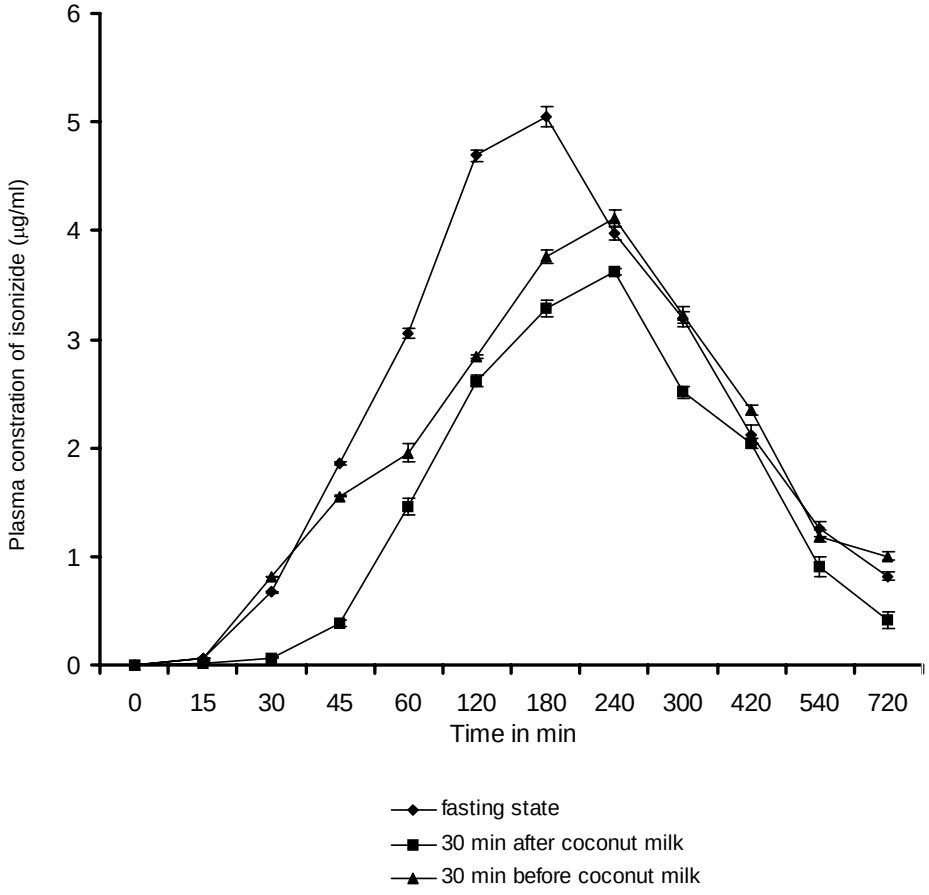

Fig. 1 : Mean plasma concentration of isoniazide (µg/ml) achieved on administration of drug under various conditions

***Rate of Absorption* :**

Isoniazide is racially absorbed in the body after administration on fasting condition, evident from mean value of T_{max}, 178.50 ± 2.53 minutes. T_{max} value was found to be higher, 273.42 ± 3.34 minutes ($p<0.001$) when administered 30 minute after coconut milk. Similarly administration of drug 30 minute before coconut milk has shifted T_{max} value to 239.62 ± 2.76 minutes ($p<0.001$). The mean peak plasma concentration of isoniazide under fasting condition was found to be 5.062 ± 0.04 µgm/ml in comparison to

3.654 ± 0.01 and 4.225 ± 0.02 obtained after administration of drug 30 minutes after and before coconut milk, respectively, indicating highly significant decrease ($p<0.001$).

***Extent of absorption* :**

The mean AUC_{0-t} (Area under the curve) values from 0 to 720 minutes were 1999.09 ± 11.53, 1276.88 ± 28.91 and 1609.51 ± 16.73 μgm/ml/min after administration of drug in fasting condition, 30 minutes after and before coconut milk respectively. The mean $AUC_{0-}\infty$ (*Area under the curve*) were 2131.34 ± 5.76, 1315.12 ± 10.82 and 1729.03 ± 12.14, respectively for three different conditions. Comparison of fasting state mean AUC_{0-t} and $AUC_{0-}\infty$ with that of 30 minutes after and before coconut milk shows highly significant difference, decrease which is more pronounced with 30 minutes after coconut milk. Similar types of results were obtained for area under first moment curve $AUMC_{0-t}$ (Area under the first moment curve) and $AUMC_{0-}\infty$ (Area under the first moment curve). Biological half-life of isoniazide shows a highly significant decrease when administered with coconut milk 111.77 ± 3.21 to 64.17 ± 1.98 and 82.50 ± 2.06, respectively. Inter treatment variation in elimination rate constant are not significant statistically. The percentage bioavailability of isoniazide for 30 minutes after and before coconut milk were 63.87 and 80.51, respectively, obtained on comparing with fasting state.

Discussion

Isoniazide is rapidly absorbed on fasting condition, but presence of coconut milk effects the rate and extent of absorption. Administration of isoniazide both 30 minutes before and after coconut milk significantly increases T_{max} and decreases C_{max}. Area under the curve and first moment curve were also significantly decreased with concurrent administration of drug and coconut milk. Isoniazide administration 30 minutes after coconut milk has reduced bioavailability as compared to 30 minutes before. This study explores interaction of coconut milk with isoniazide as coconut milk significantly decreases bioavailability of isoniazide. Interaction of foods with the antimicrobial agents mostly occurs at the level of absorption causing an increase, a decrease or a delay in the bioavailability. All foods, but especially carbohydrates reduce the absorption of isoniazide (Fraga *et al*.,

1997). Coconut milk not only delays the absorption of isoniazide, but also affect the amount absorbed. Coconut milk contains 62-70 % of carbohydrate and 58-61 % of fat. High carbohydrate and fat content may be responsible for decreased absorption of isoniazide in presence of coconut milk. This study signifies that isoniazide should not be taken concurrently with carbohydrate or fat rich food to avoid interaction which may destabilize maintenance of adequate plasma concentration needed for clinical efficacy.

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