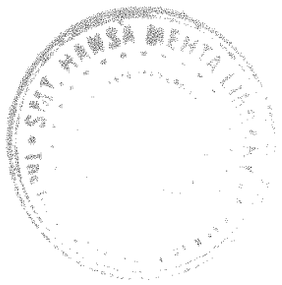


1. Introduction

There are several routes by which drugs are commonly administered. The administration of drug is to obtain a local or systemic effect. If a systemic effect is desired, the oral route is often preferred; this is the most *common route* for administration. For patient oral formulations are *convenient* as it is easy to *handle* and *administer* in the correct dose. In addition, manufacturers usually have a preference for oral formulations owing to their *low costs* of production. Due to all these reasons, majority of therapeutic agents today are administered orally. Thus an orally administered drugs needs to reach the systemic circulation in order to arrive at the site of action in adequate quantities, as determined by the potency of the drug.

However, the oral dosage forms have to disintegrate, whereupon drug molecules have to be dissolved in gastrointestinal fluids, permeate intestinal membrane, and pass through major metabolizing organ in the body, the liver, before they can reach their target through the systemic circulation. Therefore in a process of drug development it is important to find a drug that, apart from having a specific effect on the target receptor, must have good *solubility* and *bioavailability*, so it can overcome all the sequential hindrances to reach from a formulation to systemic circulation (1).

Solubility is very important parameter for a drug molecule to reach at site of action. Adequate levels of drug solubility are required, because solubility determines the amount of drug available for absorption (2). Several terms such as absorption, permeability and bioavailability need to understand to know how drug molecules are reaching the systemic circulation. Oral drug absorption is referred to as drug transfer across the apical membrane of an enterocyte, because the apical membrane is considered to be the rate limiting step for permeation of a membrane. While permeability is a general term describing how readily the drug is transferred through a membrane. The Bioavailability (F) is defined as the fraction of the dose that reaches the systemic circulation and can be described according to equation 1.1,

Equation 1.1 $$F = f_a * (1 - E_g) * (1 - E_h)$$

Where f_a is the fraction absorbed over the intestinal epithelia, E_g is the gut wall extraction and E_h is the hepatic extraction.

The Biopharmaceutical Classification System (BCS), which has been developed to provide a scientific approach for classifying drug compounds based on solubility as related to dose and intestinal permeability in combination with the dissolution properties of the oral immediate release dosage form (3). BCS can point to the solubility and permeability as important factors for the intestinal absorption of orally administered drugs (4).This classification divides the compounds into four classes (Table 1.1). The limit between having a high and low permeability has been set to a fraction absorbed (f_a) of 90 %. While, if it is to be classified as a highly soluble compound, the highest dose to be given should be soluble in 250 ml of aqueous media in the range from pH 1 to pH 7.5 (4, 5).

Table 1.1 The Biopharmaceutical Classification System (BCS).

Class I High solubility High permeability	**Class II** Low solubility High permeability
Class III High solubility Low permeability	**Class IV** Low solubility Low permeability

The BCS classification have certainly reduced costs and time in the drug development process, both directly and indirectly, and reduce unnecessary drug exposure in healthy volunteers. It has been shown to be very useful for identifying the rate-limiting step and predicting intestinal drug absorption based on primary biopharmaceutical properties such as solubility and effective intestinal permeability (P_{eff}).

Methods for permeability/bioavailability determinations

Permeability data from humans is based on bioavailability studies. The best and safest way to classify drugs as highly permeable is an absolute bioavailability study with a bioavailability greater than 90 %. There are various methods (described below) which have been accepted by FDA for permeability determinations (6).

(a) Extent of absorption in humans

⇒ Mass balance pharmacokinetic studies

⇒ Absolute bioavailability studies

(b) Intestinal permeability methods

⇒ *In vivo* intestinal perfusions studies in humans

⇒ *In vivo* or *in situ* intestinal perfusion studies in animals

⇒ *In vitro* permeation experiments with excised human or animal intestinal tissue

⇒ *In vitro* permeation experiments across epithelial cell monolayers

Good data was found in papers using the intestinal perfusion technique in humans, as these provided direct permeability data. Data from experiments with cell culture monolayers (mostly Caco-2 cells) were often available. However, absolute values reported can vary by orders of magnitude among laboratories (7).

