Literature Review
Bioavailability represents the quantity of the active component absorbed from the pharmaceutical formulation reaching the blood circulation. It depends on many factors viz., gastrointestinal pH's; gastric emptying, gastrointestinal transit, interaction with food etc. In addition the presystemic elimination of drugs also reduces the quantity of drug reaching the circulation.

### 3.1 Honey

#### 3.1.1 Introduction

Honey is thick, golden in appearance and sweet. Nutritionally, honey is little different from sugar, being basically a concentrated solution of fructose and glucose, with only physiologically insignificant traces of vitamins and minerals.

Honey is a saccharine fluid made by the hive-bee, Apis mellifera Linn., order Hymenoptera, family Apidae, from the nectar of flowers. The best honey is derived from flowers such as clover and heather and separated from the cut comb portion either by draining or by means of a centrifuge. Honey obtained by expression is liable to be contaminated with the wax. In some instances bees collect the other sweet plant juices, such as the honeydew formed from the leaves of trees such as pine, lime and sycamore. The nectar of certain flowers (e.g. species of Eucalyptus or Banksias) may give the honey a somewhat unpleasant odour and taste. Nectar from Datura stramonium is poisonous. The character of honey, when freshly prepared, is a clear, syrupy liquid of a pale yellow or reddish-brown color. On keeping, it tends to crystallize and become opaque and granular. The odour and taste depend on the flowers used in its preparation.
3.1.2 Composition

Honey is a natural mixture of carbohydrates (80% wt/wt), mainly consisting of glucose (27-35%), fructose (37-47%) and sucrose (0.2-2.5%) and water. It also contains small quantities of dextrin, formic acid, volatile oil, wax and pollen grains. All types of natural honey contain fructose in excess of glucose.

3.1.3 Use of honey in ancient medicine

It has been reported that honey has been used from ancient times. In Eastern Spain, a place known as the Arana or Spider Cave, has a painting of 10000 years old and is the earliest first proof for the hunting of honey by humans. The veddas or wild men of Sri Lanka respect honey so highly that they regularly risk their lives to obtain it. The veddas sometimes fill a hollow tree trunk with honey and then place flesh in it as a means of preserving the meat for times of scarcity.

Many Australian indigenous tribes regard the honey of the native bee as 'the supreme delicacy'. They eat the whole thing, after removing the contents, honey, wax, dead bees and brood, with relish. Honey features prominently in religious ceremonies. For example, in 1200 BC King Rameses III offered to the Nile God tens of thousands of jars, amounting to about 15 tons, of honey. Some authors reported that people believe Egyptians honey was a luxury item, sold at price only the wealthiest could afford. The poor peoples do with concentrated fruit juices, especially date juice, as their sweetener.
Pythagorus is said to have lived largely on honey and bread; and the bodies of his countrymen who died some distance from home were sometimes preserved in honey 48. In ancient time one could enjoy honey in a still wider range of dishes. In salad dressings honey balanced the acidity of the vinegar and it was a necessary ingredient of many sauces 49. Half of the 468-odd recipes in a late Roman cookery book have honey as an ingredient 49. Refined sugar was known and used in medicines but had no place in cooking 50. Mead was an alcoholic drink made by fermenting the final washings of honey from the comb in a solution with water. Although it is almost unknown today, it was very widely enjoyed from the very early middle ages until as late as the 17th century 43 especially in those areas where grapes were not available to produce grape wines 51.

Honey was prescribed by the physicians of many races of people for a wide variety of ailments 52. The Egyptians, Assyrians, Chinese, Greeks and Romans all used honey, in combination with other herbs, and on its own to treat wounds and diseases of the gut 53. In India lotus honey is said to be a panacea for eye diseases 54. The ancient usage of honey for cough and sore throats 52 and also in the treatment of leg ulcers 35, prevention of corneal scarring 54 and gastric ulcers 36 has also continued into the medicine of modern times.

Ancient physicians who prescribed honey for various disease based on practical knowledge. They had no knowledge of the principles involved in its medicinal action 56. Aristotle wrote about honey being a salve for wounds and sore eyes 56. In ancient times honey form Attica had a special reputation as a curative substance for eye disorders. In 50 AD it was reported that honey was good for sunburns and spots on the face and for all rotten and hollow ulcers, and that it heals inflammation of the throat and tonsils and
cure coughs and mollifies the prepuce so that it can be pulled back over the bared glans penis $^{52,57}$.

The use of honey as a therapeutic substance has been re-discovered by the medical profession in more recent times, and it is gaining acceptance as an antibacterial agent for the treatment of ulcers, bed sores, and other surface infections resulting from burns and wounds $^{58,59}$.

3.1.4 Use of honey in modern medicine

There has been a rebirth in the usage of honey as a medicine in more recent times. In outlining the resurgence of its usage in modern professional medicine, the author referred to honey as “a remedy rediscovered” $^{53}$. It is widely available in most of the communities. Though the mechanism of action of several of honey’s properties remains obscure and needs further investigation, the time has now come for conventional medicine to rediscover its medicinal property and give its due appreciation.

Because of the severe side effects of modern pharmaceuticals there is increasing interest in the use of alternative therapies $^{60,61}$. However, some practitioners consider that the treatment with honey is not worthy of consideration as a remedy in modern medicine. An editorial in Archives of Internal Medicine assigned honey to the category of “worthless but harmless substances” $^{62}$. Other medical professionals have clearly shown that they are unaware of the research that has demonstrated the rational explanations for the therapeutic effects of honey $^{63,64}$. Modern physicians generally require a rational explanation for its medicinal action before a traditional or complementary, medicine is given any consideration. Some important reports are found scattered in wide range of journals and some of the explanations
for the medicinal effects of honey are to be found in articles, which is unrelated to honey 64.

3.1.4.1 Honey in treatment of wounds

In modern medicine honey has been reported to treat various types of wounds. The medical literature on treating wounds with honey has been recently reviewed in specialist wound care journals, with focus on the medical evidence on the clinical aspects 60.

