Effect of honey on carbamazepine kinetics in rabbits

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The study was undertaken to determine the effect of honey on carbamazepine kinetics in rabbits. The study was done on three occasions in each animal. Study I was carried out after single dose administration of carbamazepine (80 mg/kg, po) along with saline (2.34 ml/kg, po). After a wash out period of one week, the second study was carried out by co-administration of carbamazepine with honey (2.34 ml/kg, po). After this, the animals continued to receive honey (2.34 ml/kg, po) once daily, for 7 days. On the eighth day of honey treatment, the carbamazepine kinetics was studied again. Pharmacokinetic analysis revealed that single as well as multiple dose honey treatment showed a significant decrease in area under the plasma time concentration curve (AUC) when compared with saline treated control. A significant increase in the clearance (CL/F) rate of carbamazepine was observed only after multiple dose honey treatment. Both single and multiple dose honey treatment did not show any significant effect on other pharmacokinetic parameters like $t_{1/2}$, $C_{max}$, $T_{max}$ and $Vd$ when compared with saline treated group. Data thus obtained suggested that honey decreases the bioavailability of carbamazepine.

Carbamazepine is used in the treatment of grand mal epilepsy, trigeminal neuralgia and occasionally used in treating manic-depressive illness. It is metabolized by CYP3A enzyme, which is present in the intestinal wall and in the liver.

In India, from olden days, honey is used to mask the bitterness of the drugs. It is advocated as a rapid source of energy and also as a panacea for various illnesses. It is a saccharine substance made by the hive bee, *Apis mellifera* Linn. (Order Hymenoptera, Family Apidae), and other species of Apis, from nectar of flower. Honey consists of about 70 to 80% mixture of glucose, fructose, small amount of sucrose (1.1 to 4.4%) and dextrin (0.06 to 1.24%) and trace elements like hydrogen peroxide, lysozymes and other organic substances. A small amount of volatile oil, wax and pollen grains are also usually present. Although humans commonly consume honey, its effect on drug metabolism is not studied extensively.

In the present study we have investigated the influence of honey on the pharmacokinetics of carbamazepine in rabbits.

Materials and Methods

Chemicals—Pure reference standard of carbamazepine was obtained from Sigma Chemicals (St. Louis U.S.A) and carbamazepine tablet was obtained from Sarabhai Piramal Pharma Ltd., Gujarat, India. HPLC grade methanol, acetonitrile, chloroform and analytical grade potassium phosphate, phosphoric acid, sodium hydroxide were from S.D Fine Chemicals (India). Double distilled water was used as solvent.

Honey was obtained from Periyakulam Sarvodaya Sangh, Periyakulam, India, and was tested for its purity in Public Health Laboratory, Pondicherry, India. It was within PFA (Prevention of food adulteration act-1955, India) values (moisture 24% , ash 0.3%, sucrose 2.4%, reducing sugar 71.6%, fructose/glucose ratio 0.97).

Animals

Angora grey rabbits (n=6, 2 to 2.5 kg), obtained from Livestock Research Station, Kattupakkam, India, were used in this study. They were housed in individual cages and had free access to food and water.

Experimental design

The pharmacokinetic evaluation of carbamazepine was done on each animal on three occasions.

The first study was done after single dose administration of carbamazepine (80 mg/kg, po) along with saline (2.34 ml/kg, po). After a wash out period of one week, the second study was done with single dose administration of carbamazepine and honey (2.34 ml/kg, po). After the second study, the animals continued to receive honey (2.34 ml/kg, po) once daily for 7 days. On the next day the third pharmacokinetic study was carried out after oral dosage of carbamazepine and honey.

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During each pharmacokinetic study, the animals were fasted overnight and blood samples were collected (approximately 1 ml) into screw capped eppendorf tube from a heparinized 22 G I.V cannula (Mediflon, India) inserted into the marginal ear vein. The blood sampling times were 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 14 and 24 hr after carbamazepine administration. The plasma was separated and stored at \(-20^\circ\text{C}\) until analysis.

Samples and data analysis

The plasma carbamazepine level was estimated by High Performance Liquid Chromatography (HPLC) method\(^8\) with a slight modification. An ODS column was used and the mobile phase was methanol, acetonitrile, phosphate buffer (21:14:65). The flow rate was 1.5 ml/min. Sample detection was performed with an UV-detector (SPD-6AV-Shimadzu, Japan). The concentration of the drug was determined using peak area ratio method. The assay limit of detection was 50 ng/ml. The inter day and intra day co-efficient of variation (CV) at the concentration 5, 10 and 20 \(\mu\text{g/ml}\) were all less than 5% \((n=10)\).