Factors influencing bioavailability of drugs

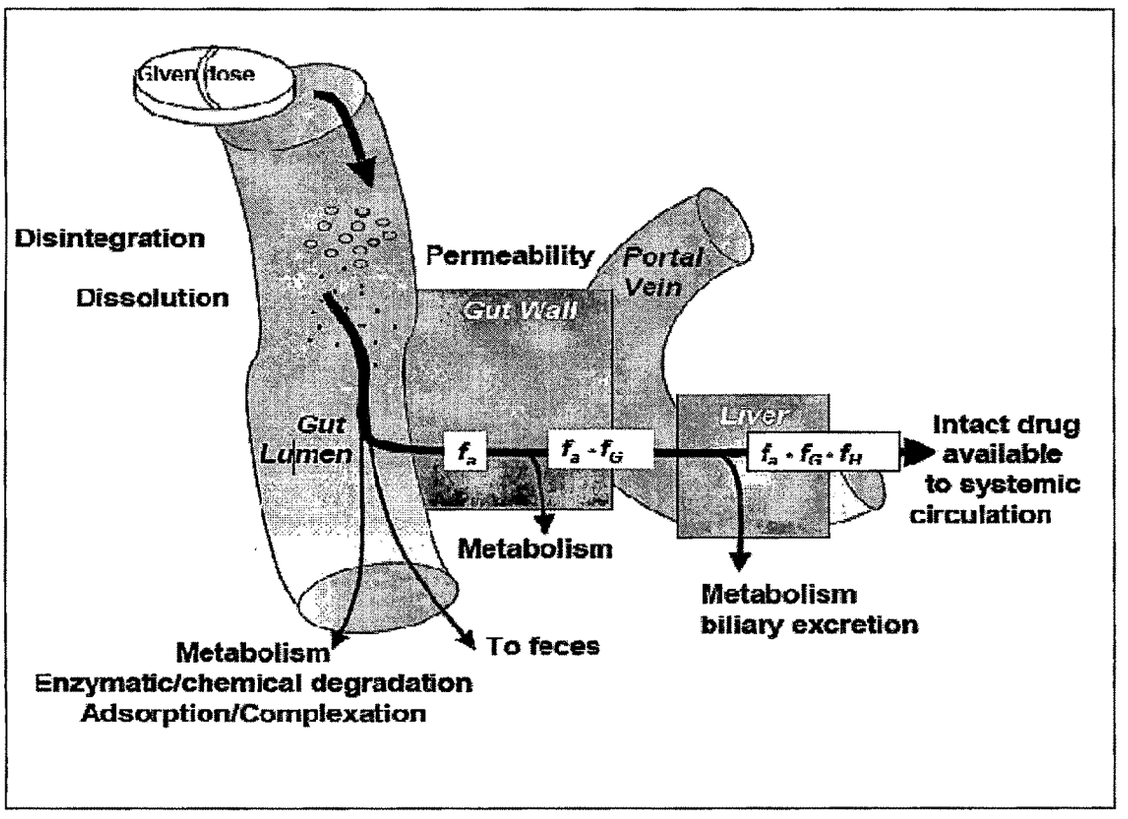
In cases where the bioavailability was lower than 90 %, the influence of the following mentioned factors has to be taken into account. There are several factors (Figure 1.1) which influence the permeability/bioavailability of the compounds, such as

1. Biopharmaceutical factors/ Factors related to the drug and to the dosage form
2. Pharmacokinetic factors/ Factors related to patient
3. Physiological factors related to Absorption,
   - Gastrointestinal barrier
   - Gastrointestinal (GI) pH
   - Gastrointestinal motility and emptying
   - Vascularity and Blood flow
   - Instability of drugs in the GI tract

- Donnan Effect
- Drug interaction and Complexation
- Malabsorption

4. Drug Dispositioning effect and variation in plasma level
   - Presystemic metabolism
   - Partition in the body fat
   - Metabolism and biotransformation
   - Excretion
5. Factors related to patients
   - Age
   - Sex
   - Diseased condition
   - Genetic make up

Figure 1.1 The schematic illustration of factors influencing the route of the systemic circulation after oral administration of formulation (Modified from 1).



Strategies to improve bioavailability of drugs

As described earlier, oral delivery remains the most favourable and preferred route for drug administration. Currently more than 60 % of drugs are marketed as oral products. These drugs cannot be effectively delivered by the oral route of administration in their original form due to above all factors. Overcoming these barriers is currently one of the most challenging goals in oral drug delivery (8,9,10). Several strategies have been employed to improve the bioavailability of drugs after oral administration. Following are some of the strategies may be applied alone or in combination to provide a solution to the problem of poor bioavailability (11).

1. Manipulation of physico-chemical factors of drug
    - # Micronisation of active drug
    - # De-aggregation of micronised particles by protective colloids
    - # Selection of correct Polymorphic form of the drug
    - # Lipophilicity and Enzyme susceptibility
2. Improvement of solubilization of active drug
    - # Chemical derivatization (salt or ester formation)
    - # Use of inclusion compounds and Complexation
    - # Manipulation of solid phase
3. Novel drug delivery carrier system/ Dosage form designing
    - # Film coating , enteric coating
    - # Development of liposomes and nanoparticles
    - # Targetted drug delivery
4. New concepts
    - # Receptor reorganisation
    - # Utilization of bioenhancers which alter the physiology of absorbing membrane
    - # Utilization of chemicals which alter the metabolism of drug by inhibiting Cytochrome 450 (CYP450) enzymes or P-gp efflux proteins
    - # Co administration of chemicals with drug which exerts specific physiological action or has capacity to exert a series of physiological actions which improves the bioavailability of the drug of interest

Although some of these approaches have been demonstrated to be successful in laboratory scale research, they still present challenges in terms of long-term safety and reproducibility in the clinical situation. Even various sophisticated novel drug delivery approaches have been attempted [12] to improve bioavailability, which has less market potential due to its high cost of manufacturing and lack of stability.