Honey has been used successfully for various wounds and the action varies widely. In the numerous wounds on which honey has been reported to be of use successfully are: abrasions 65, amputations 66, abscesses 67, bed sores (pressure sores, decubitus ulcers) 65, burns 66, burst abdominal wounds following caesarean delivery 69, cancrum 68, cervical ulcers 70, chilblains 71, cracked nipples 70, diabetic foot ulcers 72 and other diabetic ulcers 73, fistula 74, foot ulcers in lepers 72, infected wounds arising from trauma 75, large septic wounds 76, leg ulcers 71, malignant ulcers 68, sickle cell ulcers 68, skin ulcers 72, surgical wounds 74, tropical ulcers 68, wounds to the abdominal wall and perineum 77 and varicose ulcers 78.

It has been reported that honey has been successfully used to treat Fournieris Gangrene 68, a rapid spreading infection that is usually managed by aggressive surgical removal of infected tissue. In another report, honey has been effectively used for the wounds from surgery for cancer of the vulva, 77 which are difficult to treat because they are in a position where it is difficult to prevent infection. In several reports, it has been noted that honey dressing rapidly healed the wounds. One report refers to wounds becoming closed in a spectacular fashion in 90% of cases, sometimes in a few days 79.
Another refers to healing being surprisingly rapid, especially for first and second-degree burns.

Clinical observations made are that open wounds heal faster and are really faster for closure by stitching when dressed with honey (than when dressed conventionally). In one case, a patient with multiple ulcers on both legs had one leg dressed with honey and the other treated conventionally (with fibrinolysis and calcium alginate dressing): the ulcers on the leg treated with honey healed much more rapidly.

In another case, a patient with a ling abdominal wound that had become infected following surgery had one end of the wound dressed with honey and the other end dressed with Debrisan (a hydrocolloid wound dressing material). It took 16 days with the Debrisan to reach the stage, and regrowth of skin over the healing wound as compared to 8 days with honey. Stronger evidence is provided from the statistically significant results from randomized controlled clinical trials. A trial comparing honey-impregnated gauze with a commonly used polyurethane film dressing as a cover for partial thickness burns in two groups of 46 patients found faster healing with honey.

In another trial comparing honey with silver sulfadiazine, cover for partial thickness burns, in two groups of 52 patients, faster healing was found with the honey and 87% of those treated with honey healed within 15 days compared with 10% of those treated with silver sulfadiazine. Controlled trials on wounds of animals, with microscopic examination of the wound tissues, confirmed the faster rates of healing with honey.
In a trial comparing honey with silver sulfadiazine on deep burns on the skin of pigs, complete re-growth of skin over the burns was achieved within 21 days with honey whereas it took 28-35 days with silver sulfadiazine. A sugar solution was also compared in this trial. It gave the same rate of healing as honey, but microscopic examination of the tissues showed a better quality of healing with the honey and cellular evidence of a more advanced state of healing. In a study comparing honey with sugar solution on superficial burns on the skin of rats, healing was seen by microscopic examination of the tissues to be more active and advanced with honey than with the sugar solution. Other studies on animals have compared honey with saline, a standard moist dressing for wounds. In a study on infected full-thickness skin wounds on buffalo calves, honey gave the faster rate of healing compared with ampicillin ointment and saline. Honey has been used successfully in 143 patients with chronic foot ulcers in lepers and diabetic foot ulcers. There was only one failure in one report (a Buruli Ulcers: treatment) when honey was discontinued after 2 weeks because the ulcers was rapidly increasing in size. Over all among 470 cases treated with honey, there were only five cases where successful healing was not achieved.

3.1.4.2 Antibacterial activity of honey

The effect of honey as a dressing on infected wounds was attributed at least partly to its antibacterial properties. Honey is reported to be very effective in cleaning up infected wounds and to stop the advance of the infection without removing the dead tissue.

The speed with which wounds dressed with honey become clear of infection is remarkable. Wounds have been reported to become sterile in 3-6 days and 7 days. The most important role for honey in wound care is...
in the treatment of wounds infected with antibiotic-resistant bacteria \(^{67}\). Several authors have reported the cleansing effect of honey on wounds \(^{65,73,67}\). Honey has a debriding effect on wounds so that surgical debridement is unnecessary \(^{65,64,83}\). Dead tissue separates easily from the wound bed after honey has been applied to a wound \(^{74,68}\).

Infected wounds can be malodorous, especially those infected with anaerobic bacteria. Honey has been reported to give rapid deodorization of offensively smelling wounds \(^{68,64,63}\). When a wound heals the dead or damaged tissue is replaced by the growth of new connective tissue and a new outer layer of skin (epithelium) spreads over the surface of the wound. The new connective tissue grows in a granular fashion (around newly formed blood vessels) and hence is termed granulation. Many have reported that honey promotes the formation of clean healthy granulation tissue \(^{66,73,91,71}\) and growth of epithelium over the wound \(^{90,68,63}\). This clinical observation of stimulation of tissue growth have been confirmed by microscopic examination of wound tissues, in animal studies where the effect of honey on wound healing has been clearly shown \(^{50,61,66}\).

3.1.4.3 Honey in the treatment of inflammation and edema

The inflammation of surrounding tissues that results from infection of a wound, or directly from the damage to tissues caused by burns, is the major cause of the pain and discomfort associated with wounds. The process of inflammation involves blood capillaries opening up and allowing plasma from the blood to flow out into the surrounding tissues. This causes swelling of the tissues (edema), the pressure giving rise to damage and discomfort in the healing area. It also causes plasma to exude from open wounds, sometimes in large quantities. Honey has been reported to reduce inflammation \(^{60,91,71}\), edema \(^{68,92,93}\) and exudation \(^{68,80,90}\).
In an animal study, the anti-inflammatory effect of honey has also been observed by microscopic examination of wound tissues, where reduction in the number of white blood cells involved in inflammation could be seen. It has been reported that this reduction in inflammation seen when honey is applied to wounds must be a direct anti-inflammatory effect, not just a result of removal of inflammation causing bacteria.

Honey generally causes no pain on dressing or causes only momentary stinging, is non-irritating, and does not cause any allergic reaction. In several reports where honey was used on wounds, the authors have reported that honey has no harmful effects on tissues. Over all the reports of honey being used on wounds, with a total of more than six hundred cases, none of them have been reported of any harmful effects of honey on tissues. Nor have any adverse effects been noted in any of the studies in which honey has been applied to wounds on animals. The pain or discomfort usually associated with changing dressings is minimized when honey dressing is used, which are easy to apply and remove. Also there is no bleeding when removing dressings.