A model independent formula was used to calculate the pharmacokinetic data. The area under the plasma drug concentration (AUC) was calculated by trapezoidal rule. The plasma clearance (CL/F) was calculated from the formula: Dose/AUC where F is the fraction of the drug absorbed. The apparent volume of distribution (Vd/F) was determined using the formula: Dose/AUC \(\times K_d\). The elimination half life \((t_{1/2})\) was calculated using the formula: \(0.693 \times \text{Vd} / \text{CL}\). The \(C_{\text{max}}\) (the maximum plasma concentration reached) and the \(T_{\text{max}}\) (time to reach \(C_{\text{max}}\)) were read directly from the actual plasma data of 6 rabbits.

The differences between the pharmacokinetic parameters of the study were analysed using repeated measure analysis of variance test and the post-test analysis was done by Tukey’s multiple comparison test. \(P<0.05\) was considered to be indicative of significance.

Results

The plasma concentrations of carbamazepine was found to be significantly reduced when carbamazepine was given along with single or multiple doses of honey (Fig. 1).

Administration of honey has significantly reduced the AUC of carbamazepine when compared to saline treatment \((F=10.67, P<0.003)\). The reduction in AUC was more after multiple dose \((P<0.01)\) than single dose \((P<0.05)\) administration of honey (Table 1).

The clearance rate of the drug was significantly increased \((P<0.05)\) by multiple doses administration of honey. Even with single dose administration of honey the CL/F showed 60% increase when compared to

![Fig. 1 — Effect of honey (Hn) on plasma concentration of carbamazepine (CBZ). Each point represents the mean \( \pm \text{SE}. \) \((n=6). \) \(*P<0.05, \) \(**P<0.01\) when compared to CBZ alone.](image-url)
The volume of distribution of carbamazepine was higher after treatment with honey when compared to saline treatment. However this was not statistically significant. The $C_{\text{max}}$, $T_{\text{max}}$ and $t_{1/2}$ were not significantly affected after the administration of honey when compared to saline administration (Table 1).

### Discussion

The major finding of our study is that the oral administration of honey reduced the AUC of carbamazepine. It suggests that the bioavailability of carbamazepine is reduced by single or multiple doses administration of honey. Theoretically, this may be due to increase in the metabolism of carbamazepine or decrease in its absorption or both.

It is known that carbamazepine is mainly metabolized by CYP3A4 enzyme in human. In the rabbit it is metabolized by CYP3A6 enzyme. The rabbit isofom CYP3A6 and the human isofom CYP3A4 have similar P-450 predominance and substrate specificity and both are induced by rifampicin. The CYP3A sub-family of enzymes is present in the intestinal wall and in the liver of the rabbit. Cytochrome P450 enzyme can be induced by many drugs and chemicals. Even single dose administration of phenobarbital, nicotine, zolazepan, talazol and aromatic hydrocarbons like benzene, toluene or m-xylene was shown to increase the content of cytochrome P450 in the liver and gut wall.

Honey consists of mainly fructose and glucose. Fructose is known to modify the cellular lipid metabolism and cause increase in Cytochrome P-450 level. Hence it is possible that honey has induced the CYP3A sub-family of enzymes, which may be responsible for the decrease in AUC. This is supported by the observed increase in CL of carbamazepine after the administration of honey.

The reduction in AUC could also be due to the decrease in the oral absorption of carbamazepine. However the absorption kinetic parameters, viz., $t_{1/2}$ and $C_{\text{max}}$ are not significantly altered. This suggested that the reduction in the plasma levels of carbamazepine might not be due to impaired absorption but due to the increased metabolism. This hypothesis is supported by our unpublished study wherein single oral dose of honey significantly increased the metabolism of diltiazem (also a substrate for CYP3A sub-family of enzymes).

In a pilot study, the effect of repeated withdrawal of blood (3 times) from the same animal was investigated by administering carbamazepine along with saline, following the same experimental protocol of honey study. There was no difference in carbamazepine kinetics between the three studies (data not shown). It suggested that repeated withdrawal of blood from the same rabbit did not alter the pharmacokinetic parameters of carbamazepine.

It is concluded that honey treatment decreases the bioavailability of carbamazepine. This effect may be due to the enzyme induction or due to some other unknown mechanism.

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### References