Utilization of bioenhancers which alter the physiology of absorbing membrane

In recent years a variety of bioenhancers has been developed to improve the absorption characteristics of poorly absorbed drugs whose absorption is limited by polarity, ion charge and molecular size. These bioenhancers are certain non-toxic excipients which reversibly, specifically or non-specifically increases the permeation of low permeable drugs. These bioenhancers include surfactants, bile salts, chelating agents, and fatty acids [13,14]. These bioenhancers are also called as absorption enhancers or permeation enhancers or penetration enhancers. These bioenhancers are classified on the basis of mechanism of action and their structure (14) described in Table 1.2.

These all are synthetic or semi synthetic bioenhancers. Epithelial permeability of these enhancers have long been studied, their use has been limited due to a large body of data suggesting a there are more toxicity than efficacy. The development of safer and effective bioenhancer is an active area of research for use in clinical practice.

Table 1.2 The Classification of various bioenhancers.

No.	Bioenhancers	Examples
1	Surfactant	Sodium laural sulphate, Tween 80, Polysorbates etc.
2	Fatty acids and derivatives	Sodium caprate, Acylcarnitine, Caprylic acid, Lauric acid, oleic acid etc.
3	Bile acids and derivatives	Sodium glycocholate, Sodium deoxycholte, Sodium glycodihydrofusidate etc.
4	Chelating agents	EDTA, Citric acid, Salicylates
5	Sulfoxides	Dimethyl sulfoxides, Decylmethyl sulfoxide
6	Polyols	Propylene glycol, Polyethylene glycol, Glycerol etc.
7	Monohydric alcohols	Ethanol, Isopropanol
8	Steroidal Detergents	Saponins and pentacyclic triterpenoids
9	Others	Chitosan and derivatives, Cyclodextrins, Acyl acarnitine and Alakanoycholines, Terpenes, Urea & its derivatives

Herbal origin bioenhancers

There are certain herbal origin bioenhancers which are called as natural bioenhancers. When these natural bioenhancers mixed with drug enhances the effectiveness of drug without affecting drug's properties. These natural bioenhancers are effective and have less toxicity than the synthetic bioenhancers. Due to their high efficacy as bioenhancers, they can reduce the dose of drug and drug resistance is also minimized. Toxicity is reduced due to reduce dose of drugs, e.g. specifically anticancer drugs. The classification of natural bioenhancers is on the basis of chemical properties, and is described in Table 1.3.

The concept of natural bioenhancers is derived the traditional old system of Ayurveda (science of life). In Ayurveda, black pepper, long pepper and ginger are collectively termed as Trikatu. In Sanskrit "Trikatu" means three acrids. The action of these bioenhancers first

documented by Bose (15) who described that addition of long pepper to vasaka (*Adhatoda vasica*) leaves increased antiasthmatic properties of vasaka leaves.

Natural bioenhancers are drug facilitators they are the molecules which by themselves do not show typical drug activity, but when used in combination enhance the activity of drug molecules. In general, these substances can promote the permeability by opening the tight junctions between the intestinal epithelial cells or by inhibiting the efflux transporters located at the apical membranes of the enterocytes (16). The modulation of efflux systems is of particular interest, since many pharmaceutically acceptable excipients can inhibit secretory transporters, including *P*-glycoprotein and several multi-drug resistance associated proteins (MRPs) at very low concentrations (17,18). They act as bioenhancers by several ways including:

- → Altering the physiology of the absorbing membrane
- → Altering the metabolism of drug by inhibiting cytochrome P450 enzymes or P-gp efflux proteins (drug transporters)
- → By increasing the blood supply to the gastrointestinal tract, decreasing the hydrochloric acid secretion which prevents breakdown of some drugs or by increasing the emulsifying content of the gut
- → Acting as receptor for drug molecule
- → Making target cells more receptive to drug and so on

Table 1.3 The herbal origin bioenhancers.

Bioenhancers	Source	Brief mechanism
Piperine (Alkaloid) (19)	*Piper spp.*	Inhibit certain metabolizing enzymes, modulate P-gp efflux
Capsaicin (Alkaloid)	*Capsicum spp.*	inactivate CYP450 2E1 by irreversibly binding to the active sites of the enzyme
Flavonoids (20) (polyphenolic compounds)	Secondary metabolites of plants	modulate P-gp efflux, inhibit the CYP450 metabolizing enzymes
Furanocoumarins, Flavonoids (21)	Grape fruit Juice	Inhibit CYP450 isoenzymes
Bergapten (22)	Seville orange juice	Inhibit CYP450 3A4 activity
Quercitin	Citrus fruits, *Ginkgo biloba*, St. John's Wort	Modulator of P-gp efflux pump
Flavonolingnans (Milk thistle)	*Silybum marianum*	Inhibit P-gp efflux pump
Catechin (23)	Green Tea	Inhibit P-gp efflux pump
Curcumin	*Curcuma longa*	Inhibit P-gp efflux pump
Resveratrol (24)	eucalyptus, spruce, and lily	Inactivation of CYP450 3A4
Saponins (25)	Glycyrrhiza glabra	Act on paracellular route
Saponins	*Sarsaprila spp*	Act on transcellular route
Ginsen triterpenoids	Ginseng	moderate inhibitory effect on the P-gp efflux pump
Volatile oils and Fixed oils	Peppermint oil and sunflower oil	
Lectins	*Solanum tuberosum*	immobilizes the drug on the intestinal surface for long time
Niaziridin (26)	*Moringa olifera*	