3.1.4.4 Honey in the treatment of diarrhoea

The Roman physicians used honey as a cure for diarrhoea. Water and honey is also used by many veterinarians for treatment of diarrhoea in small animals, and dosage with an 8% (v/v) solution of honey has been reported to be effective for the treatment of chronic diarrhoea in a horse. Honey has been used successfully at a concentration of 5% (v/v) in place of glucose as a rehydration fluid (solution of electrolytes), in a clinical trial conducted on 169 infants and children admitted into hospital with gastroenteritis.
3.1.4.5 Honey in the treatment of peptic ulcers

Honey has been traditionally used for the treatment of peptic ulcers. Also there are numerous reports of oral dosage of honey being successfully used in modern times to treat upper gastrointestinal dyspepsia, including gastritis, duodenitis and ulceration, particularly in Russia and Arabic countries. A clinical trial has been reported in which 45 patients with dyspepsia were given no medication other than 30 ml of honey before meals 3 times daily. After treatment with honey the number of patients passing blood (from peptic ulcers) in their faeces had decreased from 37 to 4; the number of patients with dyspepsia had decreased from 41 to 8; the number of patients with gastritis or duodenitis seen on endoscopy had decreased from 24 to 15; the number of patients with a duodenal ulcer seen on endoscopy had decreased from 7 to 2.

The healing effect of honey on gastric ulcers has also been shown in a trial carried out on rats with ulcers caused by aspirin. After 3 days of treatment the control group of 10 rats (given saline) had 15 ulcers whereas the group of 10 rats given honey from sugar fed bees had 8 ulcers and the group of 10 rats given floral honey had 3 ulcers. The differences between these numbers were statistically significant. In a similar study the gastric ulcers in the rats, caused by indomethacin, the healing rate achieved with the honey in this study was 70% measured as the number of ulcers in the honey treated group compared with an untreated control group. Other studies with rats have shown that honey also has a preventative action, protecting the stomach from ulceration by indomethacin and alcohol.

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3.1.4.6 Honey in the treatment of eye disorders

In ancient times honey from Attica had a special reputation as a curative substance for eye disorders 52. In India (1945) lotus honey was said to be a panacea for eye diseases 34. Honey is also a traditional therapy in Mali for measles, it being put in the eyes to prevent the scarring of the cornea, which occurs in this infection 54. Meier has referred to honey being used to treat eyes discharging pus 104. The use of honey to treat blepharitis (inflammation of the eye-lids), catarrhal conjunctivitis, and keratitis (inflammation of the cornea) has also been reported 105. Another report has described the use of honey in place of petroleum jelly in a 3% eye ointment for the treatment of three cases of keratitis 106. The same paper reported the successful treatment with the honey ointment of 28 patients with various ailments of the cornea, syphilitic keratitis, injuries to the cornea, and lime burns of the corneas 106. Honey has been used undiluted or as a 20 –50 % solution in water for chemical and thermal burns to the eye, conjunctivitis and infections of the cornea 106.
3.2 Cytochrome P 450 enzyme

3.2.1 Introduction to Cytochrome P 450 and types

The cytochrome P 450 (CYP) enzyme system consists of a super family of hemoproteins, mainly localized in the liver and, to a minor extent, in the small intestine, that catalyze the oxidative metabolism of a wide variety of exogenous chemicals including drugs, carcinogens, toxins and endogenous compounds such as steroids, fatty acids and prostaglandin’s. It plays an important role in phase-I metabolism of drugs.

The name cytochrome P 450 is derived from the fact that these proteins have a heme group and an unusual spectrum. These enzymes are characterized by a maximum absorption wavelength of 450 nm in the reduced state in the presence of carbon monoxide. Naming a cytochrome P 450 gene includes root symbol “CYP” for humans (“Cyp” for mouse and Drosophila), an Arabic numeral denoting the CYP family (e.g. CYP1, CYP2), letters A,B,C indicating subfamily (e.g. CYP3A, CYP3C) and another Arabic numeral representing the individual gene/isoenzyme/isozymes/isoform (e.g. CYP3A4, CYP3A5). Of the 74 gene families so far described, 14 exist in all mammals. These 14 families comprise of 26 mammalian subfamilies. In human liver there are at least 12 distinct CYP enzymes.

A study based on the metabolic clearance of 315 different drugs concluded that 56% of the drugs was primarily cleared through the action of the cytochrome P450 enzymes. Among them CYP3A4 was the most important (50%) followed by CYP2D6 (20%), CYP2C9 and CYP2C19 (15%) and the remaining metabolism carried out by CYP2E1, CYP2A6, CYP1A2 and unidentified P450’s. All these enzymes are inducible except CYP2D6.
It has been reported that from about 30 isozymes, only six isoenzymes from the families CYP1, 2 and 3 are involved in the hepatic metabolism of most of the drugs. These include CYP1A2, 3A4, 2C9, 2C19, 2D6 and 2E1. This review mainly concentrate on CYP3A, 2E1 and C19 because the drugs used in the study are mainly metabolized by these enzymes.

Drug interactions involving enzyme induction are not as common as inhibition based drug interactions. Exposure to environmental pollutants as well as large number of lipophilic drugs can result in induction of CYP enzymes. The most common mechanism is transcriptional activation leading to increased synthesis of more CYP enzyme proteins. If a drug induces its own metabolism, it is called autoinduction as in the case with carbamazepine. If induction is by other compounds it is called foreign induction.

A drug may inhibit the CYP isoenzyme whether or not it is a substrate for that isoenzyme. If the two drugs are substrate for the same CYP isoenzyme then metabolism of one or both the drugs may be delayed. For example both erythromycin and midazolam are substrates for CYP3A4 isoenzymes, which results in competition for the enzyme sites, and inhibition of midazolam metabolism.

