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RESEARCH ARTICLE

Effect of Honey on CYP3A4 Enzyme and P-Glycoprotein Activity in Healthy Human Volunteers

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ABSTRACT

The activity of cytochrome p450 isozyme 3A4 (CYP3A4) enzyme and P-glycoprotein (P-gp) is modulated by grapefruit juice and herbal drugs. CYP3A4 is the major phase I drug metabolizing enzyme and P-gp is an ATP-dependent drug efflux pump that regulates the intestinal absorption of orally administered drugs. Honey is commonly consumed as a dietary supplement. However, its influence on human CYP3A4 and P-gp activity is not yet well documented. Therefore, we investigated the influence of a 10-day honey administration on CYP3A4 and P-gp activity in healthy volunteers using carbamazepine and digoxin as their probe drugs respectively. A within-group pharmacokinetic study was done in 12 healthy volunteers. They were administered single oral dose of carbamazepine (200 mg) and digoxin (0.5 mg) before and after 10 days of honey (10 ml twice daily) intake. Blood samples (5ml) were collected at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 4, 8, 12, 24, 48 and 72 h after drug administration. Concentration of carbamazepine and digoxin in plasma was measured by HPLC and RIA method respectively. Ten days of honey administration did not significantly alter the C_{max}, T_{max} and $AUC_{(0-t)}$ of carbamazepine (probe drug for CYP3A4) and digoxin (probe drug for P-gp). Our results suggest that honey may not significantly modulate the CYP3A4 enzyme and P-glycoprotein activity. The coadministration of honey with drugs may not result in significant drug interactions.

Keywords: *Honey, CYP3A4, P-glycoprotein, carbamazepine, digoxin*

Honey is a natural saccharine product made by honeybees from the nectar of flowers [1]. Being a natural source of fructose and glucose with some oligosaccharides, proteins, vitamins and minerals, honey has become a dietary supplement for healthy individuals [2]. Honey is also consumed by many patients with diabetes, hypertension and epilepsy who receive drugs for their ailments. This increases the possibility of honey-drug interaction. Most of the herb-drug interactions occur at the level of metabolism and drug transport mediated by CYP 450 group of drug metabolizing enzymes and P-glycoprotein (P-gp) respectively [3].

Among the CYP group of drug metabolizing enzymes, CYP3A4 is the major phase I drug metabolizing enzyme. It is present in the liver, jejunum, colon and pancreas. It has broad substrate specificity and is responsible for metabolism of more than 50% of administered drugs [4]. There are few studies showing the effect of honey on CYP3A4. Animal studies have shown that multiple doses of honey induced CYP3A4 activity [5,6]. In a study done in humans, single oral dose of honey failed to show any significant effect on CYP3A4 [7]. The effect of multiple doses of honey on CYP3A4 in humans has not been reported to date. It has been well documented that the CYP3A4 enzyme is involved in the metabolism and elimination of carbamazepine [8]. The pharmacokinetics of carbamazepine is influenced by alterations in the catalytic activity of CYP3A4 [9]. Hence, carbamazepine is used as a probe drug for assessing the CYP3A4 enzyme activity in our study.

P-glycoprotein (P-gp) is an ATP dependent drug efflux pump. It plays an important role as a secretory system in the intestinal barrier and regulates the intestinal absorption of orally administered drugs [10]. Many clinically important drugs viz., digoxin, losartan, erythromycin and rifampin are substrates for P-gp. Some of them besides being a substrate also induce or inhibit the P-gp activity. Drugs like fexofenadine, digoxin and loperamide are used as probe drugs to assess P-gp activity [11]. Among them, digoxin is most commonly used [12]. The effect of various dietary derivatives and herbal

Table 1. Pharmacokinetic parameters of carbamazepine (200 mg single oral dose) before and after 10 days of honey administration

Pharmacokinetic parameters	Before honey	After honey
C_{max} (µg.ml $^{-1}$)	4.1 ± 0.28	4.2 ± 0.31
T_{max} (h)	10.1 ± 1.60	9.0 ± 0.90
$AUC_{(0-72)}$ (µg.h.ml $^{-1}$)	203.1 ± 15.30	208.2 ± 17.20

Values are shown as mean ± SEM. (n =12)

products on the P-gp activity has also been studied. In an *in vitro* study using various fruit extracts, it was found that extracts of strawberry, orange, apricot and mint inhibited the intestinal P-gp [13]. In another *in vitro* study using rat small intestine, extracts of grapefruit juice and orange juice inhibited the transport activity of P-gp [14]. In a study done in humans, grapefruit juice had no effect on P-gp activity [15]. Another human study revealed that St. John's Wort, an herbal product induced P-gp activity [16]. This shows that P-gp is a potential target for drug interactions exhibited by herbal compounds. The effect of honey on P-gp activity has not been studied so far.