Many of the natural bioenhancers act on certain transporters. These transporters are ideally situated to act as first line defence by limiting the absorption of potentially toxic 'foreign' compounds. Transporters may also play a crucial role in limiting drug absorption through drug secretion into the intestinal lumen. Some of these transporters are efflux drug transporters which are principally ABC proteins such as P-glycoprotein (P-gp) and MDR (Multi-drug Resistance Protein). Several active uptake transporters are located in the epithelium of the small intestine. On the apical membrane of the enterocytes, a peptide transporter (hPepT1) takes care of the transport of di- and tripeptides. Following are some of the transporters,

P-glycoprotein

In 1976 Juliano and Ling found a correlation between the degree of drug resistance of Chinese hamster ovary cells and the presence of a 170 kDa membrane glycoprotein. The glycoprotein appeared to be unique to mutant cells displaying altered drug permeability, so they named it P-glycoprotein where the "P" stands for "permeability" (27). P-gp is a phosphor-glycoprotein belonging to the ATP (adenosine triphosphate)-binding cassette (ABC) transporter super-family. ABC transporters have a highly conserved ATP binding region, which is characteristic for these transporters. Over 200 ABC transporters are known and they exist in a wide variety of species, ranging from bacteria to humans, and are found in association with importation or export nutrients, amino acids, sugars, peptides or hydrophobic substances (28).

The P-glycoprotein can transport compounds with a broad range of chemical structure out of a cell through the consumption of energy. This phenomenon has been referred to as multi-drug resistance – MDR (29), a name which has been given to the genes encoding for P-gp. Humans have one gene for multi-drug resistance, the MDR1. It was discovered that P-gp was expressed not only in tumor cells but in many normal cells in various organs such as the liver, kidney, intestine and brain in humans and animals (30).

It has been suggested that the function of P-gp is to protect the body and important organs, such as the brain, from naturally occurring toxic substances. The function of P-gp in the intestine is not fully understood but it has been suggested that intestinal efflux transport by

P-gp and other efflux proteins can affect the rate and extent of drug absorption and metabolism in rat and human intestine (31, 32) Intestinal transporters, such as P-gp, could also cause drug-drug interactions due to changes in drug pharmacokinetics (33). It could also be possible to increase the bioavailability after oral administration and transport across the blood-brain barrier for some drugs and to further understand drug-drug interactions.

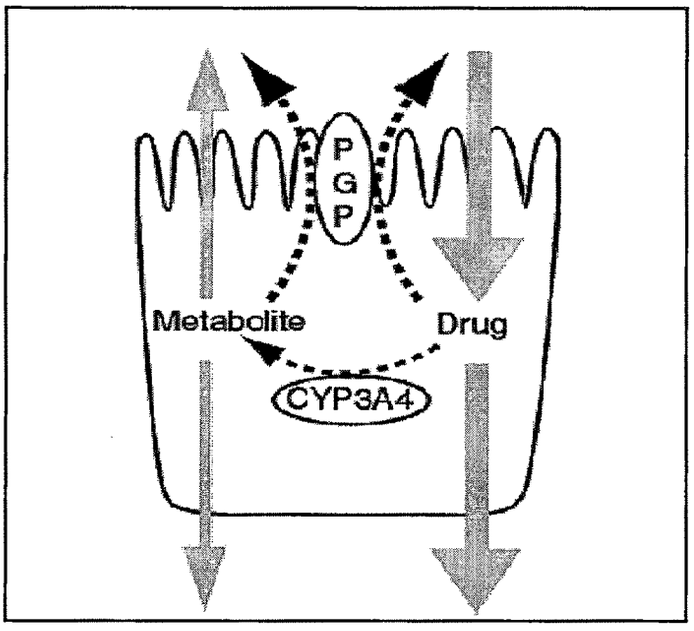
Cytochrome P450 (CYP) enzymes

The liver is the major site of metabolism for orally administered drugs, but it has been shown that intestinal metabolism can also be important for drug bioavailability (34,35). The cytochrome P450 (CYP) enzymes mediate phase I metabolism, which includes the oxidation, reduction or hydroxylation of drug compounds. Metabolism is generally the modification of molecules to make them more water soluble, to facilitate excretion from the body. In humans, it has been shown that CYP3A4 is the most important phase I enzyme involved in drug metabolism as approximately 50 % of registered drugs are CYP3A4 substrates (36). The CYP3A subfamily constitutes 20 % of CYP content in liver and 50-70 % of CYP content in the intestine (37). Metabolism is often measured using liver microsomes, but these results can't be extrapolated to determine the intestinal metabolism as there might be a lack of correlation between hepatic and intestinal CYP isoforms.