**Enzyme Induction:**

Enzyme induction results in an absolute increase in enzyme synthesis. It was first recognized in 1940's when hepatic blood flow was increased or the enzyme is stimulated. It has been reported that in animal models, phenobarbital, a well-known enzyme inducer, increases liver weight in a dose dependent manner.
The onset and duration of the induction depends both on the kinetics of the drug and on the half-life of the inducers. For example, rifampicin (shorter half life drug) results in enzyme induction (CYP3A, CYP2C) with in 24 hrs, \(^ {115}\) where as phenobarbital (longer half life drug) requires approximately one week to induce (CYP3A, CYP1A2, CYP2C) enzymes and the effect may be remaining after phenobarbital is discontinued \(^ {114}\).

Important inducers other than the pharmacologically active drugs include the polycyclic aromatic hydrocarbons in cigarette smoke, which cause the induction of CYP1A2 resulting in higher dosing requirements of drugs like theophylline in smokers. It has been reported that chronic alcohol use induces CYP2E1 \(^ {116,113}\).

Enzyme induction is also influenced by age and liver disease. The drug metabolizing capacity may decrease with age, which has been confirmed by reports that drug metabolism in elderly subjects (more than 60 yrs.) is not influenced by polycyclic aromatic hydrocarbons in cigarette smoke, as it has been reported in younger subjects \(^ {116}\). Also patients with cirrhosis or hepatitis may be less susceptible to enzyme induction \(^ {117}\).

*Enzyme inhibition:*

Enzyme inhibition occurs most often as a result of competitive binding at the enzyme's binding site. It has been reported that competitive inhibition depends on the affinity of the substrate for the enzyme being inhibited, the concentration of substrate required for inhibition, and the half-life of the inhibitor drug. For example chloramphenicol (which is metabolized by CYP2C9) and cimetidine (CYP1A2) inhibits the enzyme responsible for drug metabolism with in 24 hrs of a single dose, but amiodarone (CYP2C9) inhibitory action take months because of its long half-life \(^ {118}\).
A drug which is not a substrate, can also inhibit the activity of a non-substrate isozyme. For example, quinidine is metabolized by CYP3A4/5, but powerfully inhibits CYP2D6.  

The onset and the termination time to maximum drug interaction is also reported for drug inhibition. For example, with the cimetidine and theophylline interaction, a maximum of 2 days has been reported for the increase in the theophylline concentration, because of half-life.

The inhibition can also occur as a result of non-competitive mechanism, which can occur as a result of inhibitor inactivation of enzyme with normal substrate binding. It has been reported that the duration of this type of interaction will be longer.

Another enzyme inhibition factor is the hepatic extraction ratio of the affected drug. It has been reported that the drugs with systemic clearance of low-extraction ratio are affected to a greater extent than those with high-extraction ratio.

3.2.2 Cytochrome P450 3A4

Enzymes of the CYP3 family are the predominant phase I drug-metabolizing enzymes found in humans. It has only one subfamily i.e. CYP3A. It includes four genes viz. CYP3A4, CYP3A5, CYP3A7 and CYP3A3. Among them CYP3A4 is the most important.

**Distribution:** It accounts for 30% to 40% CYP enzyme present in the liver. High levels are also present in the small intestinal epithelium, particularly in the apical region of mature enterocytes of the microvillus, and
metabolizes more than 50 to 70% of drugs thus making it a major contributor to presystemic elimination of orally administered drugs. About 30% of the CYP3A protein is present in the kidney, mainly in the collecting ducts.

**Substrates:** CYP3A enzyme substrate is very broad. It catalyzes the oxidation reactions of many clinically important drugs. CYP3A substrates include alfentanil, alprazolam, astemizole, atorvastatin, buspirone, caffeine, cerivastatin, cisapride, clarithromycin, codeine, cyclosporin, dextromethorphan, diazepam, diltiazem, erythromycin, felodipine, fentanyl, finasteride, haloperidol, indinavir, lidocaine, lovastatin, methadone, midazolam, nisoldipine, ondansetron, pimozide, propranolol, quinidine, ritonavir, salmeterol, saquinavir, sildenafil, simvastatin, tacrolimus, tamoxifen, terfenadine, trazodone, verapamil, vincristine, zaleplon and zolpidem.

An important characteristic of CYP3A enzymes is the significant variation among the individuals in expression of hepatic CYP3A proteins as well as in the clearance of many CYP3A metabolizing drugs. It has been reported that CYP3A4 levels show wide interindividual variations in the liver (10-100 folds) and in the small intestine (up to 30 fold). The risk of adverse drug reactions related to large differences in CYP3A levels is particularly important for substrates with narrow therapeutic indices (e.g., cyclosporin A, chemotherapeutics).

**Inducers:** CYP3A enzymes in general are markedly affected by various factors like temperature, pressure and pH. It has been reported that CYP3A4 enzyme denature to an inactive form at 3000 bar pressure.
The expression of CYP3A mRNA protein is increased within 24 h following treatment with the inducing agents. The major inducers of CYP3A4 subfamily are barbiturates, carbamazepine, glucocorticoids, modafinil, rifampicin, St. John’s wart and troglitazone.

**Inhibitors:** Many compounds which are metabolized by CYP3A act as inhibitors. The common feature is the relatively large molecular size. The major inhibitors are cimetidine, ciprofloxacin, clarithromycin, diethyl-dithiocarbamate, fluvoxamine, grapefruit juice, indinavir, interleukin-10, ketoconazole, mibefradil, mifepristone, nefazodone, nelfinavir, norfloxacin, norfluoxetine and ritonavir.
| Substrates | alfentanil, alprazolam, astemizole, atorvastatin, buspirone, caffeine, cerivastatin, cisapride, clarithromycin, codeine, cyclosporine, dextromethorphan, diazepam, diltiazem, erythromycin, felodipine, fentanyl, finasteride, haloperidol, indinavir, lidocaine, lovastatin, methadone, midazolam, nisoldipine, ondansetron, pimozide, propranolol, quinidine, ritonavir, salmeterol, saquinavir, sildenafil, simvastatin, tacrolimus, tamoxifen, terfenadine, trazodone, verapamil, vincristine, zaleplon, zolpidem. |
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3.2.3 Cytochrome P 450 2C19

The CYP2C subfamily of P450, is an important cytochrome enzymes that metabolize approximately 20% of clinically used drugs. There are four members of the subfamily, CYP2C8, CYP2C9, CYP2C19, and CYP2C18. Of these CYP2C8, CYP2C9, and CYP2C19 are of clinical importance. CYP2C19 isoenzyme exhibits genetic polymorphism.