Since we wanted to know whether honey, a natural dietary supplement, will interact with concomitantly administered drugs, we investigated the effect of multi dose administration of honey on CYP3A4 and P-gp activity in humans using carbamazepine and digoxin as the probe drugs respectively. Carbamazepine is a CYP3A4 substrate but it is not a substrate for P-gp [17]. On the other hand, digoxin is a substrate for P-gp only and not a substrate for CYP3A4 [18]. Hence any change in the pharmacokinetic profile of carbamazepine and digoxin due to honey administration may reflect the change in the activity of CYP3A4 and P-gp respectively.

MATERIALS AND METHODS

A within group pharmacokinetic study was done in 12 healthy male volunteers (Age 20-45 years). The mean age of the volunteers was 27.4 ± 1.96 yrs (mean ± SEM) and their mean body mass index was 23.2 ± 0.94 Kg/m^2 (mean ±SEM). The study was approved by institutional ethics committee. A written informed consent was taken from all the volunteers. The health of the volunteers was assessed by doing a thorough physical examination and by performing ECG, liver and kidney function tests. Volunteers suffering from chronic diseases or taking concomitant medications were excluded from the study. Similarly, regular users of alcohol and/or tobacco, those with history of vomiting after digoxin intake, seizures and drug allergy were also excluded.

Study design

On day 1, single oral dose of 200 mg carbamazepine (Tegrital, Novartis [India] Limited) and 0.5 mg digoxin (Lanoxin, Burrough's Wellcome, [India] Limited) were administered to the volunteers at 7 AM who were fasted overnight. They were not allowed to take food for further 2 h. Blood samples were collected from indwelling venous catheter using heparinised disposable syringes just before and at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 4, 8, 12, 24, 48, 72 h after administration of drugs. A standardized breakfast and lunch were given to all the volunteers. From day 5 to day 14, the volunteers were administered 10 ml of honey (Periyakulam Sarwodaya Sangh, Khadi Vastralaya, Theni District, Tamilnadu, South India; Lot No.4/2002) twice daily in empty stomach with 200 ml of water. On day 15, the volunteers were given single oral dose of 200 mg carbamazepine and 0.5 mg digoxin. The blood samples were collected as mentioned before. After separation of the plasma, the samples were stored at −20 ^{0}C till the drug assays were done. The study protocol is shown as a flow chart in Figure 1.

The honey used in the present study was tested for its purity in Public Health Laboratory, Pondicherry, India. It was found to be within PFA (Prevention of food adulteration act-1955, India) values. It was composed of reducing sugar 71.6%, moisture 24%, sucrose 2.4% and ash 0.3%. The fructose/glucose ratio was 0.97%.

Drug assays

Serum carbamazepine concentration was estimated using a HPLC method [19]. The plasma sample (900 µl) and internal standard (900 µl) were taken in a 2 ml micro centrifuge tube. After vortex mixing, 600 µl was transferred to a conical flask, into which 4:1 mixture of chloroform: methanol was added. After mixing in an orbital shaker, the contents of conical flask were transferred to centrifuging tubes. After centrifugation at 2500 rpm for 10 min, the upper protein layer was transferred into evaporating tubes for evaporation at 50 ^{0}C. The dried evaporated samples were reconstituted in 400 µl

Table 2. Pharmacokinetic parameters of digoxin (0.5 mg single oral dose) before and after 10 days of honey administration