Overlapping effect of CYP3A4 and P-gp

CYP3A4 and P-gp have complete overlap in substrate specificity and they are commonly localized near the apical membrane of an enterocyte (38,39). They are regulated by the orphan nuclear pregnane X receptor (PXR) (40). It has been suggested, that CYP3A4 and P-gp cooperate in transport and metabolism of drugs in the intestinal membrane. According to this theory, P-gp slows down drug penetration rate and increases drug exposure to the CYP3A4 enzyme by recycling the drug in an enterocyte (41,42). It has also been suggested that this interplay includes P-gp mediated active transport of the metabolites formed to the intestinal lumen (Figure 1.2) (31).

Figure 1.2 The schematic illustration of how CYP3A4 and P-glycoprotein might co-operate in the enterocyte.

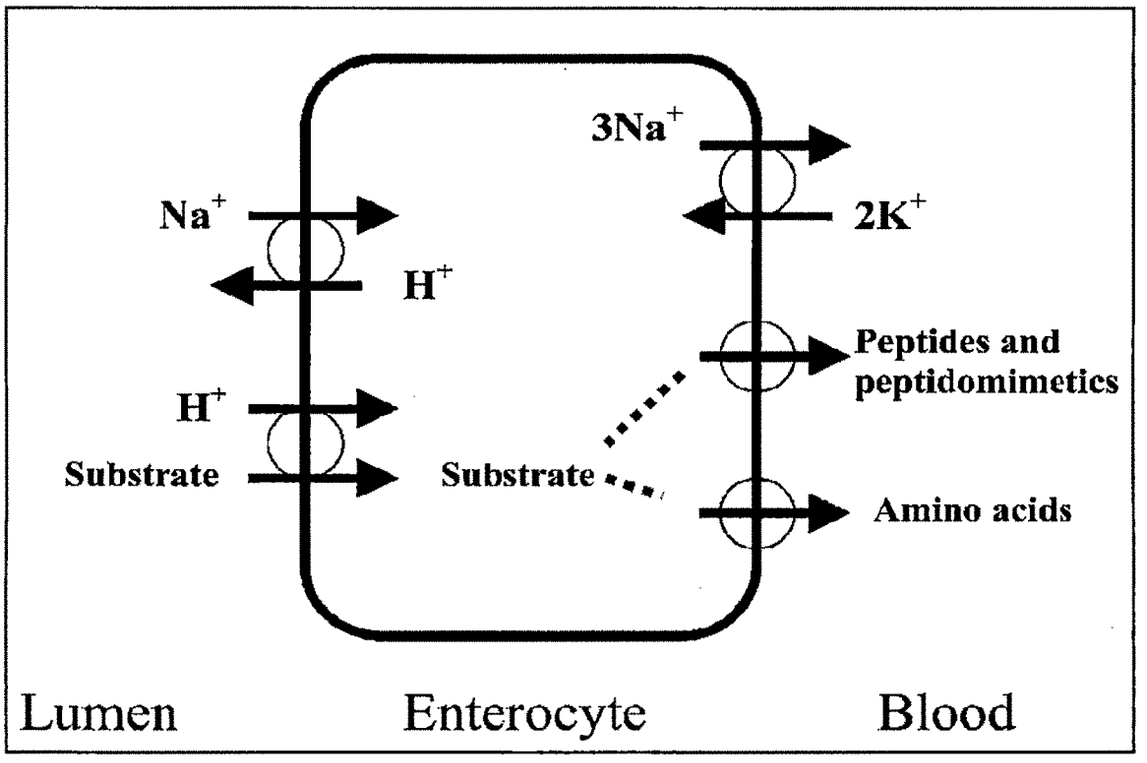


Peptide transport system

Di- and tripeptides and their structural analogues are actively transported into the intestinal epithelial cells by peptide transporter (43). Following the cloning of the di/tripeptide transporter it was possible to demonstrate that PepT1 was responsible for the carrier-mediated uptake of these analogues (44,45).

PepT1 is a member of a family of transport proteins, termed POT (Proton-dependent Oligopeptide Transporters), (46) in humans only two members of the POT family has been shown to possess transport activity, namely hPepT1 and hPepT2. PepT1 is expressed in the small intestine (47). Peptides are transported from the intestinal lumen into the cells via the apical, proton-dependent peptide transporter PepT1. Once inside the cells, peptides can be hydrolysed by cellular enzymes and cross the basolateral membrane as amino acids or they can be effluxed into the blood via a basolateral peptide transport system. A proton gradient across the apical membrane is maintained by the activity of an apical Na^+/H^+ exchanger, which in turn is energised by the basolateral Na^+/K^+-ATPase. The apical proton gradient enhances the uptake of peptide substrates (48).

Figure 1.3 The PepT1 peptide transport pathway.