Distribution: CYP2C19 is the second most abundant cytochrome in the liver, representing about 20% of the total liver CYP, and it has been studied extensively and found to metabolize many drugs. It is one of the inducible forms of cytochrome P 450. CYP2C19 is also distributed in the duodenum and small intestine.

Substrate: CYP2C19 is one of the common isoenzyme involved in the first pass drug elimination. It metabolizes variety of drugs which includes amitriptyline, citalopram, clomipramine, cyclophosphamide, diazepam, hexobarbital, indomethacin, lansoprazole, S-mephenytoin, R-mephobarbital, moclobemide, nelfinavir, nilutamide, omeprazole, pantoprazole, phenytoin, phenobarbitone, progesterone, proguanil and propranolol.

Mephenytoin has long been used as the standard CYP2C19 phenotyping probe, but problems such as sample stability and adverse effects have developed interest in investigating a potential alternative, such as omeprazole.
**Inducers:** Several environmental factors such as smoking, diet and co-administration of medications are reported to affect the CYP2C19 activity. Apart from this the main inducers are prednisone, rifampin, norethindrone, artemisinin and carbamazepine.

**Inhibitors:** Its inhibitors include felbamate, fluoxetine, fluvoxamine, lansoprazole, modafinil, omeprazole, ticlopidine, cimetidine and ketoconazole.
Table 2: Substrate, Inducers and inhibitors of cytochrome P 450 2C19 enzyme

<table>
<thead>
<tr>
<th>Cytochrome P 450 2C19</th>
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<tbody>
<tr>
<td><strong>Substrates</strong></td>
</tr>
<tr>
<td>amitriptyline, citalopram, clomipramine, cyclophosphamide, diazepam, hexobarbital, indomethacin, lansoprazole, S-mephenytoin, R-mepobarbital, moclobemide, nelfinavir, nilutamide, omeprazole, pantoprazole, phenytoin, phenobarbitone, progesterone, proguanil, propranolol.</td>
</tr>
<tr>
<td><strong>Inducers</strong></td>
</tr>
<tr>
<td>prednisone, rifampin, norethindrone, artemisinin, carbamazepine</td>
</tr>
<tr>
<td><strong>Inhibitors</strong></td>
</tr>
<tr>
<td>felbamate, fluoxetine, fluvoxamine, lansoprazole, modafinil, omeprazole, ticlopidine, cimetidine, ketoconazole.</td>
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3.2.4 Cytochrome P 450 2E1

The pharmacokinetics of many drugs varies due to changes in the expression of cytochrome P-450 (CYP) enzymes in the liver and other tissues. CYP2E1 is one of the major isoenzyme involved in the drug metabolism and it is clinically and toxicologically important.

**Distribution:** CYP2E1 accounts for 10% of the total liver CYP. It is also expressed in the small intestine. In contrast to many other CYP isoenzymes, CYP2E1 in human population is not studied extensively.

**Substrate:** Cytochrome P450 (CYP) 2E1 catalyzes the metabolism of a wide variety of therapeutic agents, procarcinogens, and low molecular weight solvents. CYP2E1-catalyzed metabolism may cause toxicity or DNA damage through the production of toxic metabolites, oxygen radicals, and lipid peroxidation. It also plays a role in the metabolism of endogenous compounds including fatty acids and ketone bodies. CYP2E1 mRNA and protein levels are altered in response to pathophysiologic conditions by hormones including insulin, glucagon, growth hormone, and leptin, and growth factors including epidermal growth factor and hepatocyte growth factor, providing evidence that CYP2E1 expression is under tight homeostatic control. It metabolizes a wide variety of chemicals with different structures, in particular small and hydrophobic compounds, including potential cytotoxic and carcinogenic agents. Its main substrates are acetaminophen, aniline, benzene, chlorzoxazone, ethanol, halothane, N, N-dimethyl formamide and theophylline. It is also involved in the metabolism of low molecular weight toxins, fluorinated ether volatile anesthetics and procarcinogens.
**Inducers:** Alcoholic liver disease was attributed exclusively to dietary deficiencies. Chronic ethanol consumption increases CYP2E1, resulting in increased generation of toxic acetaldehyde and free radicals and multiple ethanol-drug interactions. Isoniazid is also reported to induce the CYP2E1 level.

**Inhibitors:** The main inhibitors of CYP2E1 are diethyl-dithiocarbamate and disulfiram.
Table 3: Substrate, Inducers and inhibitors of cytochrome P 450 2E1 enzyme

<table>
<thead>
<tr>
<th></th>
<th>Cytochrome P 450 2E1</th>
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<tbody>
<tr>
<td><strong>Substrates</strong></td>
<td>acetaminophen, aniline, benzene, chlorzoxazone, ethanol, halothane, N, N-dimethyl formamide, theophylline</td>
</tr>
<tr>
<td><strong>Inducers</strong></td>
<td>isoniazid, ethanol</td>
</tr>
<tr>
<td><strong>Inhibitors</strong></td>
<td>diethyl-dithiocarbamate, disulfiram</td>
</tr>
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</table>
3.3 Drug efflux mechanism (P - Glycoprotein)

P-glycoprotein (P-gp), a 170 kD glycoprotein, is a member of a phylogenetically highly conserved superfamily of ATP-binding cassette (ABC) transport proteins and shares extensive similarity with numerous bacterial and eucaryotic ABC transport proteins \(24',248\). It consists of two homologous halves joined by a linker region. Each halves has a short hydrophilic N-terminal segment, six putative transmembrane domains as predicted on the basis of hydropathy plots, and a hydrophilic C-terminal region \(24\). The physiological role of the P-gp is not completely understood \(249\).