Pharmacokinetic parameters	Before honey	After honey
C_{max} (µg.ml $^{-1}$)	2.6 ± 0.22	2.5 ± 0.18
T_{max} (h)	1.5 ± 0.26	1.2 ± 0.14
$AUC_{(0-4)}$ (ng.h.ml $^{-1}$)	6.1 ± 0.44	6.2 ± 0.24
$AUC_{(0-72)}$ (µg.h.ml $^{-1}$)	28.9 ± 8.80	27.6 ± 2.20

Values are shown as mean ± SEM. (n =12)

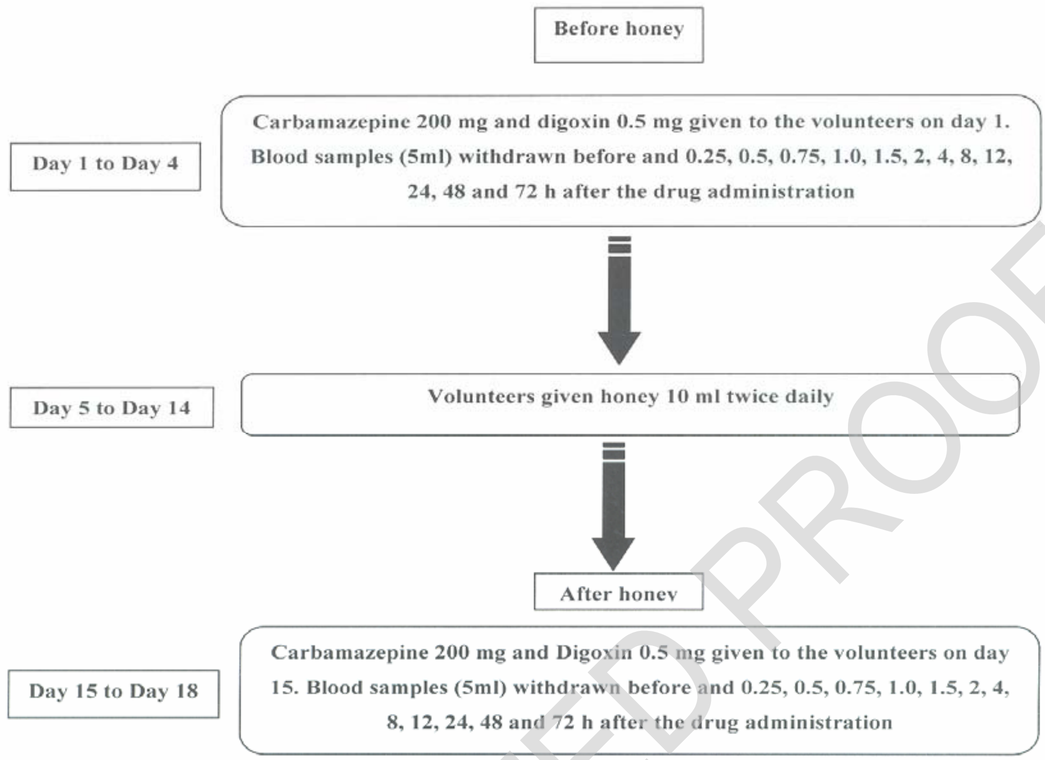

Fig 1. The study plan described as a flow chart.

of mobile phase composed of acetonitrile: methanol: phosphate buffer (12.5:25:62.5, v/v/v) and injected into HPLC. The inter-day coefficient of variation for carbamazepine HPLC assay was less than 7%.

The digoxin concentration in plasma was measured according to the manufacturer's directions, in duplicate using RIA kits (Orion diagnostics, Finland; Lot No. 1588501). Into the appropriate labeled test tubes, 25 µl of calibrators, plasma samples (unknown concentration of digoxin) and 100 µl of antiserum solution were added. All the tubes were mixed on a vortex mixer and then incubated for 1 h at room temperature. One ml of separation reagent was added to all the test tubes and mixed on a vortex mixer. They were centrifuged for 15-20 min at 2000 g. After centrifugation, the supernatant part was decanted and the head of each tube was tapped firmly against absorbent paper. Radioactivity in each tube was counted using gamma counter for 1 min. The measurement range of the kit was 0.5-8.0 nmol/l. The detection limit of the kit was 0.1nmol/l.