1.1 Aims and Objectives

The main objective was to investigate the effect of natural bioenhancers such as Piperine (PI) and Glycyrrhizic acid ammonium salt (GA) on permeability and bioavailability of class III drugs. Various methods were used to know the mechanism by which natural bioenhancers are affecting the bioavailability. In the present study, PI and GA effectively combined with BCS class III drugs, to enhance permeation properties of the same, thereby to improve bioavailability and therapeutic efficacy of drugs.

Prime objectives of the present work were,

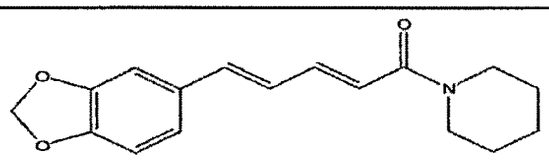
1. To develop and screening of suitable method for combination of drugs (BCS class III drugs such as atenolol and lisinopril) with the natural bioenhancers, Piperine and Glycyrrhizic acid ammonium salt.
2. To set evaluation parameters for bioenhancers-drug binary systems.
3. To investigate *ex-vivo* permeation pattern of drugs and drugs in presence of bioenhancers in binary systems.
4. To perform *in vitro* cell line study (Caco-2 cells) of drugs and drugs in presence of bioenhancers in binary systems.

   Specifically,

   a. To assess the effect of bioenhancers on the epithelium and integrity of tight junctions.
   b. To determine the influence of bioenhancers on permeation of drugs is dependent on its concentrations.
5. To develop simple and cost effective bio-analytical method for the developed bioenhancers-drug binary systems.
6. To perform in vivo studies of the bioenhancers-drug binary systems using rat as an animal model.
7. To draw significant conclusions by statistical treatment to data obtained.

1.2 Bioenhancers Profiles

Piperine (1-piperoyl piperidine)

Parameter	Properties
Source	Piperine is an alkaloid found naturally in plants belonging to the *Piperaceae* family, such as *Piper nigrum* L, commonly known as black pepper, and *Piper longum* L, commonly known as long pepper (49).
Description	Piperine is naturally a more yellowish powder, while after synthesis it has a stronger green tint to it. It is trans-trans stereoisomer of 1-piperoylpiperidine.
IUPAC name	Piperine (1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine
Molecular structure	
Molecular formula	$C_{17}H_{19}NO_3$
MW	285.34
MP	128° to 132° C
Solubility	Piperine is soluble in alcohol, chloroform, ether, benzene and hot water. It is not very reactive unless in a solution.
UV maxima (nm)	340 nm

Piperine is used world wide as spices and as ingredients in traditional systems of medicine. Black pepper and long pepper have been used in Ayurvedic medicine for the treatment of various diseases (50). One such preparation is known by the Sanskrit name trikatu and consists of black pepper, long pepper and ginger (51). Another preparation, known by the Sanskrit name pippali, consists of long pepper. Piperine in higher doses shows many pharmacological activities, while in low doses it is acting as bioenhancer.

Pharmacological activity

→ Piperine has chemopreventive action it is known to exert various protective effects by altering the metabolism of carcinogens, thereby preventing initiation stage of carcinogenesis (52,53).

→ Piperine has been proved to inhibit the cytochrome P450 enzymes in hepatic and pulmonary tissues (54).

→ The fruit of *Piper longum* is an important component of indigenous Indian medicines for dyspnoea, cardiac diseases, piles, bronchitis and fever (55).

→ Piperine is stimulant and nervine. It stimulates CNS activity in the brain stem, reverses respiratory depression (56). The stimulating activity has been postulated to occur in the brain stem controlling vital centers such as respiratory, circulation and the biological cycle of sleeping and waking.

→ Piperine belong to a class of substances that are anticonvulsant (57) as well anti-ulcer activity (58).

→ Piperine also reduces pain perception through reduction of nerve transmission of Substance P, probably in the spinal cord (59).

→ Piperine has anti-allergic and anti-inflammatory effects. It has been shown to lower the level of liver lipid peroxides known to correlate positively with the degree of inflammation. Piperine lowers the level of mast cell degranulation.

Piperine as bioavailability enhancer

Piperine has been patented and reported as bioavailability enhancer for some of food nutrients and drugs. Piperine acts as bioenhancer in low dose range such as 1-20 mg only, which is safe and not showing any toxic effects. It may acts as bioenhancer by various known and unknown causes, such as:

It binds to certain enzymes of liver and prevents their biotransformation, thus increasing their bioavailability (60).

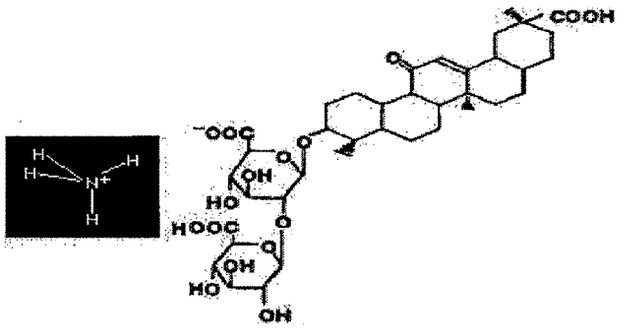
P-glycoprotein and CYP3A4 are present in the gut wall mucosa and cause metabolism in the enterocytes, can play an important role for low or variable oral bioavailability of drugs. Piperine inhibits the functions of both P-glycoprotein and

CYP3A4 and increase permeation of drugs which are substrate of P-glycoprotein and CYP3A4 (61).