**Distribution:** P-gp is expressed in a broad spectrum of tissues including the adrenals, bladder, cells of the blood-brain barrier, kidney, liver, lungs, pancreas, rectum, spleen, the oesophagus, stomach, jejunum and colon \(23,27,28\). In the intestine, P-gp is located almost exclusively within the brush border on the apical (luminal) surface of the enterocytes where it pumps xenobiotics from the enterocytes back into the intestinal lumen \(28,25\). The presence of P-gp in intestinal epithelium and in the endothelium of brain and testis capillaries suggests that P-gp is involved in the defense against xenobiotics \(28,251\). The high activity in the epithelium of the pregnant uterus also fits such a function \(262\). The presence in the epithelium of excretory organs, such as liver and kidney, suggests a role in the excretion of xenobiotics and possibly of normal metabolites \(263,254\). The presence of P-gp in the adrenal cortex led to speculation that this P-gp is required for steroid excretion \(255\).
**Substrates:** After exposure to cytotoxic drugs such as vinca alkaloids, anthracyclines, taxoids or actinomycin D, cells that express the MDR phenotype can over-express P-glycoprotein. As a result, these cells become resistant to the selective agent and cross-resistant to a broad spectrum of structurally and functionally dissimilar drugs such as, for example antibiotics and alkaloids 256.

P-gp mediated transport has been shown for excretion of xenobiotics via the canalicular membrane of hepatocytes into the bile, via the (luminal) brush border membrane of enterocytes into the gut lumen and via the (luminal) brush border membrane of proximal tubule cells into the urine 257. Substrates for P-gp cover a broad range of structures with diverse therapeutic indications. There are no clear structural features defining P-gp substrates; however, the molecules tend to be large and amphipathic, containing one or more aromatic rings 2.

The important substrates are aldosterone 258, amiodarone 259, azidopine260, bepredil 261, bisantrene 262, catharantine 263, cefazolin, cefoperazone, cefotetan 264, cepharanthine 265, cinchonidine 266, dexamethasone266, dibucaine 267, digoxin 266, diltiazem 267, dipyridamole 268, domperidone 266, emetine 269, fluphenazine 270, gallopamil, hydrocortisone 268, ivermectin, loperamide 268, methadone hydrochloride 256, methylreserpate 271, monensin 267, morphine 266, nicardipine 267, ondansetron 266, perphenazine 270, phenoxazine 272, phenytoin 266, prazosin 273, progesterone 267, quinidine 259, rhodamine 123 273, spiperone 267, tamoxifen 267, terfenadine 274, thioridazine 268, topotecan 268, trifluoperazine 270, triflupromazine 270, verapamil 259, vindoline 263 and yohimbine 263.
**Inducers:** An increase in P-gp activity can result in lowered intracellular drug concentration and increased drug resistance. It can extrude a range of hydrophobic drugs from the cell against a concentration gradient. P-gp over expression is induced not only by chemical compounds but also by physical stress, such as X-irradiation, ultraviolet light irradiation and heat shock.

Apart from this the important inducers are actinomycin D, clotrimazole, colchicines, daunorubicin, doxorubicin, epothilone A, erythromycin, etoposide, isosafrole, midazolam, nifedipine, phenobarbital, reserpine, rifampicin, taxol and vincristine.

**Inhibitors:** Many compounds with relatively low toxicity can inhibit drug transport by P-gp. They are called reversal agents and being tested in clinical trails, to increase the response to chemotherapy of treatment-resistant tumors. As toxicity of the early reversal agents did not allow efficient blockade of P-gp, more efficient and less toxic reversal agents, such as the cyclosporin A analogue, SDA PSC 833, have been developed and are entering clinical trials. The use of these effective new reversal agents requires more knowledge of the physiological function of P-gp and of the pharmacological consequences of blocking the function in normal organs.
Table 4: Substrate, Inducers and Inhibitors of P-glycoprotein

<table>
<thead>
<tr>
<th>P-glycoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>aldosterone, amiodarone, azidopine, bepredil, bisantrene, catharantine, cefazolin, cefoperazone, cefotetan, cepharanthine, cinchonidine, dexamethasone, dibucaine, digoxin, diltiazem, dipyridamole, domperidone, emetine, fluphenazine, gallopamil, hydrocortisone, ivermectin, loperamide, methadone hydrochloride, methylreserpat, monensin, morphine, nicardipine, ondansetron, perphenazine, phenoxazine, phenytoin, prazosin, progesterone, quinidine, rhodamine 123, spiperone, tamoxifen, terfenadine, thioridazine, topotectan, trifluoperazine, triflupromazine, verapamil, vindoline, yohimbine.</td>
</tr>
<tr>
<td>Inducers</td>
</tr>
<tr>
<td>actinomycin D, clotrimazole, colchicines, daunorubicin, doxorubicin, epothilone A, erythromycin, etoposide, isosafrole, midazolam, nifedipine, phenobarbital, reserpine, rifampicin, taxol, vincristine.</td>
</tr>
<tr>
<td>Inhibitors</td>
</tr>
<tr>
<td>SDA PSC 833, cyclosporin A</td>
</tr>
</tbody>
</table>
3.4 Factors affecting the bioavailability of drugs

3.4.1 Physicochemical factors

Drug absorption, after oral administration, involves complex array of interactions between the drug, its formulation, and the gastrointestinal (GI) tract. The presence of food within the GI tract significantly alters the transit profiles, pH, and its solubilization capacity. The main rate controlling factors affecting the oral absorption are unstirred water layer, membrane limitation, or flow limitation. These are much affected by the physicochemical and pharmaceutical properties of the drug: pKa, water/lipid solubility, structural resemblance to endogenous substrates for transport proteins, and the physiology of the GI tract.

3.4.1.1 Formulation factors

The nature of the drug and its formulation interactions are of clinical concern for orally administered drugs that possess a narrow therapeutic index. The physicochemical properties of the drug and factors influencing oral input are the rate-limiting processes of absorption. Gastro intestinal regional differences in membrane permeability are fundamental in developing the extended release dosage forms as well as to predicting interaction effects on absorption from immediate release dosage forms. Some of the common formulation ingredients like tween 80, oleic acid, mixed micellar solution etc., are also reported to inhibit the cytochrome P 450 activity, especially CYP3A isoenzyme.
3.4.1.2 Dissolution rate

The absorption of drugs from the gastrointestinal tract is often depends on the dissolution rate of the dosage form. Drug diffusivity, solubility in the gastrointestinal contents, the surface area of the solid wetted by the luminal fluids and the gastrointestinal hydrodynamics all play a role in determining the in vivo dissolution rate. Solubility in the gastrointestinal contents is determined by aqueous solubility, crystalline form, drug lipophilicity, solubilization by native surfactants, co-ingested foodstuffs, and pKa in relation to the gastrointestinal pH profile. Other physiological factors that can engage a role in dissolution rate are the viscosity of the luminal contents and mixing and flow patterns within the gut \(^{291}\). It has been reported that dissolution rate of the orally administered drug is also affected by the type, amount, and timing of foods consumed \(^{292}\). To better understand the in vivo dissolution of drugs, dissolution tests at different conditions have to be done \(^{291}\).