Calculation of pharmacokinetic parameters:

The pharmacokinetic analysis was done using model independent formulae. The peak plasma concentration (C_{max}) and the time to reach peak plasma concentration (T_{max}) were read directly from the actual plasma concentration data. The area under the plasma concentration versus time curve [$AUC_{(0-t)}$] was calculated by trapezoidal rule.

Statistical analysis

Pharmacokinetic data was expressed as mean ± SEM. The normality of the data was assessed by the Kolmogorov –Smirnov test. The C_{max}, T_{max} and $AUC_{(0-72)}$ were analysed by paired Student's '*t*' test. All the statistical analyses were carried out by using GraphPad Instat (version 3.05, 2000, San Diego, USA) software system. $p<0.05$ was considered statistically significant.

RESULTS

Effect of honey on carbamazepine pharmacokinetics

The plasma carbamazepine concentration measured up to 72 h was not significantly altered by honey administration (Figure 2). After ten days of honey administration, there was no statistically significant change in the mean values of C_{max}, T_{max} or $AUC_{(0-72)}$ (Table 1).

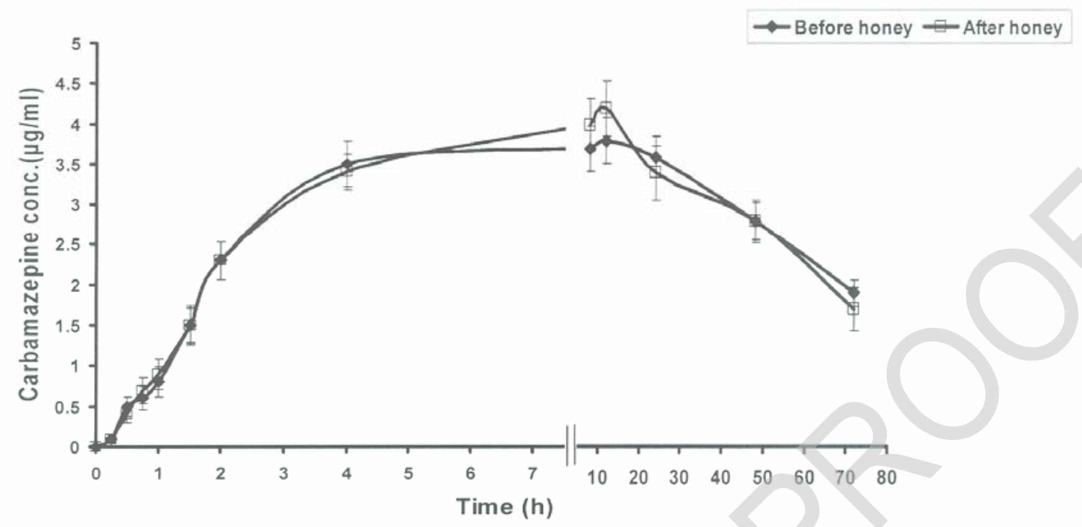

Fig 2. Concentration versus time profile of plasma carbamazepine (AUC $_{0-72}$) before and after honey. Values are shown as mean ± SEM.

Effect of honey on digoxin pharmacokinetics

The plasma digoxin concentrations measured up to 72 h were not significantly altered by honey administration (Figure 3). There was no statistically significant change in the mean values of C_{max}, T_{max}, AUC $_{(0-4)}$ or AUC $_{(0-72)}$ (Table 2).

DISCUSSION

Herbal extracts of garlic [20], grapefruit juice [21], St. John's Wort [22] and milk thistle [23] modulate the activity of CYP3A4 resulting in drug interactions. The extracts of certain herbs used in traditional Chinese medicine like Angelica dahurica [24], Angelica sinensis [25] and Glycyrrhiza glabra [26] modulate the CYP3A4 activity. Herbal extracts of Curcumin [27], hawthorn [28], ginseng [29], green tea [30], milk thistle [31], piperine [32], and grapefruit juice [14], orange juice [14] and St. John's Wort [22] modulate P-gp activity.