Piperine stimulates thyroid gland and action of thyroid hormones with a stimulation of tissue oxygen uptake. Cellular studies showed an increase in the activity of mitochondrial enzymes, ATP_{ases} which release stored energy from ATP. These cause diet induce thermogenesis. It stimulates metabolism and thermogenesis in conjunction with increased nutrient absorption (62).

Effect on gastro intestinal tract: It Increases the amount of mucosal pancreatic secretions reduces gastric acid secretions and ulcerations, increases glucose absorption. It also interacts with the cells of small intestine and increasing their ability to absorb more amino acids.

Glycyrrhizic acid ammonium salt

Parameter	Properties
Source	Glycyrrhizic acid obtained in form of a mixed K-Ca-Mg salt (glycyrrhizin) in the dried rhizomes and roots of licorice plants of the *Glycyrrhiza glabra L.* and *Glycyrrhiza uralensis F.* species. It is triterpene glycoside which is converted to glycyrrhetinic acid (the aglycone) and two moles of gucuronic acid (the glycone) on hydrolysis (63).
Description	White to pale yellowish powder.
IUPAC name	3-*O*-(2-*O*-β-D-Glucopyranuronosyl-α-D-glucopyranuronosyl)-18β-glycyrrhetinic acid ammonium salt.
Molecular structure	
Molecular formula	$C_{42}H_{61}O_{16}{\cdot}NH_4$
MW	839.96
MP	209° C
Solubility	Glycyrrhizic acid ammonium salt is soluble in water and alcohol.
UV maxima (nm)	254 nm

Glycyrrhizinic acid is potentially 50 times sweeter than sucrose. Licorice (liquorice in British spelling) is the alteration of Glycyrrhiza glabra, a Mediterranean perennial plant with blue pealike blossoms. Glycyrrhiza is the active principle for sweetening, flavoring and pharmaceutical applications.

Pharmacological activity

→ It has corticosteroid-like structure, thus is useful as an anti-inflammatory agent. The activity has been compared with glucocortocoid hormone. It has been proposed that glycyrrhizin promotes regeneration of inflammatory tissue (64).

→ It is effective in treatment of peptic ulcer and antiviral activity as well effective as inhibitor of tumour promoters.

→ Glycyrrhizin has demonstrated heptoprotective activity in animal models against carbon tetrachloride induced toxicity (65).

→ It is useful as co-emulsifier to treat skin disorders and in cosmetics and highly effective for acute and chronic dermatitis.

→ It is used in brewing and for confectionery and tobacco flavourings. It is frequently used in medicines to mask the unpleasant flavours (66).

→ It is highly effective for coughs and used as a mild laxative. It promotes the ejection of mucus or exudate from the lungs, bronchi, and trachea; sometimes extended to anti-tussives.

Glycyrrhizic acid ammonium salt as bioavailability enhancer

Glycyrrhizic acid ammonium salt has been patented (67) and reported as bioavailability enhancer for some of drugs. It acts as bioenhancer by several known and unknown causes.

Glycyrrhetinic acid, one of the main constituents of licorice root, induces an oxidative stress in liver mitochondria responsible for the induction of membrane permeability transition by interacting with the mitochondrial respiratory chain (68).

It causes activation of calmodulin dependent kinase and leads opening of the paracellular pathways (69).

1.3 Selection of Drugs

Cardiovascular diseases are diseases of the heart (cardiac muscle) or blood vessels (vasculature). WHO reports that 1 out of every 3 deaths in India is due to heart diseases. Amongst several risk factors associated with this growing menace, hypertension has been established as a key risk factor for heart diseases (70).

When the heart pumps blood into the arteries, the blood flows with a force pushing against the walls of the arteries. *Blood pressure* is the product of the flow of blood times the resistance in the blood vessels. High blood pressure is also called *Hypertension.*

Hypertension does not mean excessive emotional tension, although emotional tension and stress can temporarily increase blood pressure. Normal blood pressure is below 120/80; blood pressure between 120/80 and 139/89 is called "pre-hypertension", and a blood pressure of 140/90 or above is considered high (71). Hypertension can be classified,

→ Essential (primary): There is no specific medical cause to explain a patient's condition
→ Secondary: The high blood pressure is a result of (*i.e.*, secondary to) another condition

There are several causes responsible for primary hypertension includes obesity, salt sensitivity, renin homeostasis, insulin resistance, genetics, and age. While secondary hypertension is due to several diseased conditions such as polycystic kidney disease, aldosteronism, cushing's syndrome, genetic and drugs consumptions.