3.4.1.3 Partition coefficient

Lipophilicity, often expressed as partition coefficients (log D) in octanol/water, is an important physicochemical parameter influencing processes such as oral absorption, brain uptake and various pharmacokinetic (PK) properties. Increase in the partition coefficient values increases the oral absorption, plasma protein binding and volume of distribution. However, more lipophilic compounds also become more susceptible to cytochrome P 450 metabolism, leading to higher clearance of drugs \(^{293}\). It has been reported that the inhibitory potency of alcohol is linearly related to partition coefficient. It also states that the modification of partition coefficient will lead to inhibition of drug metabolism mainly by cytochrome P 450 \(^{294}\).
Molecular size and hydrogen bonding capacity are two other properties often considered as important for membrane permeation and pharmacokinetics. Increase in the molecular weight often gives higher potency, which leads to either higher lipophilicity and hence poorer dissolution/solubility, which limits oral absorption.

3.4.1.4 Particle size

The uptake of micro- and nanospheres, consisting of natural or synthetic polymeric materials from the gastrointestinal tract has been demonstrated by numerous authors over the past two decades. Factors such as particle surface charge and hydrophilic/hydrophobic balance of these polymeric materials are important parameters to be considered for the gastrointestinal absorption. The use of particulate carrier systems for the delivery of peptides and other hydrophilic macromolecules via the oral route remains a challenging task due to morphological and physiological absorption barriers in the gastrointestinal tract. Apart from particle size, type and composition of the polymers used for micro- or nanoencapsulation are crucial for an uptake and transport across mucosal barriers. It has been reported that smaller particle size improves lung targeting of hydrofluoroalkane which helps in reduction in the hydrofluoroalkane dose when compared with chlorofluorocarbon dose. In another report the amphotericin B (drug of choice for the treatment of fungal infections) encapsulation by the liposomes has been evaluated for its pharmacokinetic activity. It states that the drug kinetic mainly depends on the composition and particle size of the liposomes, smaller the particle size of the liposomes the drug remains in the blood for longer time. The result was coinciding with another report which showed that smaller particle size shows more rapid dissolution of the oral contraceptive tablet (norethindone) in vitro.
3.4.2 Effect of Macro-nutrients on drug absorption

Macronutrients and micronutrients of food alter drug metabolism. The important macronutrients are carbohydrates, lipids and proteins.

3.4.2.1 Carbohydrates

High dietary carbohydrates decreases hepatic metabolism of drugs both in vitro and in vivo. Carbohydrates are hydrolyzed in the intestinal lumen by specific enzymes to monosaccharides before transport across the brush border membrane of epithelial cells into the cell interior.

It has been reported that excess of carbohydrates intake delays gastric emptying rate, increase the gastric pH and increase the luminal fluid volume resulting in decrease / increase in absorption of drugs e.g. phenytoin, indinavir etc.

Fructose containing media which was incubated with adult rat cultured hepatocytes was reported to show an increased level of cytochrome P 450, relative to cells incubated with equimolar glucose. By alteration in the fructose concentration, partial inhibition of cytochrome P 450 level is achieved in the culture media. But in contrast, glucose at comparable or even greater concentrations has no measurable effect.

In another report it has been documented that administration of simple carbohydrates such as glucose and fructose influences the hepatic drug metabolism. It has been reported that fructose administration showed induction of insulin resistance over nitric oxide.
Glucose is a universal nutrient. Its increased consumption decreased the metabolism of numerous drugs in humans and animals. Enzyme assays of hepatic CYP1A2, 2C6, 2C11 and 3A2 showed significant decreases in activity from glucose-treated rats compared to control.

The above report correlates with the results of another study where it has been reported that the oral glucose treatment caused a statistically significant decrease in the AUC of propranolol when this drug was given by oral intubation.

It has been reported that change in glucose metabolism is subsequently affected along with rapid loss of cytochrome P450 levels during the early hours of the cultured hepatocytes in the monolayer formation. It also states that this loss can be reverted by the addition of nitric oxide inhibitors during liver perfusion and early culture hours, which allows catalytically active P450 to be preserved at levels close to those of the intact liver.

### 3.4.2.2 Lipids

Lipid per oxidation enhancement is the main reason for microsomal enzyme induction in the chronic ethanol interaction. The effect was primarily on the specific cytochrome P450 i.e CYP2E1 which is responsible for the enhancement of oxidation of ethanol.

Different studies have revealed that microsomal oxidation is impaired by total parenteral nutrition and that the effect will be changed when the caloric source changes from carbohydrates to lipid, especially when administered as medium-chain/long-chain triglycerides mixtures.
The quantity and quality of dietary fat affects lipid composition alters the physical and biochemical characteristic of the intestinal membrane thereby directly affecting drug entrance into the membrane and the stability of the membrane.\textsuperscript{313}

3.4.2.3 Protein

Dietary protein, cruciferous vegetables, charcoal-broiled beef containing polycyclic aromatic hydrocarbons, and methylxanthines can influence drug metabolism by the mixed function oxidase system and conjugating enzymes.\textsuperscript{314} In has been documented that high-protein diets enhance the metabolism of numerous drugs viz. tetracycline, cholestyramine etc.\textsuperscript{315}

Low intake of protein was reported to cause a decrease of about 20 to 40% in phenazone and theophylline clearance and elimination of those drugs can be accelerated by a protein-rich diet. In the same way, protein deficiency induced by either vegetarian food or undernourishment will have the opposite pharmacokinetic consequences.\textsuperscript{316}
3.5 Pharmacokinetics profile

3.5.1 Carbamazepine

Carbamazepine is chemically related to tricyclic antidepressants and used mainly for grandmal epilepsy and for the treatment of trigeminal neuralgia $^{317,318}$. It is absorbed slowly and erratically after oral administration from the gastro intestinal tract. Peak plasma concentration is usually observed at 1 to 5 hr after oral ingestion $^{319}$.