Flavonoids present in herbs have been found to interact with CYP3A4 and P-gp [3]. Honey is a natural saccharine product rich in sugars and phytochemicals. The flavonoids present in honey are pinocembrine, pinobanskin, chrysin, galangin, quercetin, luteolin and

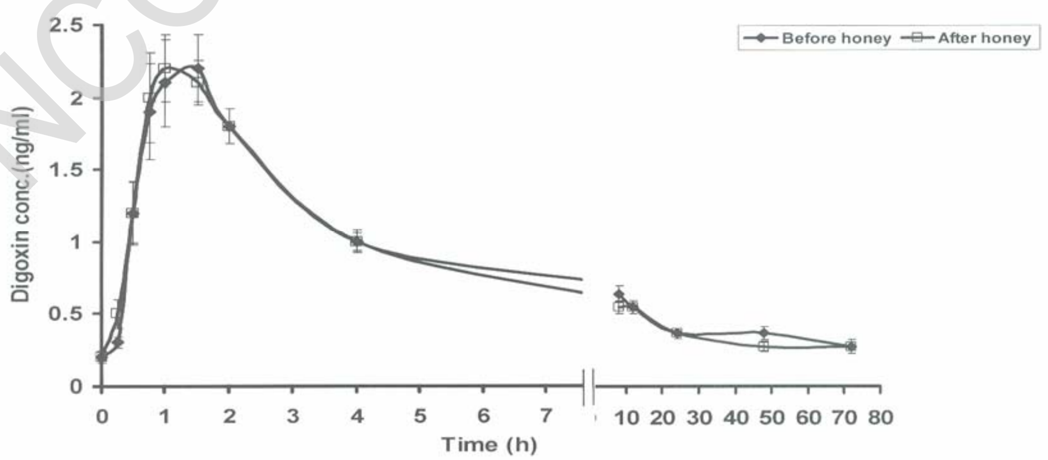

Fig 3. Concentration versus time profile of plasma digoxin (AUC $_{0-72}$) before and after honey. Values are shown as mean ± SEM.

kaempferol [2]. Studies in rabbits have shown that honey induced the metabolism of diltiazem [5] and carbamazepine [6]. In a human study, where the effect of single dose of honey on CYP3A4 was investigated using carbamazepine as a probe drug, honey failed to show statistically significant effect on carbamazepine pharmacokinetic parameters like C_{max}, T_{max} and $AUC_{(0-72)}$ [7]. Hence, we studied the effect of multiple doses of honey on carbamazepine pharmacokinetics. In our study, multiple doses of honey failed to significantly alter the pharmacokinetics of carbamazepine. Hence we assume that flavonoids present in honey may not have any significant effect on human CYP3A4 activity.

Since honey did not change the pharmacokinetics of digoxin, it is assumed that the flavonoids present in honey may not have any significant effect on P-gp also. Becquemont *et al* investigated the effect of grapefruit juice on P-gp activity in 12 healthy volunteers using digoxin as a probe drug. It was found that grapefruit juice did not significantly inhibit the intestinal P-gp activity [15]. Although the C_{max}, T_{max} and $AUC_{(0-48)}$ of digoxin did not change significantly, there was a statistically significant increase in $AUC_{(0-4)}$ of digoxin (i.e. in first 4 h) following co-administration with grapefruit juice. This correlates with observations made by Westphal *et al* that P-gp inhibitors alter the early digoxin pharmacokinetics by interfering with the absorption of digoxin [33]. In our study, 10 days of honey administration did not alter even the early absorption pharmacokinetics (AUC_{0-4}) of digoxin.

Honey and its various derivatives are natural dietary supplements consumed commonly all over the world. Healthy individuals prefer honey to maintain their health and patients with chronic illness take honey along with other medications. Hence the possibility of honey drug interactions cannot be ruled out. Apart from consuming honey as a single dose along with drugs, some patients take honey daily as a nutritional and healthy dietary supplement.

Since, *in vitro* and *in vivo* studies have reported that herbal extracts may modulate CYP3A4 and P-gp activity resulting in various types of herb drug interactions; the safety of coadministration of honey with drugs needs to be studied. This study is an attempt to investigate the same. To the best of our knowledge, this is the first study in humans where the effect of multi dose honey administration on CYP3A4 and P-gp activity has been investigated. Based upon the present study, it can be concluded that honey does not affect the CYP3A4 mediated metabolism and P-gp mediated transport of concomitantly orally administered drugs. The coadministration of multiple doses of honey with drugs may not produce significant drug interactions.

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