There are many classes of medications for treating hypertension, together called antihypertensives, which — by varying means — act by lowering blood pressure. Evidence suggests that reduction of the blood pressure by 5-6 mmHg can decrease the risk of stroke by 40 %, of coronary heart disease by 15-20 %, and reduces the likelihood of heart failure and mortality from vascular disease. These are various antihypertensive agents,

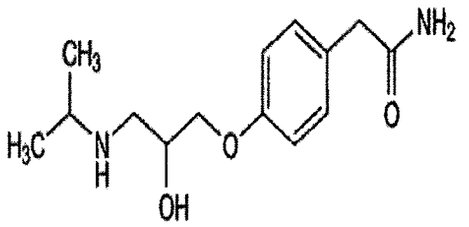
- ⇒ ACE inhibitors such as captopril, enalapril, fosinopril (Monopril), lisinopril (Zestril), quinapril, ramipril (Altace)
- ⇒ Angiotensin II receptor antagonists: eg, telmisartan (Micardis, Pritor), irebesartan (Avapro), losartan (Cozaar), valsartan (Diovan),candesartan (Amias)
- ⇒ Alpha blockers such as prazosin, or terazosin.
- ⇒ Beta blockers such as atenolol, labetalol, metoprolol (Lopressor, Toprol-XL), propranolol.
- ⇒ Calcium channel blockers such as nifedipine (Adalat) amalodipine (Norvasc), diltiazem, verapamil
- ⇒ Direct renin inhibitors
- ⇒ Diuretics: eg, bendroflumethiazide, chlortalidone, hydrochlorothiazide
- ⇒ Combination products

β adrenergic-receptor blockers have been used widely in the treatment of hypertension and are recommended as first-line drugs in hypertension guidelines. The β blockers were originally synthesised by Sir James Black for the treatment of coronary heart disease. In the present study, we selected the drugs on the basis of their classes (BCS classification, specifically class III which have high solubility and low permeability) and market potentials. Atenolol is a cardio selective β blocker, widely used in the management of hypertension, angina pectoris, cardiac arrhythmia's, and myocardial infarction (72). It is always a choice of molecule when β blockers are used for the treatment.

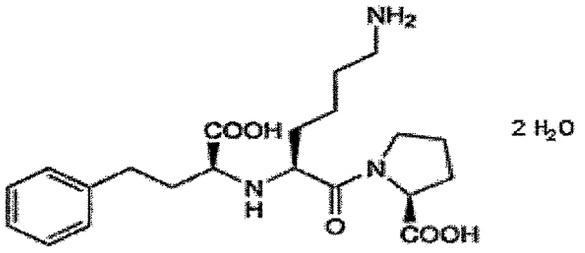
ACE inhibitors have been shown to improve survival and reduce morbidity in patients with heart failure or myocardial infarction. Lisinopril was selected as another model drug which is also BCS class III drug.

1.4 Drugs Profiles

Atenolol

Parameter	Properties
IUPAC name	4-[2-Hydroxy-3-[(1- methylethyl) amino]propoxy] benzeneacetamide
Molecular structure	
Molecular formula	$C_{14}H_{22}N_2O_3$
MW	266.3
MP	146° to 148° C
Solubility	Soluble in water, isopropanol, acetic acid and dimethyl-sulfoxide, freely soluble in methanol, very slightly soluble in acetone and dioxane
Lowest solubility	1 mg/ml
pKa	9.6 (24° C)
LogP (octanol/water)	0.23
UV maxima (nm)	Aqueous acid—274 nm, 280 nm.
Dose	25, 50, 100 mg
Bioavailability	About 50 %

Lisinopril

Parameter	Properties
IUPAC name	*S*)-1-[*N*2-(1-Carboxy-3- phenylpropyl]-l-lysyl]-lproline
Molecular structure	
Molecular formula	$C_{21}H_{31}N_3O_3 \cdot 2H_2O$
MW	441.5
MP	160 °C
Solubility	Soluble in water, methanol; insoluble in alcohol, acetone, chloroform and in ether.
Lowest solubility	33 mg/ml
pKa	2.5, 4.0, 6.7, 10.1 (25° C)
LogP (octanol/water)	1.22
UV maxima (nm)	Aqueous alkali and acid — 258 nm.
Dose	2.5 to 20 mg daily with a maximum of 40 mg daily.
Bioavailability	About 25 to 50 %

Thus pharmacokinetic data available from the literature and drugs profiles it reveals that atenolol and lisinopril are poorly bioavailable and belongs to BCS class III i.e. highly soluble and low permeable. For the present study, these both drugs atenolol and lisinopril were selected and effect of bioenhancers on its permeability was determined.

In further chapters bioenhancement of both the drugs were done using both natural bioenhancers. In the study for both the drugs binary systems of drug-bioenhancers were made with different methods. These binary systems of each method were evaluated for physical characterisation such as Differential scanning calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR).

Various methods are available for predicting intestinal absorption of drugs. Animal in vitro, in situ and in vivo techniques are available for permeation determinations. Therefore each binary system of each method was evaluated for *ex vivo* permeation study using goat as an intestinal membrane. Further binary systems were evaluated for *in vitro* permeation experiments across epithelial cell monolayers, and *in vivo* pharmacokinetic studies in animals (rat). Each of these methods has been accepted by FDA.

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