Carbamazepine is mainly metabolized by CYP3A4 in humans which is present in the intestine wall and in the liver $^{16,17}$. In the rabbit it is metabolized by CYP3A6 enzyme $^{320}$. The rabbit isoform CYP3A6 and the human isoform CYP3A4 have similar P 450 predominance and substrate specificity and both are induced by rifampicin $^{321}$. The other cytochromes involved in the metabolism of carbamazepine are CYP2C8 and CYP1A2 $^{320}$.

Its metabolism is mainly through 10,11-epoxide pathway at the cis-stilbene double bond $^{322}$. Carbamazepine is also inactivated through conjugation and hydroxylation and excreted mainly through urine $^{323}$. The drug distributes rapidly into all tissues. Binding to plasma protein occurs to the extend of about 75% $^{324}$.

Carbamazepine metabolism is induced by phenobarbital, phenytoin, valproate $^{318}$ and rifampin $^{325}$. The metabolism of carbamazepine is inhibited by propoxyphene, erythromycin $^{318}$, paclitaxel and docetaxel $^{326}$.
3.5.2 Diltiazem

Diltiazem is an benzodiazepine derivative and used as an agent to treat angina pectoris by dilating the peripheral arteries and arterioles. It is absorbed completely after oral administration and in some cases the bioavailability is reduced because of first pass hepatic metabolism. Its peak plasma concentration occurs within 2 to 3 hours. Diltiazem is distributed rapidly and its plasma protein binding ranges from 80 to 90% mainly albumin.

It is predominantly metabolized by CYP3A in the intestine and in the liver. The drug undergoes several reactions viz., deacetylation, oxidation, O- and N-demethylation and conjugation of the phenolic metabolites. Of the various metabolites the primary metabolite is desacetyldiltiazem, which is a potent vasodilator. Diltiazem is metabolized mainly by hepatic enzymes (30-40%) and by intestinal enzyme (48%) and excreted through urine.

Diltiazem metabolism is induced by simvastatin and atorvastatin and inhibited by amiloride, benzothiazepine and fluvastatin.

3.5.3 Phenytoin

Phenytoin is a hydantoin derivative. It is used for all types of partial and tonic-clonic seizures but not in absent seizures. Absorption of phenytoin after oral administration is slow and sometimes variable. Peak plasma concentration ($c_{max}$) may occur between 2 to 3 hours. It is poorly water-soluble and hence absorption is unpredictable after intramuscular injection.
It is rapidly distributed to all tissues. About 90% of phenytoin is bound with plasma protein mainly albumin. Protein binding is decreased in neonates, uremia and hypoalbuminemic states.

It is mainly metabolized by p-hydroxylation of the aromatic ring followed by conjugation. The main cytochromes involved in its metabolism are CYP2C9 and CYP2C19 enzyme. The other cytochrome involved in the metabolism of phenytoin is CYP2C6. Phenytoin is eliminated as a function of concentration i.e. in nonlinear way. Because of the saturation kinetics the half-life (t₁/₂) varies from 12-36 hours.

Phenytoin metabolism is induced by carbamazepine, chronic alcohol abuse, reserpine and sucralfate. Its metabolism is inhibited by chloramphenicol, dicumarol, disulfiram, isoniazid, cimetidine, fluoxetine and norfluoxetine.

3.5.4 Paracetamol

Paracetamol, which is also called as acetaminophen is an effective alternative to aspirin as an analgesic-antipyretic agent. It is basically a N-acetyl-p-aminophenol derivative.

Acetaminophen is almost completely absorbed after oral administration from gastrointestinal tract. The rate of absorption is determined by dissolution rate of the drug and gastric emptying time. The peak plasma concentration reaches in 30 to 60 minutes.
It is uniformly distributed throughout the body fluid and is bound to plasma protein at 20 to 50 % depending upon the concentration. Acetaminophen is mainly metabolized about 60% through glucuronidation and about 35% through sulfatation and a small fraction is oxidized by cytochrome P450 (CYP) 2E1 which is present in the liver and the intestine. It also undergoes N-hydroxylation to form an intermediate, N-acetyl-benzoquinoneimine, which is highly reactive and produces hepatotoxicity. The other cytochrome involved in the metabolism are 3A and 1A2.

This toxic metabolite is normally eliminated after getting conjugated with glutathione. More than 90 % of the drug is recovered in the urine after oral administration. In addition to metabolism in the liver, 5-10% of the drug is eliminated through the kidney. The elimination half-life is 2-2.5 hours.

The metabolism of acetaminophen is inhibited, by inhibition of glucuronidation and CYP2E1, by disulfiram and induced, by induction of CYP2E1 and glucuronidation, by chronic ethanol, isoniazid, Phenytoin, barbiturates and carbamazepine.

3.5.5 Digoxin

The most commonly used digitalis glycoside for congestive cardiac failure is digoxin. Absorption of digoxin from the gastro intestinal tract is a passive process that depends on the lipid solubility of the drug. Digoxin absorption after oral administration varies widely between 75% to 90%.
Digoxin is slowly distributed in the tissues even after intravenous therapy and 20 to 30 % is plasma protein bound. Therefore there is a lag period of several hours between drug administration and therapeutic effect.

Digoxin is a substrate for P-glycoprotein. Its metabolism is strongly affected by P-glycoprotein which is present in the apical membrane of mucosal cells in the intestine and in the brush border of renal proximal tubules.

About 80% of the digoxin is excreted unchanged in the urine with a clearance rate that is proportional to the glomerular filtration rate, resulting in approximately one third of the body drug concentration, with an elimination half-life of 12 to 14 hrs in rabbits and in humans it ranges from 36 to 48 hours.

Digoxin concentration was increased, by inhibition of P-glycoprotein, by amiodarone, cyclosporin, itraconazole, flecainide and verapamil. The drug concentration was decreased, by induction of P-glycoprotein, by omeprazole, lansoprazole and pantoprazole and Saint John's Wart.